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Small molecule inhibitors of reactive oxygen species production

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KEYWORDS. Reactive oxygen species (ROS); ROS inhibitors; small molecules; monoamine oxidase (MAO); xanthine oxidase (XO); NADPH oxidase (NOX); nitric oxide synthase (NOS).

ABSTRACT: Reactive oxygen species (ROS) are involved in physiological cellular processes including differentiation, proliferation, and apoptosis by acting as signaling molecules or regulators of transcription factors. The maintenance of appropriate cellular ROS levels is termed redox homeostasis, a balance between their production and neutralization. High concentrations of ROS may contribute to severe pathological events including cancer, neurodegenerative and cardiovascular diseases. In recent years, approaches to target the sources of ROS production directly in order to develop tool compounds or potential therapeutics have been explored. Herein, we briefly outline the major sources of cellular ROS production and comprehensively review the targeting of these by small molecule inhibitors. We critically assess the value of ROS inhibitors with different mechanisms-of-action, including their potency, mode-of-action, known off-target

effects and their clinical or preclinical status, while suggesting future avenues of research in the field.

1. INTRODUCTION

Reactive oxygen species (ROS) are chemical species derived from the partial reduction of O_2 that can be either free radicals, including superoxide ($O_2\bullet^-$), hydroxyl (OH•) and hydroperoxyl (HOO•) radicals, which are characterized by having an unpaired electron, or non-radical species with a high oxidation potential, such as hydrogen peroxide (H_2O_2).[1,2] ROS produce different cellular effects according to their concentration and site of production in the cell (Figure 1).

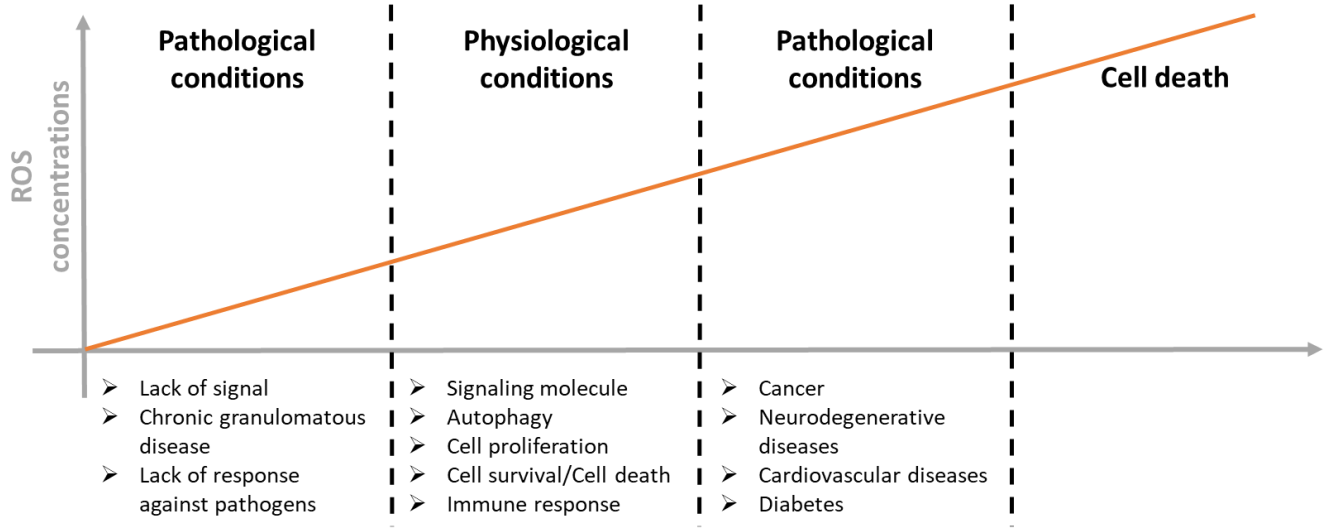

Figure 1. Concentration-dependent physiological and pathological conditions correlated to intracellular ROS levels.

At physiological concentrations, ROS play essential regulatory roles in maintaining intracellular homeostasis through signaling functions, regulation of transcription factors and protein phosphorylation. They are involved in different cellular processes including differentiation,

proliferation, growth and apoptosis.[3] Pathological responses related to ROS occur either in their absence,[3] as in the case of chronic granulomatous disease,[4] or in the case of their overproduction due to a disruption in the reduction-oxidation (redox) balance within a cell.[5] Abnormally low ROS levels impact signaling pathways and host defense and are therefore connected to specific types of autoimmune disorders or decreased defenses against bacterial pathogens.[3] In contrast, excessive ROS concentrations can create cytotoxic events due to protein, lipid and DNA damage through so-called oxidative stress, and have been reported to be involved in aging and general inflammation[6] as well as in several pathologies. For this reason, molecules that target the mechanisms behind ROS production, with the ability to restore physiological ROS levels, have considerable therapeutic potential and their development has attracted increasing attention in recent years. In this review, we discuss approaches to reduce ROS production using small molecules. We compile a comprehensive list of small molecule ROS inhibitors in various stages of (pre)clinical development and provide a detailed discussion of their modes-of-action and limitations.

1.1 ROS types. ROS are products of normal anaerobic metabolism[6] and are created either by excitation or by reduction of atmospheric oxygen (O_2, or dioxygen).[7] O_2 can be the source of radical derivatives such as superoxide ($O_2^{\bullet -}$), hydroxyl ($HO^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), carbonate ($CO_3^{\bullet -}$), and carbon dioxide radicals ($CO_2^{\bullet -}$), as well as non-radical ROS including singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hypobromous acid (HOBr) and hypochlorous acid (HOCl)[1] (Figure 2).

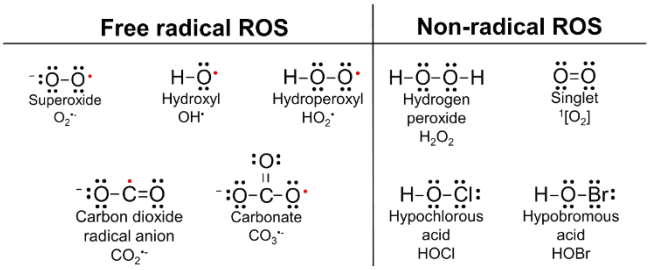

Figure 2. Lewis structures of ROS divided by free radical ROS (left) and non-radical ROS (right). Partially adapted from references 1 and 8.

The definition of a free radical is a chemical species which has one or more unpaired electrons.[1] Superoxide, $O_2^{\bullet-}$, is a radical anion which is stable for a short time, and is generated when O_2 undergoes a one electron reduction.[9] Subsequently, after a further reduction of superoxide to the peroxide anion (O_2^{2-}), hydrogen peroxide is formed by protonation. Hydrogen peroxide, through a Fenton reaction with Fe^{2+}, creates a hydroxyl radical, the most reactive oxygen radical. On the other hand, excitation of O_2 can lead to the formation of the highly reactive, non-radical, singlet oxygen.[7] The interconversion of the different ROS types can take place in the cell through non-enzymatic or enzyme-mediated mechanisms. An example is the dismutation of $O_2^{\bullet-}$ to O_2 and H_2O_2, which can progress in a spontaneous manner or catalyzed by superoxide dismutase (SOD).[10]

In addition to oxygen derivatives, the broader category of reactive species also includes reactive chlorine, bromine and nitrogen species. Reactive nitrogen species (RNS) are an important class of signaling molecules in living organisms. This class includes nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$), which is formed by the reaction of $NO^{\bullet}$ with $O_2^{\bullet-}$.[1,5] Whilst important, the discussion of RNS and related reactive species will only be addressed briefly in this review. Readers are directed to a comprehensive book chapter on the subject for further information.[11]

1.2 Main sites of ROS production and diffusion across membranes. The major cellular source of ROS are mitochondria[12] (Figure 3a). The mitochondrial electron transport chain (ETC)

in the inner mitochondrial membrane is where most mitochondrial ROS are produced, through oxidative phosphorylation (OXPHOS) generating adenosine triphosphate (ATP).[13] The process begins with electrons that are abstracted from $FADH_2$ (complex II) or NADH (complex I), produced in the tricarboxylic acid cycle, and transferred by the ETC to complex IV which, under normal conditions, produces water *via* the reduction of O_2. However, ROS are produced when the reduction does not proceed all the way to the formation of water but ends prior to the formation of superoxide. The mitochondrial membrane potential ($\Delta\psi m$) enables the generation of ATP by the action of the ATP synthase in complex V with re-direction of the protons back in the matrix. The formation of superoxide from the reduction of oxygen is generated by the existence of an electron leakage in the ETC process.[13]

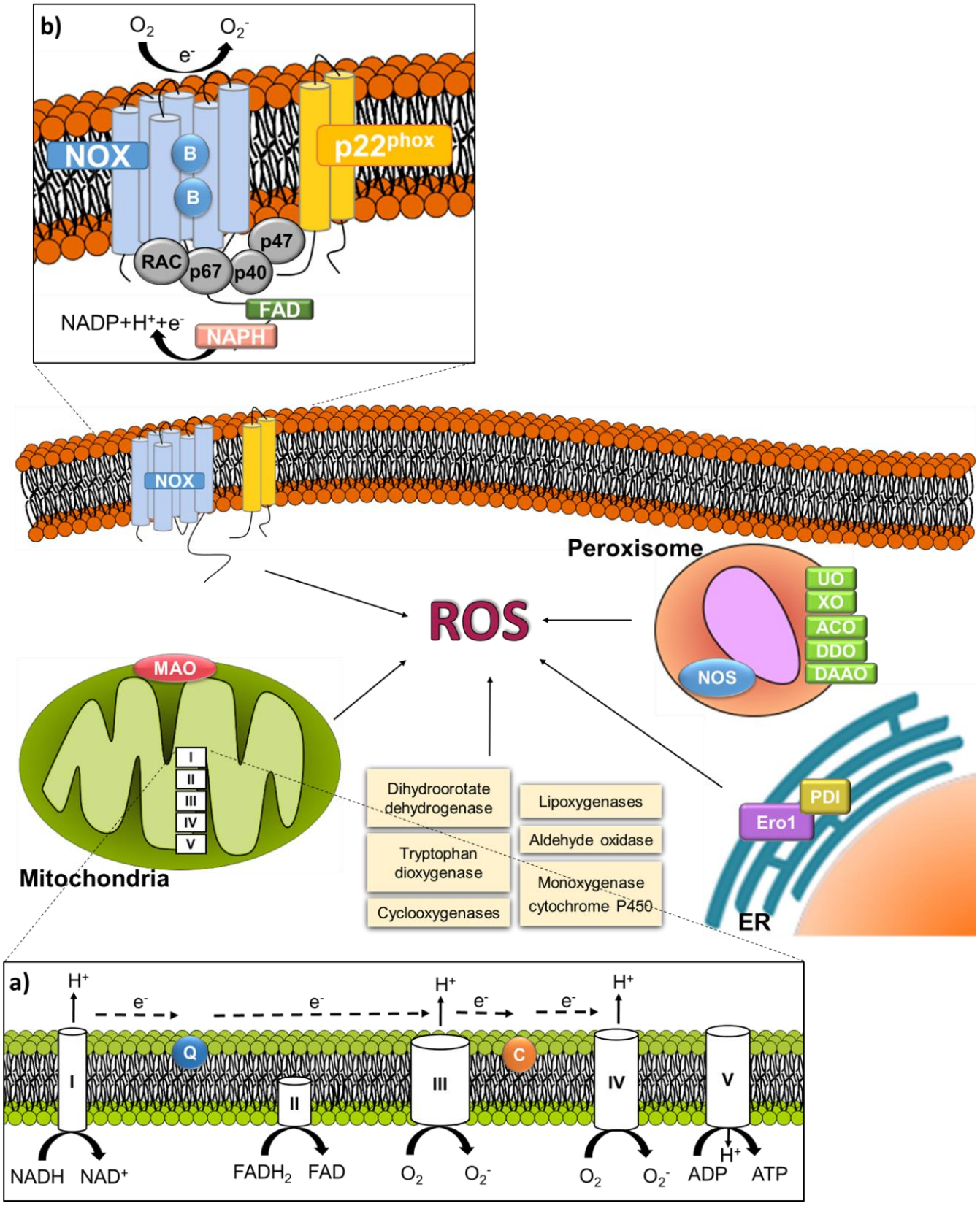
b)
O_2
O_2^-
e^-
NOX
B
B
p22phox
p47
RAC
p67
p40
FAD
NADP+H+e-
NAPH
NOX
Peroxisome
ROS
UO
XO
ACO
DDO
DAAO
NOS
MAO
I
II
III
IV
V
Mitochondria
Dihydroorotate dehydrogenase
Tryptophan dioxygenase
Cyclooxygenases
Lipoxygenases
Aldehyde oxidase
Monoxygenase cytochrome P450
PDI
Ero1
ER
a)
H^+
e^-
Q
C
I
II
III
IV
V
NADH
NAD^+
$FADH_2$
FAD
O_2
O_2^-
O_2
O_2^-
H^+
ADP
ATP

Figure 3. Overview of the main sites of endogenous ROS production. Bilayer: NADPH oxidases (NOX); Within the mitochondria: electron transport chain (ETC) – complex I to V, Monoamine oxidases (MAO); within peroxisomes: nitric oxide synthase (NOS), urate oxidase (UO), xanthine oxidases (XO), acyl-CoA (ACO), D-aspartate (DDO), and D-amino acid (DAAO) oxidases; within the endoplasmic reticulum (ER): ER oxidoreductin 1 (Ero1), protein disulfide isomerase family (PDI). The inlays describe the structure and ROS forming processes of: a) mitochondrial ETC (Q = ubiquinone, C = cytochrome c), adapted from reference 13, b) NADPH oxidase 2 (NOX2) with all associated subunits (B = cytochrome b), adapted from references 14 and 15.

In addition to mitochondria, several other ROS sources have been reported.[16] NADPH oxidases (NOX) are the only known enzymes to be exclusively dedicated to producing ROS.[14] NOX are a family of seven transmembrane proteins: NOX 1–5 and dual oxidases (DUOX) 1–2 possess different activation methods (*vide infra*) and tissue distribution. Before NOX was characterized in other tissues,[15] ROS production was thought to be limited to phagocytic cells.[15] The activation of NOX2, represented in Figure 3a as an example NOX family member, is characterized by the transient association between constitutively bound subunits p22/gp91 and cytoplasmic subunits p40, p47, p67, and rac. A conformational change occurs in response to the phosphorylation of p47, bringing p47 itself in contact with p22 and enabling the interaction of p67 with gp91, now in close proximity to p40. The last step consists of the interaction of rac with gp91 followed by p67 interaction[14] (Fig. 3b). In this context, the production of ROS by NOX is performed in two steps. The first consists of the transfer of electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to flavin adenine dinucleotide (FAD) and is followed by a second step involving the transfer of electrons to the heme group of the cytochrome b, which reduces molecular oxygen resulting in the production of $O_2^{\bullet -}$.[15]Another important class of ROS producing enzymes are

monoamine oxidases (MAO), which are specific flavoenzymes present in the outer mitochondrial membrane. Two MAO isoforms exist, MAO-A and MAO-B,[17] and their main biological role is the deamination of neurotransmitters and dietary amines.[18] MAOs are able to produce hydrogen peroxide through oxidative deamination, starting from monoamines and employing FAD as co-factor, forming aldehydes as by-products.[17]

Xanthine oxidoreductase (XOR) is a dimeric molybdenum-containing flavoprotein which, starting from hypoxanthine, forms xanthine and uric acid while catalyzing oxidation steps. In mammals, the dehydrogenase enzymatic form (XDH) can generate the xanthine oxidase form (XO). The latter is of critical importance for the formation of ROS, since it can deliver the electrons needed for the reduction of molecular oxygen and use them as an acceptor to form uric acid and superoxide/hydrogen peroxide from xanthine. XOR can also form reactive nitrogen species such as peroxynitrite via a similar mechanism.[19]

A source of reactive nitrogen species is nitric oxide synthase (NOS), the enzyme responsible for the biosynthesis of nitric oxide and RNS derivatives.[20] NOS exists in three isoforms, two constitutively expressed and regulated by Ca^{2+} and calmodulin (nNOS, neuronal nitric oxide synthase, and eNOS, endothelial nitric oxide synthase), and an inducible form, iNOS (inducible nitric oxide synthase). The isoforms have different cellular functions correlated to their different tissue localization. nNOS, or type I NOS, is the neuronal NOS and it serves as regulator of the synaptic transmission. The endothelial localization of eNOS (type III NOS), makes it responsible for the control of blood pressure, while type II NOS, known as iNOS, is induced in response to infection and inflammation in specific locations such as macrophages, monocytes, the endothelium, and smooth muscle tissue.[21] The synthesis of nitric oxide is carried out by all isoforms using molecular oxygen, NADPH and L-arginine as substrates. Electrons are transferred

from NADPH to heme in the N-terminal oxygenase domain, passing though FAD and flavin mononucleotide (FMN), also involving the cofactor (6*R*)-5,6,7,8-tetrahydro-L-biopterin (BH4). The result of this complex reaction is the formation of $NO^{\bullet}$ and L-citrulline from oxidation of L-arginine.[22]

Further sites of ROS production in the cell include peroxisomes and the endoplasmic reticulum (ER). Peroxisomes are organelles which have roles in several cellular pathways including fatty acid oxidation, glyoxylate metabolism, biosynthesis of ether-phospholipids and the pentose phosphate pathway.[5] During ordinary metabolism, peroxisomes are the sites of production of H_2O_2 due to the action of several flavoproteins, including acyl-CoA (ACO), D-aspartate (DDO), urate (UO) and D-amino acid (DAAO) oxidases, in addition to the presence of xanthine oxidases and NOS which produce superoxide and nitric oxide, respectively.[5] Inside the ER, redox signals and ROS, in particular H_2O_2, are essential for oxidative folding and the generation of post-translational modifications. The production of hydrogen peroxide occurs through a catalytic process, involving Ero1, an oxidoreductase, starting from molecular oxygen. The process ends with the formation of disulfide bonds in proteins with the action of a member of the protein disulfide isomerase family (PDI).[23,24]

Finally, a number of other cellular enzymes are known to be involved in the production of ROS, including aldehyde oxidase, cyclooxygenases, cytochrome P450 monooxygenase, dihydroorotate dehydrogenase and tryptophan dioxygenase.[25]

In addition to their site and mode of production, the half-life and ability to diffuse of ROS must also be considered. $O_2^{\bullet-}$ is characterized by instability due to its spontaneous dismutation to H_2O_2 and its high reactivity towards iron–sulfur clusters. On the contrary, H_2O_2 is quite stable, with a half-life of about 1 ms, and with a limited oxidant activity; in fact, H_2O_2 toxicity is perhaps

ascribable to its reduction to OH•. The latter is reported to react indiscriminately and not be able to diffuse far, given its half-life of 10^{-9} s.[26]

In the past, it has been assumed that ROS are able to cross biological membranes freely in order to enter cells or to reach their target. However, membrane proteins have more recently been shown to facilitate ROS diffusion and permeation.[27] For example, aquaporins (AQP) are transmembrane proteins that enhance the bilayer diffusion of several molecules including nitric oxide[28] and H_2O_2.[29] Knockout of AQP8 has been reported to impair the release of H_2O_2 from the mitochondria in hepatocytes, with subsequent accumulation of ROS and mitochondrial depolarization.[30] Extracellular $O_2^{\bullet-}$, which can be produced by NOX2 and dismutates to H_2O_2, not permeable through the membrane, can use aquaporins to cross, or chloride channels (CIC) as reported for CIC3 in endothelial cells.[31]

2. *OVERVIEW OF THE (PATHO)PHYSIOLOGICAL ROLES OF ROS*

An extensive discussion of the (patho)physiological roles of ROS is beyond the scope of this review, and they are thus only introduced briefly here. We refer readers to excellent and extensive reviews in the field for further information (references 31 and 32). Additional reviews pertaining to specific disease indications are mentioned in the following sections, where appropriate.

2.1 Physiological roles of ROS. In order for ROS to carry out their function as signaling molecules, the redox homeostasis, defined as the balance between ROS production and the antioxidant response, must be finely regulated.[2] Superoxide dismutases (SODs) are among the enzymatic antioxidants that the cell employs to reduce ROS. The role of SODs in different cellular compartments is to enhance a rapid dismutation of superoxide to hydrogen peroxide.[33] Hydrogen peroxide can be converted to water by the action of catalase, a tetrameric heme-contain enzyme, and glutathione peroxidase (GPX), which uses the reduced tripeptide glutathione (GSH) as a co-

factor to catalyze the reaction.[13] GPX is one of the major classes of selenoproteins present in the human body.[34,35] The active site is characterized by the presence of glutamine, tryptophan and selenocysteine.[35,36] Selenoproteins are known to regulate the production of thyroid hormones, growth, and the immune response and have an important role in the antioxidant response.[34] Selenoproteins, and their roles in health and disease have been comprehensively reviewed, and we therefore refer the reader to an excellent review on this class of proteins.[34]

Other examples of endogenous antioxidant enzymes are peroxiredoxin, thioredoxin, NAD(P)H dehydrogenase (quinone) 1, and glutathione S-transferase (GST).[37] The expression of these antioxidant enzyme genes is regulated by the interaction between specific transcription factors, including the nuclear factor erythroid 2-related factor 2 (Nrf2), and antioxidant response element (ARE) promoter regions.[37] One of the primary roles that ROS play inside a cell is the regulation of cell survival. Within the physiological concentration range, ROS are involved in signaling as secondary messengers in several vital processes, through the oxidation of target cysteine thiols. For example, ROS signaling mediates survival factors such as nuclear factor kappa B (NF-kB), an anti-apoptotic and pro-proliferation transcription factor. ROS also have an important role in cellular adaptation to hypoxic conditions. This occurs when cells need to adapt to a low oxygen environment to survive during pathological events including cancer or ischemia. Conversely, high ROS levels indicate the existence of a pathological situation that will lead to cell death by apoptosis, necrosis or autophagy.[38]

Autophagy is a catabolic pathway conserved across species.[39] Here, the autophagosome, a double membrane vesicle, functions to degrade misfolded or non-essential proteins and even entire organelles after fusion with the acidic lysosome that contains proteolytic enzymes. The activation of autophagy comes in response to general stress conditions like infection, starvation and

ischemia,[39] while the modulation of autophagy is regulated by redox-sensitive proteins, such as 5' adenosine monophosphate-activated protein kinase (AMPK), which undergoes S-glutathionylation of two cysteine residues. A second ROS-related regulatory mechanism involves the oxidation of a cysteine residue of autophagy-related gene 4 (ATG4), a key autophagy protease, which cleaves a precursor form of microtubule-associated protein 1A/1B light chain 3 (pro-LC3) to LC3-I, which is responsible for autophagosome formation after downstream processing. A last example of the ROS-autophagy cross-talk is related to the creation of an impaired thiol redox state due to the release of reduced GSH in the extracellular environment.[40]

ROS also influence cellular proliferation through the regulation of several growth factors including the platelet-derived growth factor (PDGF), the epidermal growth factor (EGF) and the fibroblast growth factor (FGF). These require ROS for activation and phosphorylation of a tyrosine residue in their receptor tyrosine kinase domain.[41] Once activated, the growth factors promote cellular proliferation as well as cell survival *via* different signal transduction pathways.[6] Furthermore, ROS are interconnected with vascular formation by stimulating angiogenic factors such as the specific growth factor, the vascular and endothelial growth factor VEGF, and angiopoietin.[42]

ROS are involved in several aspects of the immune response. In fact, ROS can protect the body against pathogens either in a direct way or through phagocytosis.[43] The pathogenic invasion of bacteria and fungi is followed by a burst of ROS production inside phagocytes, such as neutrophils, which together with proteases, destroy the pathogen.[44] The specific localization of NOX2 in phagocytes is of particular relevance, as it can produce ROS and $NO^{\bullet}$ to directly cause an anti-pathogen response by oxidation of proteins and DNA inside the phagosome. ROS are also involved indirectly in stimulating the release of proteins capable of proteolytic degradation, such as elastase

or cathepsin G, due to the increase of K^+ concentration in the phagosomal lumen in response to NOX2 activity.[43] Physiological concentrations of ROS are also necessary for the activation of T-cells. It has been found that ROS are involved in T-cell receptor (TCR) signaling pathways at several levels. For example the release of Ca^{2+} in response to the activation of cluster of differentiation 3 (CD3) is in turn responsible for ROS production, while mitochondrial ROS has been found to activate T-cell regulation through oxidation of interleukins 2 and 4 (IL-2 and IL-4).[45] ROS are also involved in the response to viral pathogens. The activation of ROS production by NOX2 in endosomes is stimulated by the presence of viral RNA or DNA. It has therefore long been speculated that ROS may be employed in response to viral infections.[46] In the context of COVID-19 infection, excess ROS has been identified as one of the potential trigger factors for the development of a more severe disease, especially in patients with a pre-existing alteration in the redox balance. The lower expression of SOD together with activation of ROS production by neutrophils seems to lead to alveolar damage, thrombosis and dysfunction of red blood cells.[47]

Furthermore, ROS have been shown to have significant effects in the fields of both synaptic plasticity and neurotransmission in several areas of the brain, such as the amygdala and the spinal cord. The amygdala regulates the emotional aspects of pain and neurotransmission and excitability of its central nucleus (CeA) are increased by pain neuroplasticity controlled by ROS.[48]

2.2 Pathophysiological effects correlated to high ROS levels. Several pathologies have been connected to increased ROS levels. It has been demonstrated that ROS signaling is implicated in the proliferation and cell survival of many types of cancer.[49] A characteristic of cancer is an increased production of intracellular ROS to promote tumorigenesis and differentiation.[50] On the other hand, tumor cells develop an increased resistance to high ROS levels by increasing the capacity of their antioxidant machinery.[51] This results in an altered redox homeostasis, which in

turn could be exploited as a potential target for treatment.[50] The activation of certain growth factors stimulates the release of ROS, which act as secondary messengers and modulate important signaling cascades through oxidative inactivation of phosphatases.[49] Genomic instability due to unrepaired DNA damage is a key feature of oncogenic transformation. One source of this damage is the oxidation of DNA due to high ROS levels in the tumor microenvironment.[52] The high energy level present in cancer cells is maintained thanks to a shift to anaerobic glycolysis, the so-called Warburg effect, and results in glucose consumption and subsequent lactic acid fermentation.[51] This change in cellular metabolism is required for cancer cells to adapt to hypoxic conditions. The increased expression of the gene encoding for the hypoxia-inducible transcription factor (HIF-1α) is found in this type of pathology and may lead to several oncogenic events including metastasis and angiogenesis, in addition to a more general cell proliferation/growth, as mentioned before.[3,51] Metastasis is a process that is redox-regulated, as ROS are involved in two crucial steps, the loss of cell adhesion and cell migration, which both depend on the binding between integrins and the extracellular matrix. [15,33,50]

Neurodegenerative diseases (NDs), known as progressive and incurable diseases with mostly unknown etiology, are characterized by progressive loss of cognitive function and neuronal structure of specific neurons, resulting in the accumulation of protein aggregates and neuronal death.[8] The most common NDs are Alzheimer's (AD), Parkinson's (PD) and Huntington's diseases. The link between ROS and neurodegeneration has been widely demonstrated, however it is important to highlight that oxidative stress may not be a root cause of the diseases. Nonetheless, it is considered important in disease progression.[53] In general, the fact that the neuronal cells are particularly susceptible to ROS is due to their high consumption of oxygen, a

relatively weak antioxidant defense and the abundance in the cell membrane of poly-unsaturated fatty acids, which are susceptible to peroxidation.[8,53]

Another important disease which progression have been correlated with ROS is cardiovascular disease (CVD),[54] which includes atherosclerosis and myocardial infarction among others, one of the major causes of death worldwide.[55] Increased ROS concentrations have been correlated to CVD through the activation of several signaling cascades in the coronary endothelium, a vascular tissue, during pathogenic cardiovascular events.

In the event of a stroke, the pathological role of ROS takes place during the reperfusion stage, which follows the acute event. During a stroke the blood flow in certain areas of the brain is blocked.[56] Consequently, the supply of oxygen and glucose is impaired and damage to the mitochondria may occur.[57] Even though the reperfusion is required in order to prevent the brain from further damage, this, at the same time, increases oxidative stress due to an accumulation of ROS,[57,58] resulting in cell death.[59] Several enzymes and organelles are known to be responsible for ROS overproduction during reperfusion, including NADPH,[59,60] XO,[61] as well as mitochondria at the succinate-driven reverse electron transport level.[62]

Furthermore, ROS has been recognized to be involved in diabetes-correlated pathology.[63] It has been reported that increased insulin resistance may be caused by ROS produced under diabetic conditions through glucose oxidation, lipid peroxidation and protein glycation.[64] The progression of diabetes may cause significant complications such as cardiovascular diseases, retinopathy and nephropathy.[65]

Control of free iron is another promising target in controlling ROS, as this controls the site and extent of generation of the highly aggressive hydroxyl radical. In this context ferroptosis, a recently discovered form of cell death, is worth highlighting. This iron-dependent process, which is distinct

from necrosis, apoptosis or autophagy, was first discovered in 2012.[66] It has been identified and described by employing erastin, a RAS-selective lethal compound that triggers ROS accumulation driven cell death, inhibiting the cysteine-dependent glutathione synthesis, and therefore the antioxidant defense, by blocking the cysteine/glutamate antiporter (system x_c^-).[66] In addition to the involvement of iron and the accumulation of ROS, other key features of ferroptosis are the accumulation of lipid hydroperoxides and the inhibition of the enzyme glutathione peroxidase 4 (GPX4).[67] The modulation of ferroptosis has already been linked to several disease states and will undoubtedly be the subject of future research.[68] In this context, inhibitors of ferroptosis have been reported to be efficacious in animal models of tissues damage connected to ischemia and reperfusion,[69] while activators of ferroptosis are suggested to hold promise as anti-cancer agents.[68,70]

3. SMALL MOLECULE INHIBITORS OF ROS PRODUCTION

Due to their wide-ranging roles in cellular (patho)physiology, inhibiting the production of ROS has become an important therapeutic strategy for combatting a range of diseases. The purpose of this section is to critically evaluate the most important inhibitors of ROS production that have been reported and assess their suitability as tool compounds and/or drug leads. Due to the breadth of this topic we have chosen to focus on *small molecule* inhibitors.[71] Therefore peptide-derived inhibitors such as NOXA1ds and NOXA2ds-tat will not be considered here, but have been reviewed elsewhere.[72] In addition to inhibiting ROS production directly, stimulating the cellular antioxidant system in another strategy to reduce ROS levels. This can be achieved by inhibiting the interaction between Nrf2 and Kelch-like ECH-Associated Protein 1 (Keap1), which in conditions of low oxidative stress targets Nrf2 for ubiquitination, thus preventing the induction of the antioxidant gene expression by this transcription factor.[37] This strategy will not be discussed

in this perspective, and interested viewers are referred to a recent comprehensive review on Nrf2-Keap1 inhibitors.[37]

3.1 Non-selective scavengers.

3.1.1 Endogenous scavengers with a direct mode-of-action. The main endogenous intracellular ROS scavenger is **glutathione** (GSH, **1** in Figure 4),[73] a cysteine-containing tripeptide present in all cell types.[74] The majority of GSH is localized in the cytosol, however around 10% is present in the mitochondrial compartment, where it protects from oxidative stress during electron transport and oxidative phosphorylation.[75] It has been reported that depletion of GSH leads to a permeability change of the mitochondrial membrane that results in membrane potential loss and consequent increase of ROS production.[76] In the presence of H_2O_2, GSH is oxidized by GSH peroxidase, forming glutathione disulfide (GSSG) and explaining its ROS scavenging activity.[77]

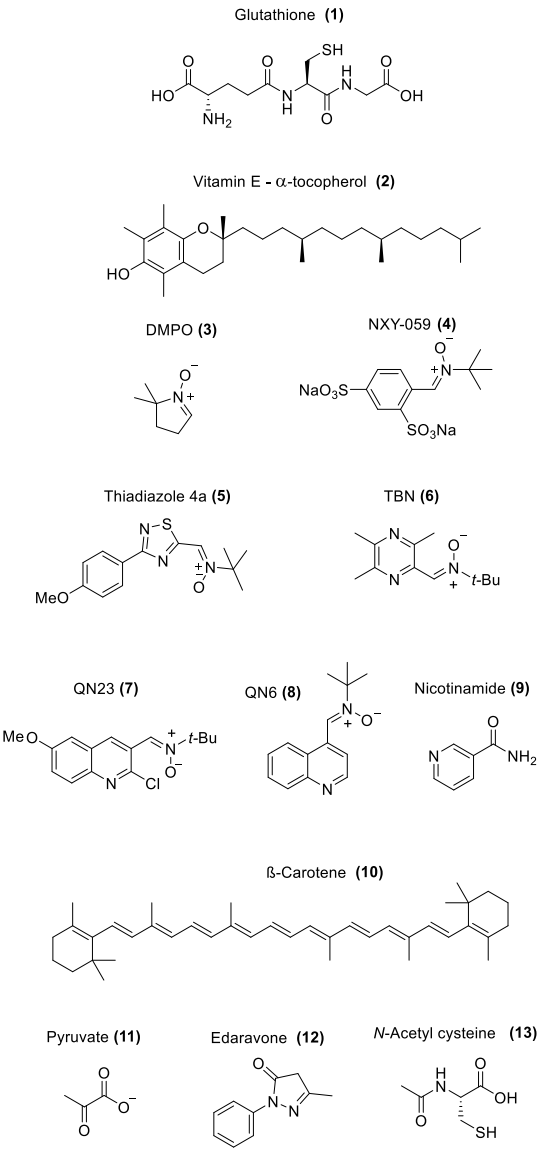

Figure 4. Small molecule scavengers of ROS.

A second ROS scavenger is **vitamin E**, in particular α-tocopherol, (**2** in Figure 4). Vitamin E is an essential component of membranes and an effective antioxidant.[78] The oxidation reaction of α-tocopherol with unsaturated lipids results in the formation of non-radical dimers, trimers and tocopherol-quinone (Figure 5).[79]

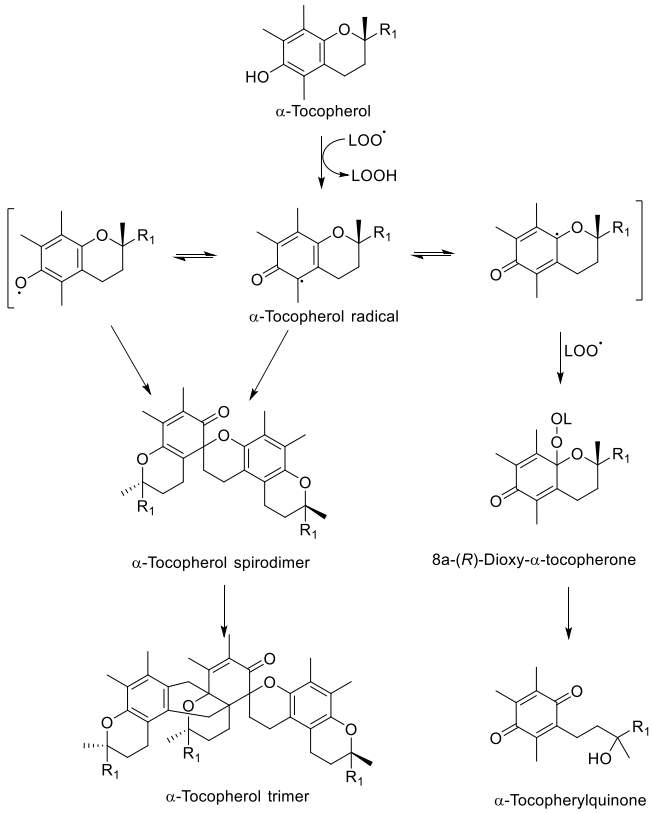

Figure 5. Mode-of-action of α-tocopherol with radical species. ROO• = lipid-peroxyl radical; ROOH=lipid hydroperoxide; R_1 = 4,8,12-trimethyltridecyl; LOOH = lipid-peroxyl radical. Adapted from reference [80].

Nitrones are a class of antioxidant compounds with reported neuroprotective effects and potential as therapeutic agents, for example in neurodegenerative diseases,[81] ischemic stroke[82,83] and traumatic injuries.[84,85] Some nitrones, including the cyclic 5,5-dimethyl-1-pyrroline *N*-oxide (**DMPO**, **3** in Figure 4), are in use as spin-trapping reagents to detect transient radical species in electron spin resonance techniques thanks to their characteristic reaction with ROS.[86,87] Radical addition to the nitrones traps the ROS (Figure 6) and subsequent decomposition proceeds with formation of NO.[82] **NXY-059** (disodium 2,4-disulfophenyl-*N*-*tert*-butylnitrone, **4** in Figure 4), part of the phenyl-*N*-*tert*-butyl nitrones (PBN),[84] has been studied in a phase III clinical trial as a potential treatment for acute ischemic stroke.[88] Unfortunately, the compound failed due to lack of

activity.[82] Other nitrones including **thiadiazole 4a** (**5**, Figure 4) and **TBN** (**6**, Figure 4), which show potential in both neurodegenerative diseases and ischemic stroke have been reported.[81,82,83] Recently, quinolylnitrones such as **QN23** (**7**, Figure 4) have emerged as alternative nitrone containing scavengers. **QN23** was found to be a neuroprotective agent *in vivo* in cerebral ischemia models and to effectively trap hydroxyl radicals.[89] Analogue synthesis and SAR analysis resulted in the discovery of **QN6** (**8**, Figure 4) as a promising starting point for future optimization of new molecules for the treatment of stroke. **QN6** demonstrated neuroprotective effect in an oxygen and glucose deprivation cell culture model, good lipophilicity, and potent activity as a hydroxyl radical scavenger.

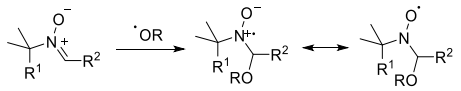

Figure 6. Mechanism of action of nitrone ROS scavengers.

Finally, a variety of natural products have been reported as putative antioxidants, the majority of which are polyphenols. The chemical biology of this compound family has been reviewed extensively[90] and will not be covered here. It is important to note that caution is warranted when working with such compounds, which have been reported as redox cyclers (i.e. pro- or anti-oxidants, depending on the environment) as well as non-specific protein precipitants.[91]

3.1.2 Scavengers with both direct and indirect modes-of-action. **Nicotinamide** (**9** in Figure 4), also known as vitamin B_3, is an endogenous metabolite that can be converted to nicotinamide adenine dinucleotide (NAD).[92] It has been found that nicotinamide may influence the activity of particular proteins, SIRT1 among others, which can modulate autophagy and apoptosis as a function of cell survival.[92] The direct ROS scavenging action of nicotinamide and its metabolites has also been reported.[93] Nicotinamide is able to decrease the intracellular ROS levels by reducing

the ETC together with the downregulation of the formation of mitochondrial permeability transition pore. The latter leads to the increase of the membrane potential.[94]

An important member of the carotenoids and the principal pro-vitamin A is **β-carotene** (**10** in Figure 4). β-carotene is also be able to modulate ROS levels by acting as an antioxidant[95] by directly quenching singlet oxygen or by reaction with, and inhibition of, lipid peroxidation.[96] The reactions between singlet oxygen and β-carotene can lead to the formation of β-carotene 5,8-endoperoxide and β-carotene 5,6-epoxide during methyl linolate chlorophyll-sensitized photo-oxidation, or the formation of cyclic monoendoperoxides and diendoperoxides in bacteriopheophytin-sensitized systems.[95] In addition to its scavenging action, pro-oxidant effects have been reported for high concentrations of β-carotene.[96]

The glycolysis product **pyruvate** (**11** in Figure 4) is reported to act as an antioxidant of hydrogen peroxide.[97] A direct scavenging action of hydrogen peroxide has been reported by a non-enzymatic decarboxylation of pyruvate to acetate which, at the same time, reduces hydrogen peroxide to water.[98] Furthermore, an indirect scavenging action involves pyruvate metabolism, which results in the reduction of GSH by the involvement of NADPH.[99]

Edaravone (**12** in Figure 4) is an approved drug for the treatment of acute brain infarction. Edaravone is also a scavenger of free radicals and a lipid peroxidation inhibitor that has a low molecular weight, is lipophilic and can cross the blood-brain barrier.[100] The mechanism of action of edaravone is illustrated in Figure 7. It has also been reported that in animal models of retinal diseases, edaravone prevents oxidative retinal damage to lipids and cell death resulting from oxidative stress.[101]

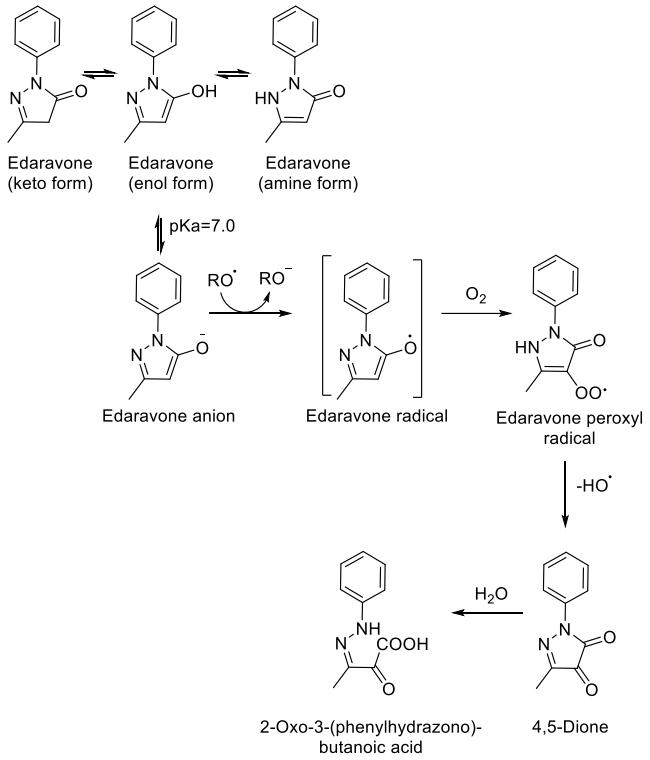

Figure 7. Hypothetical mode of action of edaravone. $RO^{\bullet}$ = radical species; RO^{-} = anion. Partially adapted from references [102] and [103].

***N*-Acetyl cysteine** (NAC, **13** in Figure 4) is a cysteine prodrug, which has been broadly employed in cellular, animal and clinical studies as an antioxidant[104] and is often considered an acetylated precursor of GSH.[105] In addition to exerting its scavenging activity by directly interacting with ROS through its thiol,[105] another scavenging mechanism-of-action for NAC has been proposed to stem from its conversion to potent ROS scavenging sulfane species, by 3-mercaptopyruvate sulfurtransferase and sulfide-quinone oxidoreductase.[104] In addition to its antioxidant properties, NAC can display other biological effects that raise questions about its utility as a tool compound. For instance it has been observed that NAC has detrimental effects on cell viability by specifically modulating the redox state of certain kinases, which results in the

activation of a cell-cycle dependent kinase inhibitor, suggesting that it is not simply a ROS scavenger.[106]

Melatonin (*N*-acetyl-5-methoxytryptamine, **14** in Figure 8) is a tryptophan-derived endogenous hormone released from the pineal gland. It is involved in several biological processes such as immunity, sleep regulation, and cardiac functions. [107] Melatonin is considered a suicidal (or terminal) antioxidant, which reduces electrophilic radicals due to the electron-rich indole functioning as an electron donor.[108,109] It has also been reported that its ring-opened metabolites, **AMK** (*N1*-acetyl-5-methoxykynuramine, **15** in Figure 8) and **AFMK** (*N1*-acetyl-*N2*-formyl-5-methoxykynuramine, **16** in Figure 8), also have ROS scavenging activity. [110] Melatonin has an additional, indirect antioxidant function by stimulating the antioxidant defense system, including SOD and GPx.[111] Several *in vivo* studies have been carried out to study the role of melatonin in protecting the brain from ischemic injuries, by initially inhibiting its release from the pineal gland.[112] It is reported that the neurological injuries caused by the ischemic event were partially recovered when melatonin was re-introduced.[112,113]

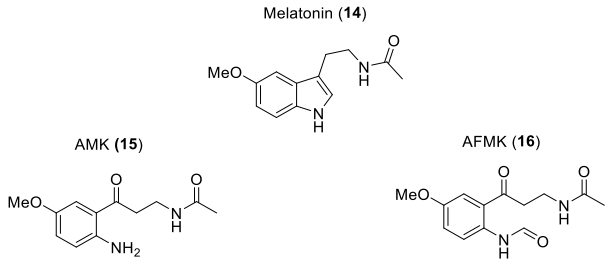

Figure 8. Melatonin and its ring-opened oxidation products.

3.2 Site-specific ROS inhibitors.

Contrary to scavengers, site-specific inhibitors block ROS production by a specific mode-of-action, without the disadvantage of wide-spread ROS depletion that could also affect physiologically beneficial ROS functions.[3]

3.2.1 NOX inhibitors. NOX have been shown to be involved in several pathologies and to be isoform-tissue specific. In order to perform detailed, targeted studies of the different roles of single NOX isoforms, more specific tool compounds are required.[72]

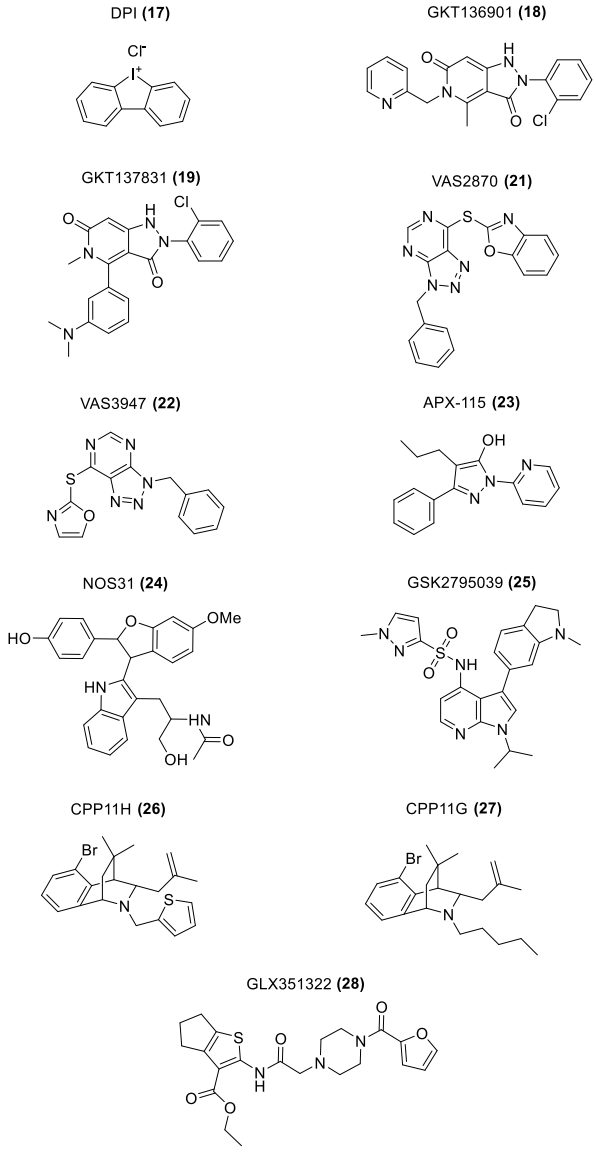

Figure 9. Structures of NOX inhibitors.

One of the compounds most widely used as a NOX inhibitor is diphenyl iodonium (**DPI**) (**17**, Figure 9 and Table 1).[114] This compound is a potent inhibitor of all NOX isoforms[115] as well as a

generic flavoprotein inhibitor, reported to be active against XO,[116] cytochrome P450,[117] NOS enzymes[118] and to interfere with the mitochondrial respiratory chain.[115] In the context of NOX inhibition, DPI reacts with the heme cofactors and FAD in the reduced state.[116] The multitude of off-target activities make DPI unsuitable for therapeutic use and as a tool for studying NOX activity.

During the last decade, a set of new, reportedly isoform-specific NOX inhibitors have been developed, even though not all have been demonstrated to be selective for a single isoform or even to be pan-NOX inhibitors. **GKT136901**[114] and **GKT137831**[119] (respectively **18** and **19**, Figure 7 and Table 1) show preferential inhibition of NOX isoforms 1 and 4, with IC_{50} values in the nanomolar range.[114,119] GKT136901 is also reported to interfere with DUOX-1 and 2[115] while activity against NOX2 has also been reported for GKT137831, however with lower potency compared to NOX1 and NOX4.[119] Both compounds were shown to be inactive against xanthine oxidase and several unrelated targets, including Ca^{2+}, K^+ and Cl^- channels, kinases, and G-protein coupled receptors.[114,119] Both compounds are orally bioavailable,[114,120] and have been reported to be effective in the prevention of hypertensive cardiac remodeling in mice (GKT137831),[120] reduce oxidative stress and albuminuria in type I (GKT137831) and II diabetes mouse models (GKT136901).[121] GKT137831 may be a promising therapeutic and is currently being tested for diabetic nephropathy in phase 2 clinical trials to reduce albuminuria (ClinicalTrials.gov reference number NCT02010242), however it has also been shown to be a potent scavenger of peroxynitrite[122] and hydrogen peroxide.[123] This is not surprising, given the fact that GKT137831 and GKT136901 both contain a dihydropyrazolone scaffold, also seen in the ROS scavenger edavarone (Figure 7), which is specifically required for its scavenging activity. Recently described issues concerning the interaction of GKT136901 with peroxidase-dependent (e.g. horseradish

peroxidase (HRP)/Amplex Red) assays have raised doubts about the correct interpretation of reported data for this compound and concerns regarding the actual mode-of-action and potency.[115] **GKT771** (**20**, Table 1, structure not disclosed) has been developed as a specific NOX1 inhibitor,[124] which does not inhibit other NOX isoforms, XO, or glucose oxidases with the same potency and does not act as a ROS scavenger. GKT771 suppressed tumor growth of colon carcinoma in mice by increasing immune cell recruitment and suppression of angiogenesis. It has been reported that the compound may act as a tumor suppressor if the immune system is intact.[124]

VAS2870 (**21** in Figure 9 and Table 1) has been defined as a pan-NOX inhibitor, because of its reported action not only against NOX2, for which VAS2870 is reported to be selective, but also against NOX1, NOX4 and NOX5.[123] Activity as a ROS scavenger has not been reported for VAS2870 and it only weakly targets XO compared to NOX.[123] VAS2870 may be potentially useful for several pathologies including neurodegeneration,[125] cardiovascular diseases,[126] tumors,[127] and thrombosis.[123] However, off-target effects have been reported and are related to the thioalkylation of cysteine residues in the ryanodine receptor Ca^{2+} channel (RyR1) by VAS2870 in skeletal muscle, which also leads to the abolishment of the nitrogen oxide regulatory activity over RyR1.[128] An analog, **VAS3947** (**22**, Figure 9 and Table 1), displays the same NOX inhibition activity and inactivity against XO and eNOS but with increased solubility compared to VAS2870.[129] To summarize, VAS compounds appear to be good tools to investigate general NOX inhibition, but less useful if isoform selectivity is required.

A similar assessment can be made with regards to **APX-115** (**23**, Figure 9 and Table 1), which is reported to be a promising compound for the treatment of type 2 diabetes.[130] APX-115, also known as Ewha-18278, has shown activity against three NOX isoforms (NOX1, NOX2 and NOX4),[130] without acting as a ROS scavenger or as an inhibitor of xanthine and glucose

oxidases.[131] This orally available compound displays good pharmacokinetics[132] and has been found in mouse studies to have a comparable efficacy to losartan, a drug used in diabetic patients for kidney protection preventing kidney injury and protecting mitochondrial and peroxisomal functions from lipid peroxidation damage[133]. It is also reported to be efficient in the protection of renal injury induced by type 2 diabetes[130] and in streptozotocin-induced type 1 diabetes[133] and has been found to be potentially useful in the treatment of osteoporosis.[131]

NOS31 (**24**, Figure 9 and Table 1), a NOX1-selective inhibitor produced from *Streptomyces* sp.,[134] was found to inhibit tumor growth of several types of cancer cell lines associated with up-regulated NOX1 expression, including stomach and colon cancer cell lines. It was shown to interfere only with cancer cell lines where NOX1 is overexpressed, and to not inhibit XO or act as a H_2O_2 scavenger. Inhibition of NOX2, NOX3, NOX4 and NOX5 is reported to be at least 14 times weaker compared to NOX1.[134] NOS31 may therefore be of interest as a tool compound, though further selectivity profiling is warranted before widespread adoption.

GSK2795039 (**25**, Figure 9 and Table 1), is a competitive inhibitor with NOX2 selectivity over other NOXs, XO and eNOS.[115,135] The activity against XO is reported to be 100-fold lower compared to NOX2. Moreover, eNOS is reported to only be 50% inhibited at 100 μM.[135] GSK2795039 inhibits the production of ROS, passes the blood–brain barrier and is orally bioavailable.[135] It has been used in several cell-based studies[136] and in *in vivo* studies[137] as a NOX2 inhibitor. While GSK2795039 is a suitable tool to study NOX2, it is important to consider possible side effects due to its weak activity against other flavoproteins and NOX isoforms.

In order to address NOX2 in a more specific way, selective NOX2 inhibitors, **CPP11G** and **CPP11H** (respectively **26** and **27** in Figure 9 and Table 1) may be used. These two bridged tetrahydroisoquinolines,[138] which were found to be inactive against NOX1, NOX4, NOX5, and

XO, nor to directly interact with ROS, are reported to block the translocation of $p47^{phox}$ from the cytosol to the plasma membrane preventing the essential interactions between $p47^{phox}$ and p22. It has also been reported to prevent inflammation of the vascular endothelium in mice and vascular dysfunction stimulated by tumor necrosis factor α (TNFα).[139] Profiling of CPP11s is still limited and their potency is somewhat low which limits their utility; however, their selectivity profile is promising and warrants further investigation.

GLX351322 (**28**, Figure 9 and Table 1) is a reported NOX4 inhibitor with 8-fold selectivity *in vitro* over NOX2. GLX351322 has no scavenging activity and is reported to prevent the death of high-glucose-induced islet cells and release of high-glucose insulin in mice in a NOX4-dependent manner.[140] A follow-up compound, **GLX7013114** (**29,** in Table 1structure not disclosed),[115] is reported to target NOX4 specifically with increased potency, and with no reported activity against NOX1, NOX2, NOX3 and NOX5, XO, or glucose oxidase.[141] Contrary to the mechanism of action proposed for VAS2870, here the isoform selectivity supposedly arises from targeting a non-conserved subunit of NOX. *In vitro* studies report that GLX7013114 may protect against cell death, enhanced by either a combination of high glucose and palmitate concentrations or the presence of cytokines.[141] Furthermore it was shown to negatively interfere with transforming growth factor β (TGFβ)-induced epithelial to mesenchymal transition in mice.[142] This compound appears to be an excellent tool for NOX4-specific studies due to its high specificity and reported absence of off-target activity.

Table 1. Summary of NOX inhibitors, their specific target(s) and clinical indications.

NOX inhibitors			
Name	**Target**	**Other activities/specificity**	**Clinical indications**
DPI (17)	NOX[115]	Inhibits all NOX isoforms, XO,[116] cytochrome P450[117] and NOS enzymes,[118] ETC[115]	
GKT136901 (18)	NOX1 (and NOX4)[114]	Peroxynitrite scavenger;[122] hydrogen peroxide scavenger;[123] Selective for NOX1 and NOX4;[114] Activity for DUOX;[115] interference with luminol and HRP/Amplex Red assays[115]	Orally bioavailable;[114] Kidney fibrosis and albuminuria[121]
GKT137831 (19)	NOX1 (NOX2 and NOX4)[119]	Selective for NOX1 over NOX4; low activity for NOX2[119]	Phase 2 clinical trials for diabetic nephropathy (Setanaxib); orally bioavailable;[120] Kidney fibrosis and albuminuria[120]
GKT771 (20)	NOX1[124]		Cancer (tumor growth suppressor and inhibition of angiogenesis)[124]
VAS2870 (21)	NOX1, NOX2, NOX4 and NOX5[123]	Slightly higher selectivity for NOX2 over the other isoforms;[123] cysteine residues thioalkylation (RyR1)[128]	Several therapeutic applications, including neurodegeneration,[125] cancer[127] and thrombosis[123]
VAS3947 (22)	NOX1, NOX2, NOX4 and NOX5[129]		Higher solubility compare with VAS2870[129]
APX-115 (23)	NOX1, NOX2 and NOX4[130]		Orally bioavailable;[132] type 2 diabetes;[130] type 1 diabetes;[133] osteoporosis[131]
NOS31 (24)	NOX1[134]	Active in NOX2, NOX3, NOX4 and NOX5 (marginal activity)[134]	Cancer (NOX1 overexpression)[134]
GSK2795039 (25)	NOX2[115,135]	Active in NOX1, NOX3, NOX4 and NOX5 in a range between 61 and 68% (activity for NOX2 98%); active in XO (100 folds lower compare to NOX2);.active in eNOS (50%inhibition at 100μM)[135]	Crosses blood–brain barrier; orally bioavailable[135]
CPP11H (26) and **CPP11G (27)**	NOX2[138]		Inflammation; vascular dysfunctions[139]
GLX351322 (28)	NOX4 (and NOX2)[140]	NOX4 with 8-fold selectivity over NOX2[140]	Diabetes[140]
GLX7013114 (29)	NOX4 [141]		

3.2.2 Mitochondrial ROS inhibitors. In this section, both mitochondria-targeted ROS scavengers, and specific inhibitors of ROS produced by the mitochondrial respiratory chain are discussed. **MitoQ** (**30**, Figure 10 and Table 2) is a ubiquinone-derived compound with demonstrated mitochondria-selective scavenging activity, which is also able to prevent lipid

peroxidation and peroxide-induced apoptosis.[143,144] Ubiquinone is an inner membrane component of the mitochondrial respiratory chain, which accepts two electrons from complex I and complex II, leading to its reduction to ubiquinol. The latter is an antioxidant that donates a hydrogen atom to a lipid peroxyl radical, decreasing mitochondrial lipid peroxidation.[143] MitoQ consists of a ubiquinone moiety attached via a 10-carbon alkyl chain to a lipophilic tetraphenylphosphonium cation (TPP), which enables mitochondrial bilayer permeation due to the mitochondrial potential, allowing MitoQ to accumulate within the mitochondria.[143,145] It has been reported that MitoQ blocks the activation of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome by the reduction of mitochondrial ROS.[144] Recently, side-effects unrelated to the antioxidant activity of MitoQ have been reported, including mitochondrial swelling and depolarization in proximal tubules of renal cells.[145] Applying the same principle of an antioxidant coupled to a TPP group, Murphy and collaborators developed **MitoTEMPO** (**31**, Figure 10 and Table 2).[146] TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxy) and TEMPO (2,2,6,6-tetramethylpiperidine-1-oxy) are both piperidine nitroxides, which are efficient antioxidants able to mimic the effect of superoxide dismutase, by oxidizing nitroxide to oxoammonium, which can itself be reduced by a molecule of superoxide to regenerate nitroxide.[147] The addition of TPP to one of the piperidine nitroxides creates specific mitochondrial antioxidants,[147] such as MitoTEMPO, by increasing the accumulation of these molecules inside the mitochondria by a factor of one hundred.[146] MitoTEMPO has been recognized to be an effective antioxidant in models of several pathologies like colitis, hypertension, toxicity-induced liver injury and related-kidney injuries.[146] MitoTEMPO may be reported as a promising therapeutic candidate,[146] however its mechanism of action as a general mitochondrial ROS scavenger makes it less valuable as a tool compound.

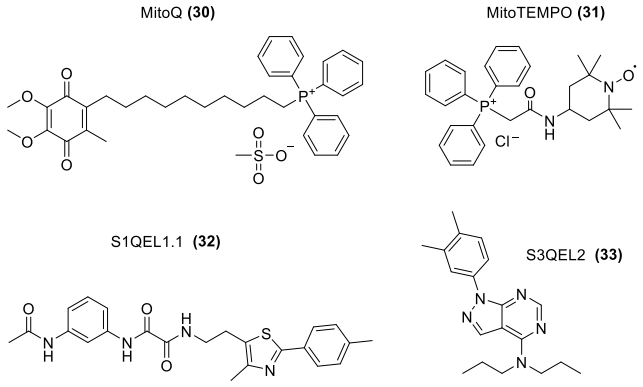

Figure 10. Structures of inhibitors of mitochondrial ROS production.

Contrary to the mitochondrial scavengers, small molecules that inhibit the specific production of ROS by the ETC have recently been reported. These compounds were identified in high-throughput screening campaigns and have been named accordingly to their specific target site: **S1QELs** and **S3QELs** (example structures respectively **32** and **33** in Figure 10 and Table 2). S3QELs are a set of molecules that inhibits the electron leak of complex III of the mitochondrial ETC, specifically in the III_{QO} site, while S1QELs suppress the electron leak from the I_Q site of complex I.[148,149,150,151] These compounds have been shown to inhibit the production of either superoxide or hydrogen peroxide with the main advantages of being selective for the electron leak, not possessing other known targets, and not suppressing normal electron flow or oxidative phosphorylation. S3QELs[148] were shown to modulate ROS-mediated signaling in response to hypoxia, by inhibiting the accumulation of HIF-1α.[151] S1QELs may be further divided in two scaffold types: S1QEL1 is characterized by the presence of a thiazole core while S1QEL2 contains a piperazine.[150] The precise mechanism of action is still unknown,[149,150] but it has been proposed that the site where S1QELs interfere with the ROS production may be I_Qr, without influencing any other components.[149] S1QELs are reported to have promising therapeutic potential, by decreasing

stem cell hyperplasia in *Drosophila* and by protecting mice against ischemia/reperfusion (I/R) injury.[148]

Table 2. Summary of mitochondrial ROS inhibitors, their specific target(s) and clinical indications.

Mitochondrial inhibitors			
Name	**Target**	**Other activities/specificities**	**Clinical indications**
MitoQ (30)	Mitochondrial ROS[143,144]	Scavenger; not target-specific	Side effects: mitochondrial swelling and depolarization in proximal tubules of renal cells[145]
MitoTEMPO (31)	Mitochondrial ROS[147]	Scavenger; not target-specific	Colitis, hypertension, toxicity-induced liver injury and related-kidney injuries[146]
S3QELs (32)	ETC complex III (III_{QO} site)[148]		Hypoxia[151]
S1QELs (33)	ETC complex I (I_{Qr} site)[150]		Hyperplasia; I/R[148]

3.2.3 MAO inhibitors. MAO inhibitors (Figure 11) have been extensively used as antidepressants, and the first drug employed for this purpose was **iproniazid** (**34** in Figure 11 and Table 3), previously used as an anti-tuberculosis agent.[152] Several side effects were observed for this and similar MAO inhibiting compounds, including the so called "cheese effect", caused by inhibitory activity against MAO-A. This particular adverse reaction occurs through the build-up of dietary tyramine, often found in cheese, which in large quantities can give rise to a noradrenaline release and increased blood pressure.[153] Currently, inhibitors of monoamine oxidase are used in neurodegenerative diseases. Both isoforms MAO-A and MAO-B are implicated in the degradation of neurotransmitters, such as dopamine and serotonin and due to the products (hydrogen peroxide or ammonia) of MAOs' oxidative deamination in the presence of biogenic amines.[152] Here we

highlight only the most important MAO inhibitors and refer the reader to a recent and comprehensive review for further details. [152]

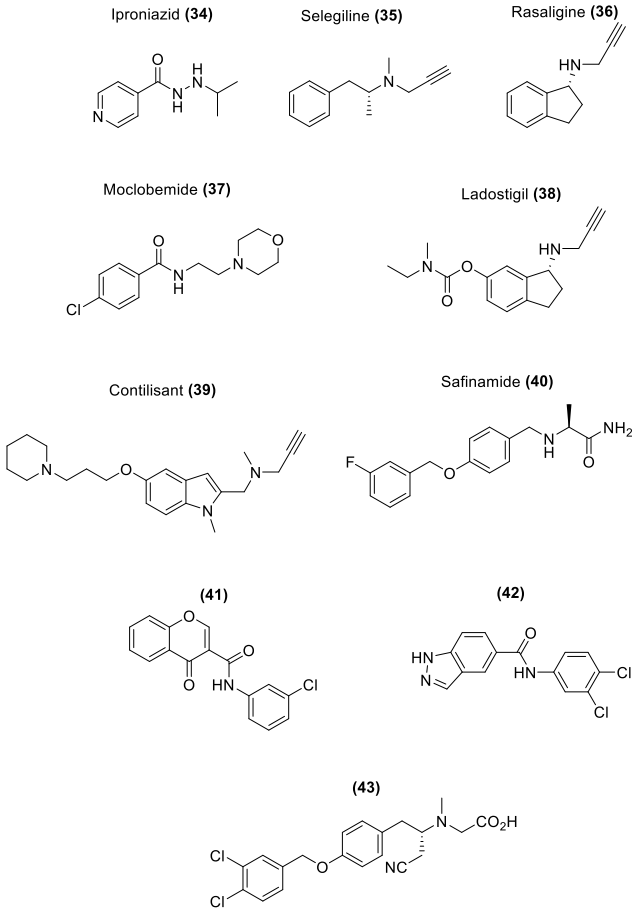

Figure 11. Inhibitors of monoamine oxidases.

The beneficial effect of several MAO-B inhibitors in slowing down the progression of PD has been reported, [154] and among all, Selegiline is one of the most promising.[155] **Selegiline** (or deprenyl, (**35** in Figure 11 and Table 3), a selective MAO-B inhibitor, has been found to increase life expectancy of patients affected by PD in combination with L-DOPA (levodopa),[156] and now is used as a treatment for PD[157]. In addition to PD, selegiline has been studied in other diseases including AD,[158] cancer,[159] and its effects correlated with vascular function in diabetes.[160] Following the results of selegiline, another irreversible MAO-B inhibitor,[161] **rasagiline** (also

named TVP-1012, **36** in Figure 11 and Table 3)[157], has been approved for the treatment of PD in 2005 in the EU.[162] This compound has shown selectivity for MAO-B over MAO-A, with an inhibitory potency more than 100-fold higher for the former compared to the latter, and it is reported to bind covalently to the FAD moiety of MAO-B.[157]

Moclobemide (**37** in Figure 11 and Table 3) is a selective MAO-A inhibitor with a reversible mode of action, developed as an antidepressant since it has been shown to improve vigilance and memory in older patients. The main advantage of this compound is that it does not display the side effects previously described including the cheese reaction, typical of nonspecific or irreversible MAO-A inhibitors.[153] Moclobemide was reported to be clinically effective against PD, particularly in combination with L-DOPA.[163] Moreover it was shown to also be active in the reduction of vascular inflammation.[164]

It is worth highlighting the MAO-A and MAO-B inhibitor **ladostigil** (TV3326, **38** in Figure 11 and Table 3). This propargylamine derivative has proven to be effective in neuroprotection and therefore employed in the treatment of PD and AD.[165] Intriguingly, the beneficial effect may be due to its polypharmacology, which includes acetylcholinesterase and butyrylcholinesterase inhibition.[166] Another multi-targeted drug with promising preclinical activity is **contilisant** (**39** in Figure 11 and Table 3). Contilisant is also described as a cell permeable antioxidant compound, which, in addition to inhibition of MAO and acetylcholinesterase, also antagonizes the histamine receptor subtype 3 (H3R)[167] and acts as a sigma 1 receptor (S1R) agonist.[168] *In vivo* studies have confirmed the potential of this compound for the treatment of AD and it is reported to be effective in restoring the cognitive deficit in mouse models of dementia.[168]

Recently the MAO-B inhibitor **safinamide** (**40** in Figure 11 and Table 3) has been approved for the treatment of mid- to late-stage PD patients in combination with L-DOPA or other treatments.

Safinamide is an α-aminoamide that has shown both dopaminergic and glutamatergic activity. While the glutamatergic action is involved in the inhibition of the glutamate release by modulation of (N-type) calcium channels and blockage of voltage-dependent sodium channels, the dopaminergic effect is correlated to the inhibition of MAO-B in the brain.[169] It has been shown to be highly selective for MAO-B over MAO-A (>700-fold)[170] and to have no thiamine correlated side effect.[171] It is a reversible MAO-B inhibitor, unlike selegiline and rasagiline.[169] The available co-crystal of safinamide in human recombinant MAO-B (PDB 2V5Z, Figure 12) shows that the molecule binds in two pockets, the substrate and the entrance pockets, which together form the protein active site. The aromatic ring of safinamide is oriented in the direction of the substrate cavity where the FAD binds.[170] Two water molecules present in the crystal structure are thought to be involved in the binding interactions.

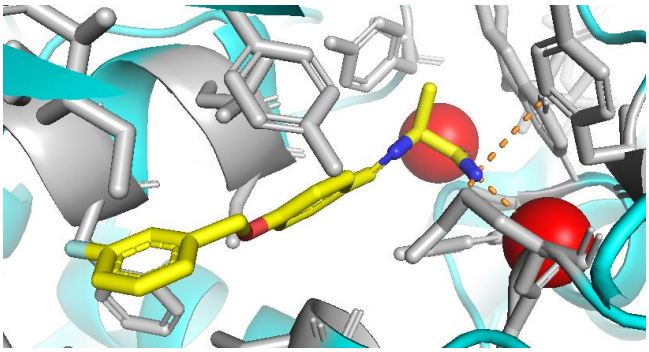

Figure 12. Safinamide in the active site of MAO-B, co-crystal structure PDB 2V5Z. The alpha-sheet and beta-barrel of the protein are shown in cyan; active site residues are in gray; H-bonds are the orange dotted line; safinamide is represented in yellow and water molecules as red spheres.

Although MAOs have been explored as drug targets for decades, more recent advances have focused on new scaffolds (**41** in Figure 11 and Table 3; **42** in Figure 11 and Table 3) with increased potency and selectivity for MAO-B,[172,173,174] as well as non blood-brain-barrier permeable

inhibitors (**43** in Figure 11 and Table 3) with applications in non-CNS related inflammatory disease models.[175]

Table 3. Summary of MAO inhibitors, their specific target(s) and clinical indications.

MAO inhibitors			
Name	**Target**	**Other activities/specificities**	**Clinical indications**
Iproniazid (34)	MAO-A and MAO-B[152]		Depression, tuberculosis;[152] side effects: "cheese effect"[153]
Selegiline (35)	MAO-B[156]		In use for PD (in combination with L-DOPA);[157] studied in AD,[158] cancer,[159] vascular function in diabetes[160]
Rasagiline (36)	MAO-B[157]	Active in MAO-A (100-fold less potent than against MAO-B)[157]	Approved for PD[162]
Moclobemide (37)	MAO-A[153]		Depression,[153] PD (in combination with L-DOPA),[163] inflammation[164]
Ladostigil (38)	MAO-A and MAO-B[165]	Inhibits acetylcholinesterase and butyrylcholinesterase[166]	PD, AD[165]
Contilisant (39)	MAO-A and MAO-B[167]	Inhibits acetylcholinesterase, antagonizes H3R[167]; S1R agonist[168]	AD[168]
Safinamide (40)	MAO-B[169]	Modulates N-type calcium channels; blocks voltage-dependent sodium channels;[169] active in MAO-A (700 fold less potent than against MAO-B)[170]	Approved for PD (in combination with L-DOPA)[169]
(41)	MAO-B[172,173]		
(42)	MAO-B[174]		
(43)	MAO-B[175]		Non-CNS related inflammatory disease[175]

3.2.4 XO inhibitors. In addition to inhibiting ROS production, XO inhibitors were originally developed and approved as therapeutics for gout, acting through reduction of uric acid levels.[176] Since XO appears to play a role in inflammatory, vascular and heart-related conditions, XO inhibitors have been touted as potential treatments for these pathologies.[177]

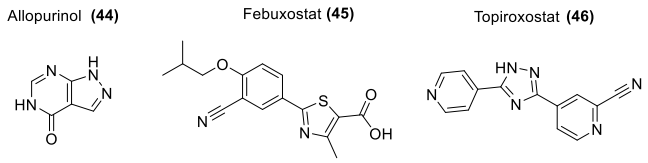

Figure 13. Structures of XO inhibitors.

The purine-like **allopurinol** (**44** in Figure 13 and Table 4)[178] was approved as treatment for gout and hyperuricemia-related conditions. Allopurinol, the hydroxypyrazolopyrimidine analogue of hypoxanthine, and its active metabolite, oxypurinol, are both XO inhibitors. Oxypurinol is formed *via* oxidation of allopurinol by XO, and is an analog of xanthine.[177] The fact that allopurinol efficiently lowers plasma levels of uric acid *in vivo* is counterbalanced by clinical side-effects such as necrosis, liver impairment, allergies, and rash, which are more probable in patients with impaired renal function,[179] as the drug is mostly excreted by the kidney.[180] Moreover, allopurinol has been shown to inhibit enzymes that are part of pyrimidine biosynthesis, including orotidylate decarboxylase[181] and orotate phosphoribosyltransferase.[182]

Febuxostat (**45** in Figure 13 and Table 4),[183] also named TMX-67 and 6720, is a non-purine like inhibitor of both the reduced and oxidized form of XO.[184] Febuxostat is characterized by oral bioavailability and a safe profile, and is found in clinical studies to lower urate in serum in a more efficient way and with a longer effect compared to allopurinol.[180] In contrast to allopurinol, no significant effects of febuxostat are reported against several purine or pyrimidine metabolic enzymes, making it a more suitable tool for exploring the roles of XO in disease.[184] Another approved non-purine XO inhibitor is **topiroxostat** (**46** in Figure 13 and Table 4), which is in use in Japan to treat hyperuricemia in both patients with and without gout.[179] Topiroxostat, or FYX-051[179] is reported to act by covalently binding to the molybdenum co-factor of the enzyme.[185] This compound reduces the level of albumin in the urine and the level of urate in the serum.[179] It is orally bioavailable and has a safe profile, due primarily to the fact that it is excreted as triazole N1-glucuronides and N2-glucuronides in urine.[186] In addition, it is reported to be only a weak inhibitor of cytochrome P450 3A4.[186] The binding mode of topiroxostat with bovine XOR is illustrated by an available crystal structure (Figure 14). The molecule binds in the xanthine hydroxylation active

site forming, *via* its metabolite trihydroxy-topiroxostat, an interaction with the molybdenum cofactor. [187]

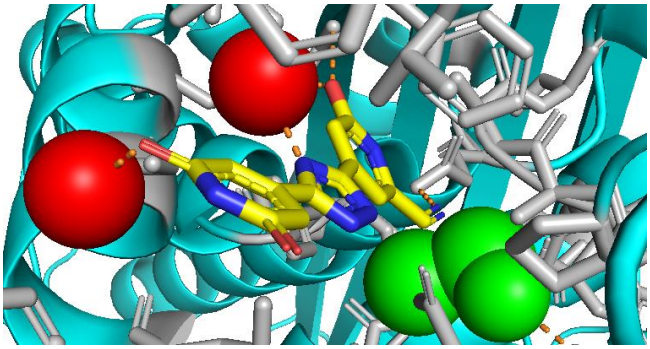

Figure 14. Co-crystal structure of topiroxostat in the active site of XO (PDB 3AM9). The α-sheet and β-barrel of the protein are shown in cyan; active site residues are in gray; H-bonds are displayed as orange dotted lines; topiroxostat is represented in yellow. Water molecules are represented as red spheres and molybdenum cofactor as green spheres.

As a general guideline regarding the use of XO inhibitors in research, non-purine like compounds may be preferable to purine-like inhibitors, in order to avoid side effects related to the metabolism of other purines or pyridines.

Table 4. Summary of XO inhibitors, their specific target(s) and clinical indications.

XO inhibitors			
Name	**Target**	**Other activities/specificities**	**Clinical indications**
Allopurinol (44)	XO[177]	Inhibits pyridine biosynthesis enzymes[182,181]	Approved drug for gout and hyperuricemia, reduces uric acid;[179] clinical side-effects (as necrosis, liver impairment, allergies and rash)[179]
Febuxostat (45)	XO[184]		Approved drug for gout, reduces uric acid; orally bioavailable; longer effect in reducing uric acid than allopurinol[180]
Topiroxostat (46)	XO[179]	Weak inhibitor of cytochrome P450 3A4[186]	Approved drug for hyperuricemia, reduces uric acid;[179] orally bioavailable[186]

3.2.5 NOS inhibitors. The primary focus of this review are inhibitors of reactive oxygen species, and as such, molecules targeting NOS and thus inhibitors of reactive nitrogen species will only be discussed briefly. We have chosen to focus on molecules that in our opinion constitute the most promising tools for studying NOS activity and function and that also show therapeutic potential. The reader is referred to references 20 and [188], for a more comprehensive coverage of NOS inhibitors.

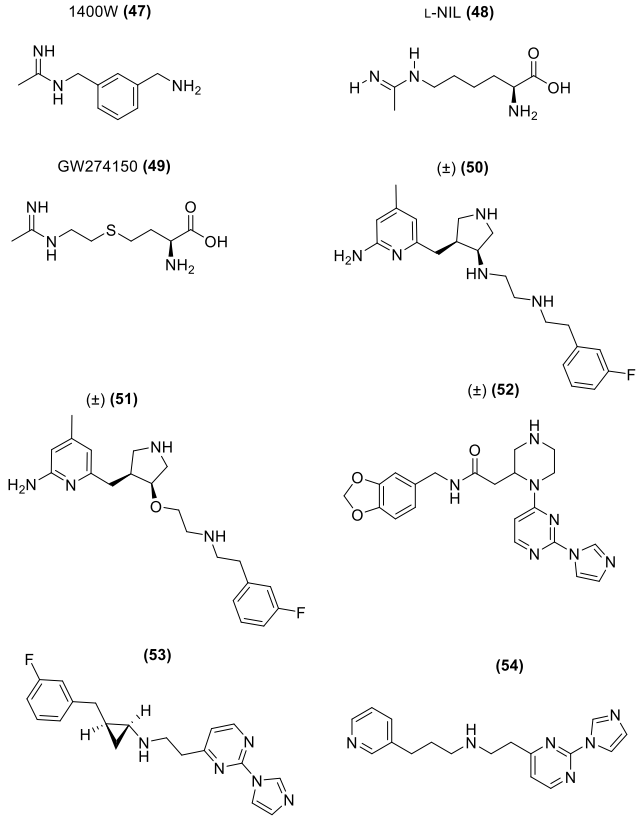

Figure 15. Structures of NOS inhibitors.

The production of nitric oxide by iNOS is reportedly correlated to neurodegenerative disorders and to a wide range of inflammatory pathologies including rheumatoid arthritis, osteoarthritis and asthma. Consequently, iNOS has become a promising drug target. However, NOS isoform selectivity is a highly desirable characteristic of iNOS-targeting to avoid side-effects from nNOS or eNOS activity, such as hypertension.[189,190] **1400W** (**47** in Figure 15 and Table 5) is a selective

human iNOS inhibitor that shows approximately 500 times lower activity in eNOS and more than 200 times selectivity over nNOS.[191] 1400W has been found to penetrate tissues[20] but unfortunately displays toxic effects in rats at high concentrations.[191]

L-NIL, or L-N^6-(l-iminoethyl)lysine,[192] (**48** in Figure 15 and Table 5), is a compound that effectively reduces kidney dysfunctions associated with I/R in rats.[193] It is reportedly an irreversible inhibitor of iNOS with moderate selectivity for iNOS over other isoforms.[192] On the other hand, another arginine analog, **GW274150** (**49** in Figure 15 and Table 5), has demonstrated higher selectivity for human iNOS over the other two isoforms eNOS and nNOS, with 248 and 81 fold–times higher potency, respectively.[189] As with 1400W, the inhibition of iNOS requires the presence of NADPH and proceeds slowly, in contrast with the fast and reversible inhibition of eNOS and nNOS.[191,194] GW274150 has shown promise in experimental pathological conditions of collagen-induced arthritis,[195] I/R-related renal dysfunction[194,196] and as a neuroprotective agent in rodent PD-models.[197] The selectivity for one isoform over the others, together with the reported bioavailability and the absence of toxic effects,[189] make this compound not only suitable as research tool but also promising as a therapeutic agent.

2-Aminopyridines have also been reported as NOS inhibitors. They interact in the conserved heme-binding pocket,[198] and have been the subject of several studies aimed at obtaining isoform-specific compounds.[199,200] As a result of these efforts, pyrrolidinomethyl-2-aminopyridines emerged as promising leads.[201] Two compounds (**50** and **51** in Figure 15 and Table 5)[202] have been reported as particularly interesting, due to their isoform selectivity for nNOS of 2000- and 2676-fold over eNOS and 293/807-fold over iNOS, but unfortunately, neither was able to pass the blood-brain barrier.[201] Another compound (**52** in Figure 15 and Table 5)[203] showed *in vivo* efficacy in rats and cellular permeability. However, it only demonstrated a slight selectivity for iNOS over

nNOS (5 fold), while retaining a good selectivity over eNOS (1000 fold). Thanks to further lead-optimization studies, two new compounds with preference for nNOS over the other isoforms have been reported: compound **53** (selectivity: eNOS=573, iNOS=119) and **54** (selectivity: eNOS=200, iNOS=33) (Figure 15 and Table 5). Notably, both compounds were found to be permeable in Caco-2 cell lines but, data pertaining to blood-brain barrier permeability was not reported. Furthermore, minor off-target activity against cytochrome P450 3A4 was reported. Compound **54** is 1296-fold selective for nNOS over P450, while for compound **53** this was only 17-fold.[201] Further studies focused on increasing the blood-brain barrier permeability of these compounds are still required. As can be deduced from the co-crystal structure PDB 4V3U, compound **50** appears to interact with a histidine residue in the hydrophilic pocket of the human form one nNOS by the nitrogen of the pyridine ring (Figure 16).[201]

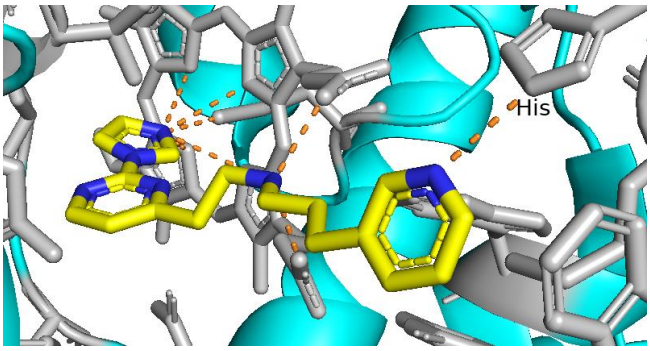

Figure 16. Co-crystal structure of **54** in the active site of nNOS (PDB 4V3U). The α-sheet and β-barrel of the protein are shown in cyan; active site residues are in gray; H-bonds are displayed as orange dotted lines; compound **54** is represented in yellow.

Table 5. Summary of NOS inhibitors, their specific target(s) and clinical indications.

NOS inhibitors			
Name	**Target**	**Other activities/specificities**	**Clinical indications**
1400W (47)	iNOS[191]	Activity in eNOS (500 times lower than iNOS) and in nNOS (200 times lower than iNOS)[191]	Toxic[186,191]
N-NIL (48)	iNOS[192]	Activity in eNOS and in lower than iNOS[192]	I/R-associated kidney dysfunctions[193]
GW274150 (49)	iNOS[189]	Activity in eNOS (248 times lower than iNOS) and in nNOS (81 times lower than iNOS)[189]	Orally bioavailable;[189] collagen-induced arthritis;[195] I/R-related renal dysfunction;[194,196] PD[197]
(50)	nNOS[201]	Activity in eNOS (2000 times lower than nNOS) and in iNOS (293 times lower than nNOS)[201]	
(51)	nNOS[201]	Activity in eNOS (2676 times lower than nNOS) and in iNOS (807 times lower than nNOS)[201]	
(52)	iNOS (and nNOS)[201]	Activity in nNOS (5 times lower than iNOS) and in eNOS (1000 times lower than iNOS)[201]	*in vivo* efficacy and cellular permeability[201]
(53)	nNOS[201]	Activity in eNOS (573 times lower than nNOS) and in iNOS(119 times lower than nNOS); activity in cytochrome P450 3A4 (1296 times lower than nNOS)[201]	
(54)	nNOS[201]	Activity in eNOS (200 times lower than nNOS) and in iNOS (33 times lower than nNOS); activity in cytochrome P450 3A4 (17 times lower than nNOS)[201]	Cellular permeability [201]

3.3 Inhibitors of ferroptosis.

Inhibitors of ferroptosis are reported to act through several modes-of-action. The most common are radical trapping antioxidants (RTAs). These directly block a radical chain reaction, resulting in the inhibition of lipid peroxidation. RTAs, such as hindered secondary amines, react with peroxyl radicals through a fast hydrogen transfer due to weak hydrogen bonds (Figure 17a). The resulting radicals react further with other peroxyl radicals to end as a non-radical species.[204] Indirect inhibitors may interfere by depleting iron or by inhibiting acyl-CoA synthetase long-chain family member 4 (ACSL4), the ligase responsible for the synthesis of esterified polyunsaturated fatty acids.[68,204]

Ferrostatin-1 (**55** in Figure 17b and Table 6) has been reported as a ferroptosis inhibitor when the cellular process was first described as a distinctive cell death mechanism by Dixon *et al.*[66] Ferrostatin-1 is a scavenger of the alkoxy radicals that are created in the reaction between ferrous iron and lipid hydroperoxides.[205] A recently published paper reports a series of ferrostatin-1 analogs with improved solubility, improved ADME (absorption, distribution, metabolism, and excretion) properties and increased metabolic stability compared to ferrostatin-1 itself. The best compounds of the series, including **UAMC-3203** (**56** in Figure 17b and Table 6), also showed a significant increase in potency in mice with iron poisoning injuries.[206] Another important ferroptosis inhibitor, which was identified in a high-throughput screening campaign is **liproxstatin-1** (**57** in Figure 17b and Table 6).[207] This compound is a derivative of spiroquinoxalinamine and act similarly to ferrostatin-1, as an RTAs inhibitor. Liproxstatin-1 was also found to be an anti-ferroptosis agent active in *Gpx4* knockout cells and pre-clinical models of hepatic tissue damage by ischemia/reperfusion.[208]

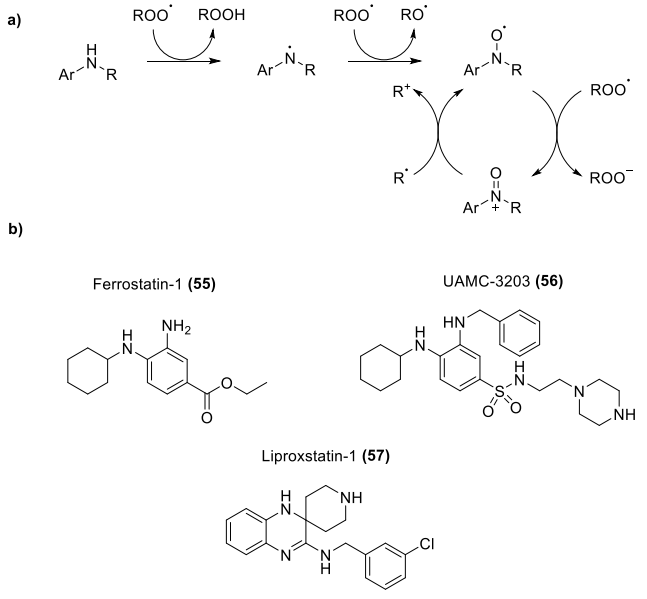

Figure 17. Mechanism and structures of radical trapping antioxidants. (a) Scavenging of peroxyl radicals by hindered secondary amines; (b) structures of ferroptosis inhibitors that act as RTAs.

A recent study reported phenoxazines as a new scaffold with improved properties compared to previously described RTAs (eg. Ferrostatin-1). **Phenoxazines** (**58** in Figure 18a and Table 6) showed increased potency as RTAs, both in solution and in a liposomal formulation, together with a comparable cell-toxicity profile and potency in inhibiting ferroptosis.[209] Moreover phenoxazine-derived nitroxides, (**PHOXNO**, **59** in Figure 18a and Table 6), appear to be particularly potent RTAs with a mode of action that involves the transfer of an electron from a superoxide radical to form oxygen and an oxoammonium ion, which is then protonated to form a hydroxylamine (Figure 18b). The nitroxide can be reformed through hydrogen transfer to a peroxyl radical, or further reactions with superoxide radicals can lead to the formation of a phenoxazine, which is itself a potent RTA.[210] This "cross-dismutation" activity is at the core of its potent biological activity. Due to the relatively recent discovery of ferroptosis and its relevance in several pathologies, we predict that the search for improved RTAs, their clinical application, and agents that target specific protein regulators of ferroptosis will continue in the near future.

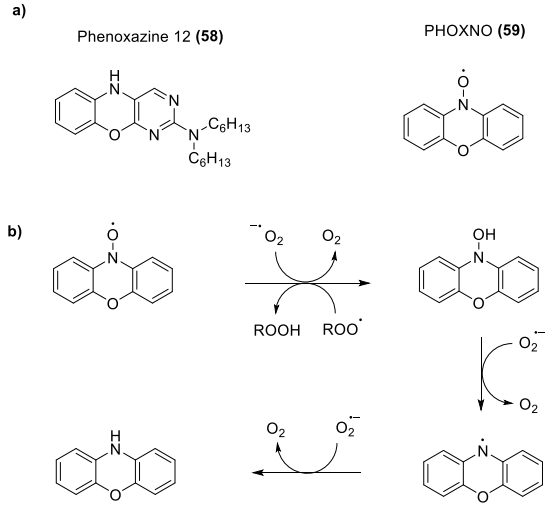

Figure 18. Structure and activity of phenoxazine derived ferroptosis inhibitors. (a) Structure of phenoxazine **12** and phenoxazine derived nitroxide; (b) suggested mechanism of superoxide and peroxyl radical quenching by PHOXNO.

Table 6. Summary of ferroptosis inhibitors, their mode-of-action and clinical indications.

Ferroptosis inhibitors		
Name	**Mode-of-action**	**Clinical indications**
Ferrostatin-1 (55)	Scavenger (RTA)[205]	
UAMC-3203 (56)	Scavenger (RTA)[206]	Iron poisoning injuries[206]
Liproxstatin-1 (57)	Scavenger (RTA)[207]	Ischemia/reperfusion[208]
Phenoxazines (58)	Scavenger (RTA)[209]	
PHOXNO (59)	Scavenger (RTA)[210]	

3.4 Inhibitors with other mechanisms of action.

In this section, we describe compounds that have been found to interfere with ROS neither by chemically scavenging ROS directly nor by interacting with one of the ROS production systems described in the sections above. Many, though not all, of these compounds possess unclear modes-of-action and, where relevant, we discourage the readers from using them as tool compounds.

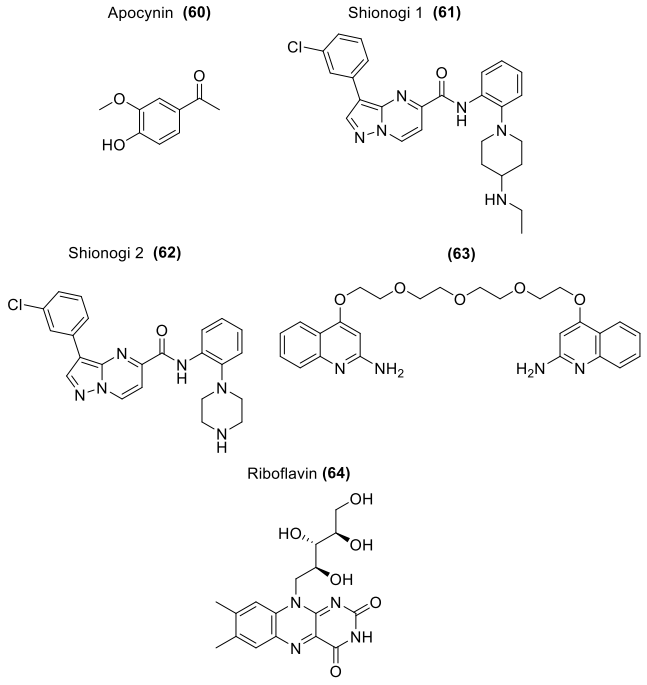

Figure 19. Structures of ROS inhibitors with other mode of action.

A well-reported and still widely used compound as NOX inhibitor is **apocynin** (**60** in Figure 19 and Table 7). The methoxy-substituted catechol (also known as acetovanillone) originates from

the roots of *Picrorrhiza kurroain*.[211] It has been reported as a NOX inhibitor that impairs the assembly of $p47^{phox}$ and $p67^{phox}$ required for NOX in phagocytes.[212] More recently, this compound has been reported to act as antioxidant scavenger in the vascular smooth muscle and endothelial cells[213] as well as a ROS inducer in non-phagocytic cells.[211] Therefore, due to the contradictory nature of the data we do not recommend its use as a ROS scavenger or NOX inhibitor.

Shionogi 1 and 2 (respectively **61** and **62** in Figure 19 and Table 7) are reported NOX2 inhibitors that do not interfere with the enzyme in a direct manner [214]. The actual mode-of-action of these compounds is the inhibition of protein kinase Cβ II (PKCβII), which consequently becomes unable to perform its task in $p47^{phox}$ translocation[214]. The resulting isoform specificity for NOX2 makes these compounds suitable for specific studies but it is important to remember that their mechanism of action does not involve direct binding and inhibition of NOX2.

Another compound that indirectly inhibits NOX2 has recently been reported (**63**, in Figure 19 and Table 7). Compound **63** is described as a reversible inhibitor of the $p47^{phox}$ and $p22^{phox}$ protein-protein interaction (PPI), required for the activation of NOX2. The compound has been developed by linking two fragments that have been identified previously during a fragment screening effort to be inhibitors of two different domains of $p47^{phox}$, SH3A and SH3B respectively.[215] While this interesting compound still requires further optimization, its mode-of-action provides one of the first examples of a PPI inhibitor that may lower ROS levels, which is a welcome addition to the catalytic site binders reported thus far.

Riboflavin (**64** in Figure 19 and Table 7) or vitamin B2, is a water soluble compound composed of a lumiflavin ring connected to a sugar side chain.[216] Its biological role consists in its conversion to flavin mononucleotide (FMN) and FAD, both important cofactors for the function of several oxidases such as NOX2, reductases and dehydrogenases.[217] A recent study demonstrated that

vitamin B2 performs its antioxidant action by stimulating SOD as well as increasing the expression of Nrf2 in a mouse model of AD.[218] Despite this, and its widespread use as a nutritional supplement, its pleiotropic effects may make it unsuitable as a tool compound in basic research.

Table 7. Summary of ROS inhibitors with other mechanism of action, their specific target(s) and clinical indications.

Other mechanisms of action			
Name	**Target**	**Other activities/specificities**	**Clinical indications**
Apocynin (60)		Scavenger[213]	
Shionogi 1 (61) and Shionogi 2 (62)	PKCβII (NOX2)[214]		
(63)	$p47^{phox}$ and $p22^{phox}$ PPI (NOX2)[215]		
Riboflavin (64)		Modulates reductases, dehydrogenases, oxidases (NOX2)[217]	AD[218]

4. CONCLUSION AND OUTLOOK

Pathologies driven or characterized by excessive ROS levels are numerous and include cancer, neurodegenerative diseases, cardiovascular diseases, hypertension and diabetes. As a result, small molecules that are able to reduce ROS levels have been investigated for several decades, leading to a number of interesting findings and the identification of various inhibitors and ROS scavengers. Non-specific scavengers have largely failed to deliver clinical benefits due to several drawbacks including the induction of reductive stress.[219] This has led to a renewed search for compounds that are able to inhibit the *production* of ROS specifically. In this context, we summarized the main intracellular ROS production sites and critically evaluated small molecules developed to modulate cellular ROS levels in pathological situations. The major sources of ROS covered include the

mitochondria, which can produce ROS from the ETC, MAO, the NOX enzymes, XO, and nitric oxide synthases (NOS).

Despite their lack of clinical efficacy, scavengers can still be used as tool compounds, but caution must be exercised when interpreting data resulting from their use. More specific radical scavengers aimed at inhibiting ferroptosis through the trapping of peroxyl radicals have been an active area of recent research, though their clinical utility remains to be determined. One of the major challenges in developing specific inhibitors of ROS production is isoform selectivity. Recent examples of reportedly isoform selective NOX inhibitors are the NOX1 inhibitor NOS31 (**24**) as well as GKT771 (**20**). GSK2795039 (**25**) appears to target NOX2 with appreciable selectivity over other isoforms and flavoenzymes. CPP11G (**26**) and CPP11H (**28**) also show good NOX2 selectivity but poor potency, suggesting that their wider adoption as tools and leads is still a way off. The most advanced NOX inhibitor in clinical trials currently is GKT137831 (**19**), which targets both NOX2 and NOX4. The most interesting compounds that specifically reduce mitochondrial ROS are the ETC complex I inhibitors S1QELs and the ETC complex III inhibitors S3QELs. Mitochondria-targeted scavengers were initially viewed favorably, but have recently been brought into question due to potential non-specific activity as redox cyclers as well as undesired side effects. XO inhibitors are already used clinically against gout and hyperuricemia and have shown good toxicity and safety profiles. Nevertheless, the non-purine like compounds, as febuxostat (**45**) and topiroxostat (**46**), are preferred over the purine analogues to avoid off-target effects. Finally, promising compounds have been developed for nNOS, including the pyrrolidinomethyl-2-aminopyridine derivatives (**50-54**) and others, like GW274150 (**49**), are described as selective iNOS inhibitors, but to the best of our knowledge, no selective eNOS inhibitors have been reported to date.

In summary, tool compounds and potential therapeutics against ROS-related pathologies exist, however finding leads against ROS-producing enzymes has been challenging due to lack of specificity, off-targets effects, toxicity or lack of blood-brain-barrier permeability. The latter is particularly relevant in diseases such as NDs. A continued and sustained effort to develop better tools and clinical candidates should eventually enable researchers in the field to determine whether ROS modulation can be used effectively as a therapeutic strategy in selected diseases.

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Author Contributions

The manuscript was written through contributions from all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interests.

Biographies

Elisa Sassetti obtained her bachelor degree in Biological Science at the University of Florence and continued her studies at the same university achieving a master degree in Biology. During the master, she performed an internship at the University of Cambridge (Department of Genetics). She worked at Fraunhofer Institute in Hamburg as researcher, within the Marie Skłodowska-Curie Actions ETN *Integrate* and she obtained a PhD in Biochemistry in 2019 from Jacobs University Bremen, working on antibiotic drug discovery. Currently she is a Euro Tech co-founded Marie Skłodowska-Curie postdoc fellow in the groups of Mads H. Clausen and Luca Laraia at DTU Chemistry. Her research is focused on studying the role of ROS in neurodegenerative diseases by finding new ROS inhibitors.

Mads Hartvig Clausen, born in 1974, studied chemistry at DTU where he obtained his PhD in 2002 with Professor Robert Madsen. Mads was a postdoctoral fellow at Harvard University from 2002–2004 with Professor Andrew Myers, where he worked on the synthesis of enediyne antibiotics. He returned to DTU as an assistant professor and group leader in 2004. In 2014, he became full professor of chemical biology. His research focuses on chemical biology, medicinal chemistry, and library synthesis. The group designs targeted prodrugs for chemotherapy and inflammatory disease, develop methodology to generate libraries of screening compounds, and are engaged in fragment-based drug discovery. The group also has a longstanding interest in

carbohydrate chemistry and oligosaccharide synthesis, with applications in plant cell wall research and immuno-oncology.

Luca Laraia studied chemistry at Imperial College London, before moving to the University of Cambridge to carry out his Cancer Research UK-funded PhD in chemical biology with Prof. David R. Spring and Prof. Ashok R. Venkitaraman. After graduating in 2014 he moved to the Max Planck Institute of Molecular Physiology (Dortmund, Germany), first as an Alexander von Humboldt postdoctoral fellow and then as project leader in the chemical biology department with Prof. Herbert Waldmann. He moved to DTU in November 2017 to take up an Assistant Professorship in chemical biology. His lab's research is focused on the synthesis of natural product inspired compound collections, identification of small molecule modulators of cholesterol levels and localization, ROS levels and autophagy, as well as chemoproteomic methods for small molecule target identification and validation.

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ABBREVIATIONS

$^1O_2$, Singlet oxygen; AFMK, N1-acfetyl-N2-formyl-5-methoxykynuramine; ACO, Acyl-CoA; ACSL4, Acyl-CoA synthetase long-chain family member 4; AD, Alzheimer's disease; ADME,

Absorption, distribution, metabolism, and excretion; AMK, N1-acetyl-5-methoxykynuramine; AMPK, 5' adenosine monophosphate-activated protein kinase; AQP, Aquaporins; ARE, Antioxidant response element; ATG4, Autophagy-related gene 4; ATP, Adenosine triphosphate; BH4, 6*R*)-5,6,7,8-tetrahydro-L-biopterin; CeA, Central nucleus; CIC, Chloride channels; CO2•, Carbon dioxide radicals; CO3•, Carbonate; COVID-19, Corona virus disease 19; CVD, Cardiovascular disease; DDO, D-aspartate; DMPO, 5,5-dimethyl-1-pyrroline N-oxide; DPI, Diphenyl iodonium; DUOX, Dual oxidase; DAAO, D-amino acid oxidases; EGF, Epidermal growth factor; eNOS, Endothelial nitric oxide synthase; ER, Endoplasmic reticulum; Ero1, Endoplasmic reticulum oxidoreductin 1; ETC, Mitochondrial electron transport chain; FAD, Flavin adenine dinucleotide; FGF, Fibroblast growth factor; FMN, Flavin mononucleotide; GPX, Glutathione peroxidase; *Gpx4*, Glutathione peroxidase 4; GSH, Reduced tripeptide glutathione or glutathione; GSSG, Glutathione disulfide; GST, Glutathione S-transferase; H_2O_2, Hydrogen peroxide; H3R, Histamine receptor subtype 3; HIF-1α, Hypoxia-inducible transcription factor; HO_2•, Hydroperoxyl; HOBr, Hypobromous acid; HOCl, Hypochlorous acid; HRP, horseradish peroxidase; I/R, Ischemia/reperfusion; IL, Interleukin; iNOS, Inducible nitric oxide synthase; Keap1, Kelch-like ECH-Associated Protein 1; LC3, Microtubule-associated protein 1A/1B light chain 3; L-DOPA, Levodopa; MAO, Monoamine oxidase; NAC, N-acetyl cysteine; NAD, Nicotinamide adenine dinucleotide; NADPH, Nicotinamide adenine dinucleotide phosphate; NDs, Neurodegenerative diseases; NF-Kb, Nuclear factor kappa B; NLRP3, NOD-like receptor family pyrin domain containing 3; nNOS p8, Neuronal nitric oxide synthase; NO•, Nitric oxide; NOS, Nitric oxide synthase; NOX, NADPH oxidases; Nrf2, Nuclear factor erythroid 2-related factor 2; O2•−, Superoxide; OH•, Hydroxyl; ONOO-, Peroxynitrite; OXPHOS, Oxidative phosphorylation; PBN, Phenyl-tert-N-butyl nitrones; PD, Parkinson’s disease; PDGF, Platelet-derived growth

factor; PDI, Protein disulfide isomerase; PKCβII, Protein kinase Cβ II; PPI, Protein-protein interaction; Redox, Reduction-oxidation; RNS, Reactive nitrogen species; ROS, Reactive oxygen species; RTA, Radical trapping antioxidant; RyR1, Ryanodine receptor Ca^{2+} channel; S1R, Sigma 1 receptor; SOD, Superoxide dismutase; TCR, T-cell receptor; TGFβ, Transforming growth factor β; TNFα, Tumor necrosis factor α; TPP, Tetraphenylphosphonium cationUO, Urate oxidase; XDH, Xanthine dehydrogenase; XO, Xanthine oxidase; XOR, Xanthine oxidoreductase; Δψm, Mitochondrial membrane potential.

REFERENCES

(1) Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*; Oxford University Press: Oxford, 2015. https://doi.org/10.1093/acprof:oso/9780198717478.001.0001.

(2) Ray, P. D.; Huang, B.-W.; Tsuji, Y. Reactive Oxygen Species (ROS) Homeostasis and Redox Regulation in Cellular Signaling. *Cell Signal* **2012**, *24* (5), 981–990. https://doi.org/10.1016/j.cellsig.2012.01.008.

(3) Briegera, K.; Schiavonea, S.; Miller Jr., F. J.; Krausea, K.-H. Reactive Oxygen Species: From Health to Disease. *Swiss Med. Wkly.* **2012**, *142* (August), 1–14. https://doi.org/10.4414/smw.2012.13659.

(4) Mauch, L.; Lun, A.; O'Gorman, M. R. G.; Harris, J. S.; Schulze, I.; Zychlinsky, A.; Fuchs, T.; Oelschlägel, U.; Brenner, S.; Kutter, D.; Rösen-Wolff, A.; Roesler, J. Chronic Granulomatous Disease (CGD) and Complete Myeloperoxidase Deficiency Both Yield Strongly Reduced Dihydrorhodamine 123 Test Signals but Can Be Easily Discerned in Routine Testing for CGD. *Clin. Chem.* **2007**, *53* (5), 890–896. https://doi.org/10.1373/clinchem.2006.083444.

(5) Fransen, M.; Nordgren, M.; Wang, B.; Apanasets, O. Role of Peroxisomes in ROS/RNS-Metabolism: Implications for Human Disease. *Biochim. Biophys. Acta* **2012**, *1822* (9), 1363–1373. https://doi.org/10.1016/j.bbadis.2011.12.001.

(6) Schieber, M.; Chandel, N. S. ROS Function in Redox Signaling and Oxidative Stress. *Curr. Biol.* **2014**, *24* (10), R453–R462. https://doi.org/10.1016/j.cub.2014.03.034.

(7) Mittler, R. ROS Are Good. *Trend Plant Sci.* **2017**, *22* (1), 11–19. https://doi.org/10.1016/j.tplants.2016.08.002.

(8) Kim, G. H.; Kim, J. E.; Rhie, S. J.; Yoon, S. The Role of Oxidative Stress in Neurodegenerative Diseases. *Exp. Neurobiol.* **2015**, *24* (4), 325–340. https://doi.org/10.5607/en.2015.24.4.325.

(9) Hayyan, M.; Hashim, M. A.; AlNashef, I. M. Superoxide Ion : Generation and Chemical Implications. *Chem. Rev.* **2016**, *116*, 3029−3085. https://doi.org/10.1021/acs.chemrev.5b00407.

(10) Zorov, D. B.; Juhaszova, M.; Sollott, S. J. Mitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release. *Physiol. Rev.* **2014**, *94* (3), 909–950. https://doi.org/10.1152/physrev.00026.2013.

(11) Hughes, M. N. Chemistry of Nitric Oxide and Related Species. In *Methods in Enzymology. Globins and Other Nitric Oxide-Reactive Proteins, Part A*; Elsevier Inc.: San Diego, 2008; Vol. 436, pp 3–19. https://doi.org/10.1016/S0076-6879(08)36001-7.

(12) Muntean, D. M.; Sturza, A.; Dănilă, M. D.; Borza, C.; Duicu, O. M.; Mornoș, C. The Role of Mitochondrial Reactive Oxygen Species in Cardiovascular Injury and Protective Strategies. *Oxid. Med. Cell. Longev.* **2016**, *2016*. https://doi.org/10.1155/2016/8254942.

(13) Li, X.; Fang, P.; Mai, J.; Choi, E. T.; Wang, H.; Yang, X. F. Targeting Mitochondrial Reactive Oxygen Species as Novel Therapy for Inflammatory Diseases and Cancers. *J. Hematol. Oncol.* **2013**, *6* (19), 1–19. https://doi.org/10.1186/1756-8722-6-19.

(14) Bedard, K.; Krause, K.-H. The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. *Physiol. Rev.* **2007**, *87*, 245–313. https://doi.org/10.1152/physrev.00044.2005.

(15) Panday, A.; Sahoo, M. K.; Osorio, D.; Batra, S. NADPH Oxidases: An Overview from Structure

to Innate Immunity-Associated Pathologies. *Cell. Mol. Immunol.* **2015**, *12*, 5–23. https://doi.org/10.1038/cmi.2014.89.

(16) Forrester, S. J.; Kikuchi, D. S.; Hernandes, M. S.; Xu, Q.; Griendling, K. K. Reactive Oxygen Species in Metabolic and Inflammatory Signaling. *Circ. Res.* **2018**, *122* (6), 877–902. https://doi.org/10.1161/CIRCRESAHA.117.311401.Reactive.

(17) Maggiorani, D.; Manzella, N.; Edmondson, D. E.; Mattevi, A.; Parini, A.; Binda, C.; Mialet-Perez, J. Monoamine Oxidases, Oxidative Stress, and Altered Mitochondrial Dynamics in Cardiac Ageing. *Oxid. Med. Cell. Longev.* **2017**, *2017*. https://doi.org/10.1155/2017/3017947.

(18) Kaludercic, N.; Carpi, A.; Nagayama, T.; Sivakumaran, V.; Zhu, G.; Lai, E. W.; Bedja, D.; De Mario, A.; Chen, K.; Gabrielson, K. L.; Lindsey, M. L.; Pacak, K.; Takimoto, E.; Shih, J. C.; Kass, D. A.; Di Lisa, F.; Paolocci, N. Monoamine Oxidase b Prompts Mitochondrial and Cardiac Dysfunction in Pressure Overloaded Hearts. *Antioxidants Redox Signal.* **2014**, *20* (2), 267–280. https://doi.org/10.1089/ars.2012.4616.

(19) Battelli, M. G.; Polito, L.; Bortolotti, M.; Bolognesi, A. Xanthine Oxidoreductase-Derived Reactive Species: Physiological and Pathological Effects. *Oxid. Med. Cell. Longev.* **2016**, *2016*. https://doi.org/10.1155/2016/3527579.

(20) Ferreira, E. I.; Serafim, R. A. M. Nitric Oxide Synthase Inhibitors. In *Nitric Oxide Synthase - Simple Enzyme-Complex Roles*; IntechOpen: Rijeka, 2017; pp 217–237. https://doi.org/10.5772/67027.

(21) Yu, X.; Ge, L.; Niu, L.; Lian, X.; Ma, H.; Pang, L. The Dual Role of Inducible Nitric Oxide Synthase in Myocardial Ischemia/Reperfusion Injury: Friend or Foe? *Oxid. Med. Cell. Longev.* **2018**, *2018*, 1–7. https://doi.org/10.1155/2018/8364848.

(22) Förstermann, U.; Sessa, W. C. Nitric Oxide Synthases: Regulation and Function. *Eur. Heart J.* **2012**, *33* (7), 829–837. https://doi.org/10.1093/eurheartj/ehr304.

(23) Chaube, R.; Werstuck, G. H. Mitochondrial ROS versus ER ROS: Which Comes First in Myocardial Calcium Dysregulation? *Front. Cardiovasc. Med.* **2016**, *3* (October), 1–5. https://doi.org/10.3389/fcvm.2016.00036.

(24) Yoboue, E. D.; Sitia, R.; Simmen, T. Redox Crosstalk at Endoplasmic Reticulum (ER) Membrane Contact Sites (MCS) Uses Toxic Waste to Deliver Messages. *Cell Death Dis.* **2018**, *9* (3), 1–14. https://doi.org/10.1038/s41419-017-0033-4.

(25) Thannickal, V. J.; Fanburg, B. L. Reactive Oxygen Species in Cell Signaling. *Am J. lung cell Mol. Physiol.* **2000**, *279* (6), L1005–L1028. https://doi.org/10.1152/ajplung.2000.279.6.L1005.

(26) D'Autréaux, B.; Toledano, M. B. ROS as Signalling Molecules: Mechanisms That Generate Specificity in ROS Homeostasis. *Nat. Rev. Mol. Cell Biol.* **2007**, *8* (10), 813–824. https://doi.org/10.1038/nrm2256.

(27) Tamma, G.; Valenti, G.; Grossini, E.; Donnini, S.; Marino, A.; Marinelli, R. A.; Calamita, G. Aquaporin Membrane Channels in Oxidative Stress, Cell Signaling, and Aging: Recent Advances and Research Trends. *Oxid. Med. Cell. Longev.* **2018**, *2018*, 1–14. https://doi.org/10.1155/2018/1501847.

(28) Herrera, M.; Hong, N. J.; Garvin, J. L. Aquaporin-1 Transports NO across Cell Membranes. *Hypertension* **2006**, *48* (1), 157–164. https://doi.org/10.1161/01.HYP.0000223652.29338.77.

(29) Chauvigné, F.; Boj, M.; Finn, R. N.; Cerdà, J. Mitochondrial Aquaporin-8-Mediated Hydrogen

Peroxide Transport Is Essential for Teleost Spermatozoon Motility. *Sci. Rep.* **2015**, *5*, 2–11. https://doi.org/10.1038/srep07789.

(30) Marchissio, M. J.; Francés, D. E. A.; Carnovale, C. E.; Marinelli, R. A. Mitochondrial Aquaporin-8 Knockdown in Human Hepatoma HepG2 Cells Causes ROS-Induced Mitochondrial Depolarization and Loss of Viability. *Toxicol. Appl. Pharmacol.* **2012**, *264* (2), 246–254. https://doi.org/10.1016/j.taap.2012.08.005.

(31) Di Pietro, S. M.; Falcón-Pérez, J. M.; Tenza, D.; Setty, S. R. G.; Marks, M. S.; Raposo, G.; Dell'Angelica, E. C. BLOC-1 Interacts with BLOC-2 and the AP-3 Complex to Facilitate Protein Trafficking on Endosomes. *Mol. Biol. Cell* **2006**, *17*, 4027–4038. https://doi.org/10.1091/mbc.E06-05-0379.

(32) Sies, H.; Jones, D. P. Reactive Oxygen Species (ROS) as Pleiotropic Physiological Signalling Agents. *Nat. Rev. Mol. Cell Biol.* **2020**, *21* (7), 363–383. https://doi.org/10.1038/s41580-020-0230-3.

(33) Chio, I. I. C.; Tuveson, D. A. ROS in Cancer: The Burning Question. *Trends Mol. Med.* **2018**, *23* (5), 411–429. https://doi.org/10.1016/j.molmed.2017.03.004.

(34) Hariharan, S.; Dharmaraj, S. Selenium and Selenoproteins: It's Role in Regulation of Inflammation. *Inflammopharmacology* **2020**, *28* (3), 667–695. https://doi.org/10.1007/s10787-020-00690-x.

(35) Arbogast, S.; Ferreiro, A. Selenoproteins and Protection against Oxidative Stress: Selenoprotein N as a Novel Player at the Crossroads of Redox Signaling and Calcium Homeostasis. *Antioxidants Redox Signal.* **2010**, *12* (7), 893–904. https://doi.org/10.1089/ars.2009.2890.

(36) Maiorino, M.; Aumann, K. D.; Brigelius-Flohe, R.; Ursini, F.; van den Heuvel, J.; McCarthy, J.; Flohé, L. Probing the Presumed Catalytic Triad of Selenium-Containing Peroxidases by Mutational Analysis of Phospholipid Hydroperoxide Glutathione Peroxidase (PHGPx). *Biol. Chem. Hoppe. Seyler.* **1995**, *376* (11), 651–660. https://doi.org/10.1515/bchm3.1995.376.11.651.

(37) Pallesen, J. S.; Tran, K. T.; Bach, A. Non-Covalent Small-Molecule Kelch-like ECH-Associated Protein 1−Nuclear Factor Erythroid 2-Related Factor 2 (Keap1−Nrf2) Inhibitors and Their Potential for Targeting Central Nervous System Diseases. *J. Med. Chem.* **2018**, *61*, 8088–8103. https://doi.org/10.1021/acs.jmedchem.8b00358.

(38) Azad, M. B.; Chen, Y.; Gibson, S. B. Regulation of Autophagy by Reactive OxygenSpecies (ROS): Implications for Cancer Progression and Treatment. *Antioxid. Redox Signal.* **2009**, *11* (4), 777–790. https://doi.org/10.1089=ars.2008.2270.

(39) Scherz-shouval, R.; Elazar, Z. Regulation of Autophagy by ROS : Physiology and Pathology. *Trends Biochem. Sci.* **2011**, *36* (1), 30–38. https://doi.org/10.1016/j.tibs.2010.07.007.

(40) Filomeni, G.; De Zio, D.; Cecconi, F. Oxidative Stress and Autophagy : The Clash between Damage and Metabolic Needs. *Cell Death Differ.* **2015**, *22* (3), 377–388. https://doi.org/10.1038/cdd.2014.150.

(41) Sundaresan, M.; Yu, Z.; Ferrans, V. J.; Irani, K.; Finkel, T. Requirement for Generation of H2O2 for Platelet-Derived Growth Factor Signal Transduction. *Am. Assoc. Adv. Sci.* **1995**, *270* (5234), 296–299. https://doi.org/10.1126/science.270.5234.296.

(42) Zhou, Y.; Yan, H.; Guo, M.; Zhu, J.; Xiao, Q.; Zhang, L. Reactive Oxygen Species in Vascular Formation and Development. *Oxid. Med. Cell. Longev.* **2013**, *2013*, 1–14. https://doi.org/10.1155/2013/374963.

(43) Yang, Y.; Bazhin, A. V; Werner, J.; Karakhanova, S.; Yang, Y.; Bazhin, A. V; Werner, J.; Karakhanova, S.; Yang, Y.; Bazhin, A. V; Werner, J.; Karakhanova, S. Reactive Oxygen Species in the Immune System Reactive Oxygen Species in the Immune System. *Int. Rev. Immunol.* **2013**, *32* (3), 249–270. https://doi.org/10.3109/08830185.2012.755176.

(44) Selemidis, S. Targeting Reactive Oxygen Species for Respiratory Infection: Fact or Fancy? *Respirology* **2019**, *24* (1), 15–16. https://doi.org/10.1111/resp.13417.

(45) Chen, X.; Song, M.; Zhang, B.; Zhang, Y. Reactive Oxygen Species Regulate T Cell Immune Response in the Tumor Microenvironment. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 1–10. https://doi.org/10.1155/2016/1580967.

(46) To, E. E.; Vlahos, R.; Luong, R.; Halls, M. L.; Reading, P. C.; King, P. T.; Chan, C.; Drummond, G. R.; Sobey, C. G.; Broughton, B. R. S.; Starkey, M. R.; Van Der Sluis, R.; Lewin, S. R.; Bozinovski, S.; O'Neill, L. A. J.; Quach, T.; Porter, C. J. H.; Brooks, D. A.; O'Leary, J. J.; Selemidis, S. Endosomal NOX2 Oxidase Exacerbates Virus Pathogenicity and Is a Target for Antiviral Therapy. *Nat. Commun.* **2017**, *8* (1), 69. https://doi.org/10.1038/s41467-017-00057-x.

(47) Laforge, M.; Elbim, C.; Frère, C.; Hémadi, M.; Massaad, C.; Nuss, P.; Benoliel, J. J.; Becker, C. Tissue Damage from Neutrophil-Induced Oxidative Stress in COVID-19. *Nat. Rev. Immunol.* **2020**, *20* (9), 515–516. https://doi.org/10.1038/s41577-020-0407-1.

(48) Beckhauser, T. F.; Francis-Oliveira, J.; De Pasquale, R. Reactive Oxygen Species : Physiological and Physiopathological Effects on Synaptic Plasticity. *J. Exp. Neurosci.* **2016**, *10 Suppl 1*, 23–48. https://doi.org/10.4137/JEN.S39887.

(49) Liou, G.-Y.; Storz, P. Reactive Oxygen Species in Cancer. *Free Radic. Res.* **2010**, *44* (5), 479–496. https://doi.org/10.3109/10715761003667554.

(50) Reczek, C. R.; Chandel, N. S. The Two Faces of Reactive Oxygen Species in Cancer. *Annu. Rev. Cancer Biol.* **2017**, *1*, 79–98. https://doi.org/10.1146/annurev-cancerbio-041916-065808.

(51) Kumari, S.; Badana, A. K.; Murali Mohan, G.; Shailender, G.; Malla, R. R. Reactive Oxygen Species: A Key Constituent in Cancer Survival. *Biomark. Insights* **2018**, *13*, 1–9. https://doi.org/10.1177/1177271918755391.

(52) Stanicka, J.; Russell, E. G.; Woolley, J. F.; Cotter, T. G. NADPH Oxidase-Generated Hydrogen Peroxide Induces DNA Damage in Mutant FLT3-Expressing Leukemia Cells. *J. Biol. Chem.* **2015**, *290* (15), 9348–9361. https://doi.org/10.1074/jbc.M113.510495.

(53) Liu, Z.; Zhou, T.; Ziegler, A. C.; Dimitrion, P.; Zuo, L. Oxidative Stress in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Applications. *Oxid. Med. Cell. Longev.* **2017**, *2017*, 1–11. https://doi.org/10.1155/2017/2525967.

(54) Panth, N.; Paudel, K. R.; Parajuli, K. Reactive Oxygen Species : A Key Hallmark of Cardiovascullar Disease. *Adv. Med.* **2016**, *2016*, 1–12. https://doi.org/10.1155/2016/9152732.

(55) Jha, J. C.; Bose, M.; Jandeleit-Dahm, M. Modulation of Oxidative Stress in Cardiovascular Diseases. In *Modulation of Oxidative Stress in Heart Disease*; Springer: Singapore, 2019; pp 237–253. https://doi.org/10.1007/978-981-13-8946-7_10.

(56) Andrabi, S. S.; Parvez, S.; Tabassum, H. Ischemic Stroke and Mitochondria: Mechanisms and Targets. *Protoplasma* **2020**, *257* (2), 335–343. https://doi.org/10.1007/s00709-019-01439-2.

(57) Revuelta, M.; Elicegui, A.; Moreno-Cugnon, L.; Bührer, C.; Matheu, A.; Schmitz, T. Ischemic Stroke in Neonatal and Adult Astrocytes. *Mech. Ageing Dev.* **2019**, *183* (September), 111147.

https://doi.org/10.1016/j.mad.2019.111147.

(58) Sims, N. R.; Yew, W. P. Reactive Astrogliosis in Stroke: Contributions of Astrocytes to Recovery of Neurological Function. *Neurochem. Int.* **2017**, *107*, 88–103. https://doi.org/10.1016/j.neuint.2016.12.016.

(59) Guan, R.; Zou, W.; Dai, X.; Yu, X.; Liu, H.; Chen, Q.; Teng, W. Mitophagy, a Potential Therapeutic Target for Stroke. *J. Biomed. Sci.* **2018**, *25* (87), 1–16. https://doi.org/10.1186/s12929-018-0487-4.

(60) Ma, M. W.; Wang, J.; Dhandapani, K. M.; Brann, D. W. NADPH Oxidase 2 Regulates NLRP3 Inflammasome Activation in the Brain after Traumatic Brain Injury. *Oxid. Med. Cell. Longev.* **2017**, *2017*. https://doi.org/10.1155/2017/6057609.

(61) Ngarashi, D.; Fujikawa, K.; Ferdaus, M. Z.; Zahid, H. M.; Ohara, H.; Nabika, T. Dual Inhibition of NADPH Oxidases and Xanthine Oxidase Potently Prevents Salt-Induced Stroke in Stroke-Prone Spontaneously Hypertensive Rats. *Hypertens. Res.* **2019**, *42* (7), 981–989. https://doi.org/10.1038/s41440-019-0246-2.

(62) Korge, P.; John, S. A.; Calmettes, G.; Weiss, J. N. Reactive Oxygen Species Production Induced by Pore Opening in Cardiac Mitochondria: The Role of Complex II. *J. Biol. Chem.* **2017**, *292* (24), 9896–9905. https://doi.org/10.1074/jbc.M116.768325.

(63) Kaludercic, N.; Di Lisa, F. Mitochondrial ROS Formation in the Pathogenesis of Diabetic Cardiomyopathy. *Front. Cardiovasc. Med.* **2020**, *7*, 1–15. https://doi.org/10.3389/fcvm.2020.00012.

(64) Asmat, U.; Abad, K.; Ismail, K. Diabetes Mellitus and Oxidative Stress-A Concise Review. *Saudi Pharm. J.* **2016**, *24* (5), 547–553. https://doi.org/10.1016/j.jsps.2015.03.013.

(65) Fakhruddin, S.; Alanazi, W.; Jackson, K. E. Diabetes-Induced Reactive Oxygen Species: Mechanism of Their Generation and Role in Renal Injury. *J. Diabetes Res.* **2017**, *2017*, 1–30. https://doi.org/10.1155/2017/8379327.

(66) Dixon, S. J.; Lemberg, K. M.; Lamprecht, M. R.; Skouta, R.; Zaitsev, E. M.; Gleason, C. E.; Patel, D. N.; Bauer, A. J.; Cantley, A. M.; Yang, W. S.; Morrison, B.; Stockwell, B. R. Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. *Cell* **2012**, *149* (5), 1060–1072. https://doi.org/10.1016/j.cell.2012.03.042.

(67) Latunde-Dada, G. O. Ferroptosis: Role of Lipid Peroxidation, Iron and Ferritinophagy. *Biochim. Biophys. Acta - Gen. Subj.* **2017**, *1861* (8), 1893–1900. https://doi.org/10.1016/j.bbagen.2017.05.019.

(68) Zheng, J.; Conrad, M. The Metabolic Underpinnings of Ferroptosis. *Cell Metab.* **2020**, *32* (6), 920–937. https://doi.org/10.1016/j.cmet.2020.10.011.

(69) Alim, I.; Caulfield, J. T.; Chen, Y.; Swarup, V.; Geschwind, D. H.; Ivanova, E.; Seravalli, J.; Ai, Y.; Sansing, L. H.; Emma, E. J.; Hondal, R. J.; Mukherjee, S.; Cave, J. W.; Sagdullaev, B. T.; Karuppagounder, S. S.; Ratan, R. R. Selenium Drives a Transcriptional Adaptive Program to Block Ferroptosis and Treat Stroke. *Cell* **2019**, *177* (5), 1262-1279.e25. https://doi.org/10.1016/j.cell.2019.03.032.

(70) Belavgeni, A.; Bornstein, S. R.; Von Mässenhausen, A.; Tonnus, W.; Stumpf, J.; Meyer, C.; Othmar, E.; Latk, M.; Kanczkowski, W.; Kroiss, M.; Hantel, C.; Hugo, C.; Fassnacht, M.; Ziegler, C. G.; Schally, A. V.; Krone, N. P.; Linkermann, A. Exquisite Sensitivity of Adrenocortical Carcinomas to Induction of Ferroptosis. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (44), 22269–

22274. https://doi.org/10.1073/pnas.1912700116.

(71) Gurevich, E. V; Gurevich, V. V. Therapeutic Potential of Small Molecules and Engineered Proteins. *Handb Exp Pharmacol.* **2014**, *219*, 1–12. https://doi.org/10.1007/978-3-642-41199-1.

(72) Chocry, M.; Leloup, L. The NADPH Oxidase Family and Its Inhibitors. *Antioxid. Redox Signal.* **2020**, *00* (00), 1–23. https://doi.org/10.1089/ars.2019.7915.

(73) Haenen, G. R. M. M.; Bast, A. Glutathione Revisited : A Better Scavenger than Previously Thought. *Front. Pharmacol.* **2014**, *5*, 1–5. https://doi.org/10.3389/fphar.2014.00260.

(74) T, R. M.; Benedetto, R. Di; Varı, R.; Filesi, C.; Giovannini, C. Novel Mechanisms of Natural Antioxidant Compounds in Biological Systems : Involvement of Glutathione and Glutathione-Related Enzymes. *J. Nutr. Biochem.* **2005**, *16* (10), 577–586. https://doi.org/10.1016/j.jnutbio.2005.05.013.

(75) Armstrong, J. S.; Steinauer, K. K.; Hornung, B.; Irish, J. M.; Lecane, P.; Birrell, G. W.; Peehl, D. M.; Knox, S. J. Role of Glutathione Depletion and Reactive Oxygen Species Generation in Apoptotic Signaling in a Human B Lymphoma Cell Line. *Cell Death Differ.* **2002**, *9* (3), 252–263. https://doi.org/10.1038/sj/cdd/4400959.

(76) Swamy, S. M. K.; Huat, B. T. K. Intracellular Glutathione Depletion and Reactive Oxygen Species Generation Are Important in α-Hederin-Induced Apoptosis of P388 Cells. *Mol. Cell. Biochem.* **2003**, *245* (1–2), 127–139. https://doi.org/10.1023/a:1022807207948.

(77) Mills, G. C. Hemoglobin Catabolism I. Glutathione Peroxidase, An Erythrocyte Enzyme Which Protects Hemoglobin From Oxidative Breakdown. *J. Biol. Chem.* **1957**, *229*, 189–198.

(78) Quintana-Cabrera, R.; Fernandez-Fernandez, S.; Bobo-Jimenez, V.; Escobar, J.; Sastre, J.; Almeida, A.; Bolaños, J. P. γ 3-Glutamylcysteine Detoxifies Reactive Oxygen Species by Acting as Glutathione Peroxidase-1 Cofactor. *Nat. Commun.* **2012**, *3*. https://doi.org/10.1038/ncomms1722.

(79) Csallany, A. S.; Chiu, M.; Draper, H. H. Oxidation Products of α-Tocopherol Formed in Autoxidizing Methyl Linoleate. *Lipids* **1970**, *5*, 63–70. https://doi.org/10.1007/BF02531096.

(80) Yamauch, R. Vitamin E: Mechanism of Its Antioxidant Activity. *Food Sct Technol. Int. Tokyo* **1997**, *3* (4), 301–309. https://doi.org/10.3136/fsti9596t9798.3.301.

(81) Cancela, S.; Canclini, L.; Mourglia-Ettlin, G.; Hernández, P.; Merlino, A. Neuroprotective Effects of Novel Nitrones: In Vitro and in Silico Studies. *Eur. J. Pharmacol.* **2020**, *871* (November 2019), 172926. https://doi.org/10.1016/j.ejphar.2020.172926.

(82) Nash, K. M.; Schiefer, I. T.; Shah, Z. A. Development of a Reactive Oxygen Species-Sensitive Nitric Oxide Synthase Inhibitor for the Treatment of Ischemic Stroke. *Free Radic. Biol. Med.* **2018**, *115* (December 2017), 395–404. https://doi.org/10.1016/j.freeradbiomed.2017.12.027.

(83) Marco-Contelles, J. Recent Advances on Nitrones Design for Stroke Treatment. *J. Med. Chem.* **2020**, *63* (22), 13413–13427. https://doi.org/10.1021/acs.jmedchem.0c00976.

(84) Clausen, F.; Marklund, N.; Lewén, A.; Hillered, L. The Nitrone Free Radical Scavenger NXY-059 Is Neuroprotective When Administered after Traumatic Brain Injury in the Rat. *J. Neurotrauma* **2008**, *25* (12), 1449–1457. https://doi.org/10.1089/neu.2008.0585.

(85) Marklund, N.; Lewander, T.; Clausen, F.; Hillered, L. Effects of the Nitrone Radical Scavengers PBN and S-PBN on in Vivo Trapping of Reactive Oxygen Species after Traumatic Brain Injury in

Rats. *J. Cereb. Blood Flow Metab.* **2001**, *21* (11), 1259–1267. https://doi.org/10.1097/00004647-200111000-00002.

(86) Villamena, F. A.; Das, A.; Nash, K. M. Potential Implication of the Chemical Properties and Bioactivity of Nitrone Spin Traps for Therapeutics. *Future Med. Chem.* **2012**, *4* (9), 1171–1207. https://doi.org/10.4155/fmc.12.74.

(87) Locigno, E. J.; Zweier, J. L.; Villamena, F. A. Nitric Oxide Release from the Unimolecular Decomposition of the Superoxide Radical Anion Adduct of Cyclic Nitrones in Aqueous Medium. *Org. Biomol. Chem.* **2005**, *3* (17), 3220–3227. https://doi.org/10.1039/b507530k.

(88) Shuaib, A.; Lees, K. R.; Lyden, P.; Grotta, J.; Davalos, A.; Davis, S. M.; Diener, H.-C.; Ashwood, T.; Wasiewski, W. W.; Emeribe, U. NXY-059 for the Treatment of Acute Ischemic Stroke. *N. Engl. J. Med.* **2007**, *357* (6), 562–571. https://doi.org/10.1056/nejmoa070240.

(89) Chioua, M.; Martínez-Alonso, E.; Gonzalo-Gobernado, R.; Ayuso, M. I.; Escobar-Peso, A.; Infantes, L.; Hadjipavlou-Litina, D.; Montoya, J. J.; Montaner, J.; Alcázar, A.; Marco-Contelles, J. New Quinolylnitrones for Stroke Therapy: Antioxidant and Neuroprotective (Z)- N- Tert-Butyl-1-(2-Chloro-6-Methoxyquinolin-3-Yl)Methanimine Oxide as a New Lead-Compound for Ischemic Stroke Treatment. *J. Med. Chem.* **2019**, *62* (4), 2184–2201. https://doi.org/10.1021/acs.jmedchem.8b01987.

(90) Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouységu, L. Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. *Angewandte Chemie - International Edition*. 2011, pp 586–621. https://doi.org/10.1002/anie.201000044.

(91) Zheng, S.; Laraia, L.; O'Connor, C. J.; Sorrell, D.; Tan, Y. S.; Xu, Z.; Venkitaraman, A. R.; Wu, W.; Spring, D. R. Synthesis and Biological Profiling of Tellimagrandin I and Analogues Reveals That the Medium Ring Can Significantly Modulate Biological Activity. *Org. Biomol. Chem.* **2012**, *10* (13), 2590–2593. https://doi.org/10.1039/c2ob25065a.

(92) Choi, H. J.; Jang, S.; Hwang, E. S. High-Dose Nicotinamide Suppresses ROS Generation and Augments Population Expansion during CD8 + T Cell Activation. *Mol. Cells* **2015**, *38* (10), 918–924. https://doi.org/10.14348/molcells.2015.0168.

(93) Sikora, A.; Szajerski, P.; Piotrowski, Ł.; Zielonka, J.; Adamus, J.; Ã, A. M.; Jerzy, G. Radical Scavenging Properties of Nicotinamide and Its Metabolites. *Radiat. Phys. Chem.* **2008**, *77* (3), 259–266. https://doi.org/10.1016/j.radphyschem.2007.05.005.

(94) Song, S. B.; Jang, S.; Kang, H. T.; Wei, B.; Jeoun, U.; Yoon, G. S.; Hwang, E. S. Molecules and Cells Modulation of Mitochondrial Membrane Potential and ROS Generation by Nicotinamide in a Manner Independent of SIRT1 and Mitophagy. *Mol. Cells* **2017**, *40* (7), 503–514. https://doi.org/10.14348/molcells.2017.0081.

(95) Nishino, A.; Yasui, H.; Maoka, T. Reaction and Scavenging Mechanism of β -Carotene and Zeaxanthin with Reactive Oxygen Species. *J. Oleo Sci.* **2017**, *66* (1), 77–84. https://doi.org/10.5650/jos.ess16107.

(96) Kawata, A.; Murakami, Y.; Suzuki, S.; Fujisawa, S. Anti-Inflammatory Activity of β-Carotene , Lycopene and Tri- n -Butylborane , a Scavenger of Reactive Oxygen Species. *In Vivo (Brooklyn).* **2018**, *32*, 255–264. https://doi.org/10.21873/invivo.11232.

(97) Varma, S. D.; Devamanoharan, P. S.; Ali, A. H. Prevention of Intracellular Oxidative Stress to Lens by Pyruvate and Its Ester. *Free Radic. Res.* **1998**, *28* (2), 131–135. https://doi.org/10.3109/10715769809065799.

(98) Crestanello, J. A.; Kamelgard, J.; Whitman, G. J. R. The Cumulative Nature of Pyruvate's Dual Mechanism for Myocardial Protection. *Journal of Surgical Research*. 1995, pp 198–204. https://doi.org/10.1006/jsre.1995.1154.

(99) Cavallini, L.; Valente, M.; Rigobello, M. P. The Protective Action of Pyruvate on Recovery of Ischemic Rat Heart: Comparison with Other Oxidizable Substrates. *J. Mol. Cell. Cardiol.* **1990**, *22* (2), 143–154. https://doi.org/10.1016/0022-2828(90)91111-j.

(100) Morimoto, K.; Hasegawa, T.; Tanaka, A.; Wulan, B.; Morimoto, N.; Okita, Y.; Okada, K. Free-Radical Scavenger Edaravone Inhibits Both Formation and Development of Abdominal Aortic Aneurysm in Rats. *J. Vasc. Surg.* **2012**, *55* (6), 1749–1758. https://doi.org/10.1016/j.jvs.2011.11.059.

(101) Inokuchi, Y.; Imai, S.; Nakajima, Y.; Shimazawa, M.; Aihara, M.; Araie, M.; Hara, H. Edaravone, a Free Radical Scavenger, Protects against Retinal Damage in Vitro and in Vivo. *J. Pharmacol. Exp. Ther.* **2009**, *329* (2), 687–698. https://doi.org/10.1124/jpet.108.148676.

(102) Watanabe, T.; Tahara, M.; Todo, S. The Novel Antioxidant Edaravone: From Bench to Bedside. *Cardiovasc. Ther.* **2008**, *26* (2), 101–114. https://doi.org/10.1111/j.1527-3466.2008.00041.x.

(103) Yamamoto, Y.; Kuwahara, T.; Watanabe, K.; Watanabe, K. Antioxidant Activity of 3-Methyl-1-Phenyl-2-Pyrazolin-5-One. *Redox Rep.* **1996**, *2* (5), 333–338. https://doi.org/10.1080/13510002.1996.11747069.

(104) Ezerin, D.; Takano, Y.; Hanaoka, K.; Urano, Y.; Dick, T. P.; Ezerin, D.; Takano, Y.; Hanaoka, K.; Urano, Y.; Dick, T. P. N-Acetyl Cysteine Functions as a Fast-Acting Antioxidant by Triggering Intracellular H2S and Sulfane Sulfur Production. *Cell Chem. Biol.* **2018**, *25* (4), 447–459. https://doi.org/10.1016/j.chembiol.2018.01.011.

(105) Zhang, F.; Lau, S. S.; Monks, T. J. The Cytoprotective Effect of N-Acetyl- L -Cysteine against ROS-Induced Cytotoxicity Is Independent of Its Ability to Enhance Glutathione Synthesis. *Toxicol. Sci.* **2011**, *120* (1), 87–97. https://doi.org/10.1093/toxsci/kfq364.

(106) Kim, K.-Y.; Rhim, T. Y.; Choi, I.; Kim, S.-S. N-Acetylcysteine Induces Cell Cycle Arrest in Hepatic Stellate Cells through Its Reducing Activity. *J. Biol. Chem.* **2001**, *276* (44), 40591–40598. https://doi.org/10.1074/jbc.M100975200.

(107) Hacışevki, A.; Baba, B. An Overview of Melatonin as an Antioxidant Molecule: A Biochemical Approach. In *Melatonin-molecular biology, clinical and pharmaceutical approaches*; IntechOpen: London, 2018; pp 59–88.

(108) Chainy, G. B. N.; Sahoo, D. K. Hormones and Oxidative Stress: An Overview. *Free Radic. Res.* **2020**, *54* (1), 1–26. https://doi.org/10.1080/10715762.2019.1702656.

(109) Martinez, G. R.; Almeida, E. A.; Klitzke, C. F.; Onuki, J.; Prado, F. M.; Medeiros, M. H. G.; Di Mascio, P. Measurement of Melatonin and Its Metabolites: Importance for the Evaluation of Their Biological Roles. *Endocrine* **2005**, *27* (2), 111–118. https://doi.org/10.1385/ENDO:27:2:111.

(110) Ma, X.; Idle, J. R.; Krausz, K. W.; Tan, D. X.; Ceraulo, L.; Gonzalez, F. J. Urinary Metabolites and Antioxidant Products of Exogenous Melatonin in the Mouse. *J. Pineal Res.* **2006**, *40* (4), 343–349. https://doi.org/10.1111/j.1600-079X.2006.00321.x.

(111) Antolín, I.; Rodríguez, C.; Sáinz, R. M.; Mayo, J. C.; Uría, H.; Kotler, M. L.; Rodríguez-Colunga, M. J.; Tolivia, D.; Menéndez-Peláez, A. Neurohormone Melatonin Prevents Cell Damage: Effect on Gene Expression for Antioxidant Enzymes. *FASEB J.* **1996**, *10* (8), 882–890. https://doi.org/10.1096/fasebj.10.8.8666165.

(112) Ramos, E.; Patiño, P.; Reiter, R. J.; Gil-Martín, E.; Marco-Contelles, J.; Parada, E.; los Rios, C. de; Romero, A.; Egea, J. Ischemic Brain Injury: New Insights on the Protective Role of Melatonin. *Free Radic. Biol. Med.* **2017**, *104* (2016), 32–53. https://doi.org/10.1016/j.freeradbiomed.2017.01.005.

(113) Kilic, E.; Özdemir, Y. G.; Bolay, H.; Keleştimur, H.; Dalkara, T. Pinealectomy Aggravates and Melatonin Administration Attenuates Brain Damage in Focal Ischemia. *J. Cereb. Blood Flow Metab.* **1999**, *19* (5), 511–516. https://doi.org/10.1097/00004647-199905000-00005.

(114) Laleu, B.; Gaggini, F.; Orchard, M.; Fioraso-Cartier, L.; Cagnon, L.; Houngninou-Molango, S.; Gradia, A.; Duboux, G.; Merlot, C.; Heitz, F.; Szyndralewiez, C.; Page, P. First in Class, Potent, and Orally Bioavailable NADPH Oxidase Isoform 4 (Nox4) Inhibitors for the Treatment of Idiopathic Pulmonary Fibrosis. *J. Med. Chem.* **2010**, *53* (21), 7715–7730. https://doi.org/10.1021/jm100773e.

(115) Augsburger, F.; Filippova, A.; Rasti, D.; Seredenina, T.; Lam, M.; Maghzal, G.; Mahiout, Z.; Jansen-Dürr, P.; Knaus, U. G.; Doroshow, J.; Stocker, R.; Krause, K. H.; Jaquet, V. Pharmacological Characterization of the Seven Human NOX Isoforms and Their Inhibitors. *Redox Biol.* **2019**, *26*, 1–15. https://doi.org/10.1016/j.redox.2019.101272.

(116) Reis, J.; Massari, M.; Marchese, S.; Ceccon, M.; Aalbers, F. S.; Corana, F.; Valente, S.; Mai, A.; Magnani, F.; Mattevi, A. A Closer Look into NADPH Oxidase Inhibitors: Validation and Insight into Their Mechanism of Action. *Redox Biol.* **2020**, *32*, 1–13. https://doi.org/10.1016/j.redox.2020.101466.

(117) Szilagyi, J. T.; Mishin, V.; Heck, D. E.; Jan, Y. H.; Aleksunes, L. M.; Richardson, J. R.; Heindel, N. D.; Laskin, D. L.; Laskin, J. D. Selective Targeting of Heme Protein in Cytochrome P450 and Nitric Oxide Synthase by Diphenyleneiodonium. *Toxicol. Sci.* **2016**, *151* (1), 150–159. https://doi.org/10.1093/toxsci/kfw031.

(118) Stuehr, D. J.; Fasehun, O. A.; Kwon, N. S.; Gross, S.; Gonzalez, J. A.; Levi, R.; Nathan, C. F. Inhibition of Macrophage and Endothelial Cell Nitric Synthase by Diphenyleneiodonium and Its Analogs. *FASEB J.* **1991**, *5* (1), 98–103. https://doi.org/10.1096/fasebj.5.1.1703974.

(119) Aoyama, T.; Paik, Y.-H.; Watanabe, S.; Laleu, B.; Gaggini, F.; Fioraso-Cartier, L.; Molango, S.; Heitz, F.; Merlot, C.; Szyndralewiez, C.; Page, P.; Brenner, D. D. Nicotinamide Adenine Dinucleotide Phosphate Oxidase (NOX) in Experimental Liver Fibrosis: GKT137831 as a Novel Potential Therapeutic Agent. *Hepatology* **2012**, *56* (6), 2316–2327. https://doi.org/10.1002/hep.25938.

(120) Zeng, S. yu; Yang, L.; Yan, Q. jiang; Gao, L.; Lu, H. qin; Yan, P. ke. Nox1/4 Dual Inhibitor GKT137831 Attenuates Hypertensive Cardiac Remodelling Associating with the Inhibition of ADAM17-Dependent Proinflammatory Cytokines-Induced Signalling Pathways in the Rats with Abdominal Artery Constriction. *Biomed. Pharmacother.* **2019**, *109* (November 2018), 1907–1914. https://doi.org/10.1016/j.biopha.2018.11.077.

(121) Sedeek, M.; Gutsol, A.; Montezano, A. C.; Burger, D.; Nguyen Dinh Cat, A.; Kennedy, C. R. J.; Burns, K. D.; Cooper, M. E.; Jandeleit-Dahm, K.; Page, P.; Szyndralewiez, C.; Heitz, F.; Hebert, R. L.; Touyz, R. M. Renoprotective Effects of a Novel Nox1/4 Inhibitor in a Mouse Model of Type 2 Diabetes. *Clin. Sci.* **2013**, *124* (3), 191–202. https://doi.org/10.1042/CS20120330.

(122) Schildknecht, S.; Weber, A.; Gerding, H.; Pape, R.; Robotta, M.; Drescher, M.; Marquardt, A.; Daiber, A.; Ferger, B.; Leist, M. The NOX1/4 Inhibitor GKT136901 as Selective and Direct Scavenger of Peroxynitrite. *Curr. Med. Chem.* **2014**, *21* (3), 365–376.

https://doi.org/10.2174/09298673113209990179.

(123) Dao, V. T. V.; Elbatreek, M. H.; Altenhöfer, S.; Casas, A. I.; Pachado, M. P.; Neullens, C. T.; Knaus, U. G.; Schmidt, H. H. H. W. Isoform-Selective NADPH Oxidase Inhibitor Panel for Pharmacological Target Validation. *Free Radic. Biol. Med.* **2020**, *148*, 60–69. https://doi.org/10.1016/j.freeradbiomed.2019.12.038.

(124) Stalin, J.; Garrido-Urbani, S.; Heitz, F.; Szyndralewiez, C.; Jemelin, S.; Coquoz, O.; Ruegg, C.; Imhof, B. A. Inhibition of Host NOX1 Blocks Tumor Growth and Enhances Checkpoint Inhibitor–Based Immunotherapy. *Life Sci. Alliance* **2019**, *2* (4), 1–16. https://doi.org/10.26508/lsa.201800265.

(125) Kleinschnitz, C.; Grund, H.; Wingler, K.; Armitage, M. E.; Jones, E.; Mittal, M.; Barit, D.; Schwarz, T.; Geis, C.; Kraft, P.; Barthel, K.; Schuhmann, M. K.; Herrmann, A. M.; Meuth, S. G.; Stoll, G.; Meurer, S.; Schrewe, A.; Becker, L.; Gailus-Durner, V.; Fuchs, H.; Klopstock, T.; de Angelis, M. H.; Jandeleit-Dahm, K.; Shah, A. M.; Weissmann, N.; Schmidt, H. H. H. W. Post-Stroke Inhibition of Induced NADPH Oxidase Type 4 Prevents Oxidative Stress and Neurodegeneration. *PLoS Biol.* **2010**, *8* (9), 1–13. https://doi.org/10.1371/journal.pbio.1000479.

(126) Liu, Z.; Tuo, Y. H.; Chen, J. W.; Wang, Q. Y.; Li, S.; Li, M. C.; Dai, G.; Wang, J. S.; Zhang, Y. L.; Feng, L.; Shi, Z. S. NADPH Oxidase Inhibitor Regulates MicroRNAs with Improved Outcome after Mechanical Reperfusion. *J. Neurointerv. Surg.* **2017**, *9* (7), 702–706. https://doi.org/10.1136/neurintsurg-2016-012463.

(127) Sancho, P.; Fabregat, I. The NADPH Oxidase Inhibitor VAS2870 Impairs Cell Growth and Enhances TGF-β-Induced Apoptosis of Liver Tumor Cells. *Biochem. Pharmacol.* **2011**, *81* (7), 917–924. https://doi.org/10.1016/j.bcp.2011.01.007.

(128) Sun, Q.-A.; Hess, D. T.; Wang, B.; Miyagi, M.; S., S. J. Off-Target Thiol Alkylation by the NADPH Oxidase Inhibitor 3- Benzyl-7-(2-Benzoxazolyl)Thio-1,2,3-Triazolo[4,5-d]Pyrimidine (VAS2870). *Free Radic. Biol. Med.* **2012**, *52* (9), 1897–1902. https://doi.org/10.1016/j.freeradbiomed.2012.02.046.

(129) Wind, S.; Beuerlein, K.; Eucker, T.; Müller, H.; Scheurer, P.; Armitage, M.; Ho, H.; Schmidt, H.; Wingler, K. Comparative Pharmacology of Chemically Distinct NADPH Oxidase Inhibitors. *Br. J. Pharmacol.* **2010**, *161* (4), 885–898. https://doi.org/10.1111/j.1476-5381.2010.00920.x.

(130) Cha, D. R.; Cha, J. J.; Min, H. S.; Kim, K. T.; Kim, J. E.; Ghee, J. Y.; Kim, H. W.; Lee, J. E.; Han, J. Y.; Lee, G.; Ha, H. J.; Bae, Y. S.; Lee, S. R.; Moon, S. H.; Lee, S. C.; Kim, G.; Kang, Y. S. APX-115, a First-in-Class Pan-NADPH Oxidase (Nox) Inhibitor, Protects Db/Db Mice from Renal Injury. *Lab. Investig.* **2017**, *97* (4), 419–431. https://doi.org/10.1038/labinvest.2017.2.

(131) Joo, J. H.; Huh, J. E.; Lee, J. H.; Park, D. R.; Lee, Y.; Lee, S. G.; Choi, S.; Lee, H. J.; Song, S. W.; Jeong, Y.; Goo, J. Il; Choi, Y.; Baek, H. K.; Yi, S. S.; Park, S. J.; Lee, J. E.; Ku, S. K.; Lee, W. J.; Lee, K. I.; Lee, S. Y.; Bae, Y. S. A Novel Pyrazole Derivative Protects from Ovariectomy-Induced Osteoporosis through the Inhibition of NADPH Oxidase. *Sci. Rep.* **2016**, *6*, 1–14. https://doi.org/10.1038/srep22389.

(132) Lee, S. G.; Lee, J.; Kim, K. M.; Lee, K. I.; Bae, Y. S.; Lee, H. J. Pharmacokinetic Study of NADPH Oxidase Inhibitor Ewha-18278, a Pyrazole Derivative. *Pharmaceutics* **2019**, *11* (9), 1–8. https://doi.org/10.3390/pharmaceutics11090482.

(133) Dorotea, D.; Kwon, G.; Lee, J. H.; Saunders, E.; Bae, Y. S.; Moon, S. H.; Lee, S. J.; Cha, D. R.; Ha, H. A Pan-NADPH Oxidase Inhibitor Ameliorates Kidney Injury in Type 1 Diabetic Rats. *Pharmacology* **2018**, *102* (3–4), 180–189. https://doi.org/10.1159/000491398.

(134) Yamamoto, T.; Nakano, H.; Shiomi, K.; Wanibuchi, K.; Masui, H.; Takahashi, T.; Urano, Y.; Kamata, T. Identification and Characterization of a Novel NADPH Oxidase 1 (Nox1) Inhibitor That Suppresses Proliferation of Colon and Stomach Cancer Cells. *Biol. Pharm. Bull.* **2018**, *41* (3), 419–426. https://doi.org/10.1248/bpb.b17-00804.

(135) Hirano, K.; Chen, W. S.; Chueng, A. L. W.; Dunne, A. A.; Seredenina, T.; Filippova, A.; Ramachandran, S.; Bridges, A.; Chaudry, L.; Pettman, G.; Allan, C.; Duncan, S.; Lee, K. C.; Lim, J.; Ma, M. T.; Ong, A. B.; Ye, N. Y.; Nasir, S.; Mulyanidewi, S.; Aw, C. C.; Oon, P. P.; Liao, S.; Li, D.; Johns, D. G.; Miller, N. D.; Davies, C. H.; Browne, E. R.; Matsuoka, Y.; Chen, D. W.; Jaquet, V.; Rutter, A. R. Discovery of GSK2795039, a Novel Small Molecule NADPH Oxidase 2 Inhibitor. *Antioxidants Redox Signal.* **2015**, *23* (5), 358–374. https://doi.org/10.1089/ars.2014.6202.

(136) Yauger, Y. J.; Bermudez, S.; Moritz, K. E.; Glaser, E.; Stoica, B.; Byrnes, K. R. Iron Accentuated Reactive Oxygen Species Release by NADPH Oxidase in Activated Microglia Contributes to Oxidative Stress in Vitro. *J. Neuroinflammation* **2019**, *16* (1), 1–15. https://doi.org/10.1186/s12974-019-1430-7.

(137) Wang, M.; Luo, L. An Effective NADPH Oxidase 2 Inhibitor Provides Neuroprotection and Improves Functional Outcomes in Animal Model of Traumatic Brain Injury. *Neurochem. Res.* **2020**, *45* (5), 1097–1106. https://doi.org/10.1007/s11064-020-02987-3.

(138) Cifuentes-Pagano, E.; Saha, J.; Csányi, G.; Ghouleh, I. Al; Sahoo, S.; Rodr´ıguez, A.; Wipf, P.; Pagano, P. J.; Skoda, E. M. Bridged Tetrahydroisoquinolines as Selective NADPH Oxidase 2 (Nox2) Inhibitors. *Medchemcomm* **2013**, *4* (7), 1085–1092. https://doi.org/10.1039/c3md00061c.

(139) Li, Y.; Cifuentes-Pagano, E.; DeVallance, E. R.; de Jesus, D. S.; Sahoo, S.; Meijles, D. N.; Koes, D.; Camacho, C. J.; Ross, M.; St Croix, C.; Pagano, P. J. NADPH Oxidase 2 Inhibitors CPP11G and CPP11H Attenuate Endothelial Cell Inflammation & Vessel Dysfunction and Restore Mouse Hind-Limb Flow. *Redox Biol.* **2019**, *22* (December 2018). https://doi.org/10.1016/j.redox.2019.101143.

(140) Anvari, E.; Wikström, P.; Walum, E.; Welsh, N. The Novel NADPH Oxidase 4 Inhibitor GLX351322 Counteracts Glucose Intolerance in High-Fat Diet-Treated C57BL/6 Mice. *Free Radic. Res.* **2015**, *49* (11), 1308–1318. https://doi.org/10.3109/10715762.2015.1067697.

(141) Wang, X.; Elksnis, A.; Wikström, P.; Walum, E.; Welsh, N.; Carlsson, P. O. The Novel NADPH Oxidase 4 Selective Inhibitor GLX7013114 Counteracts Human Islet Cell Death in Vitro. *PLoS One* **2018**, *13* (9), 1–20. https://doi.org/10.1371/journal.pone.0204271.

(142) Das, S.; Wikström, P.; Walum, E.; Lovicu, F. J. A Novel NADPH Oxidase Inhibitor Targeting Nox4 in TGFβ-Induced Lens Epithelial to Mesenchymal Transition. *Exp. Eye Res.* **2019**, *185*, 1–5. https://doi.org/10.1016/j.exer.2019.107692.

(143) Kelso, G. F.; Porteous, C. M.; Coulter, C. V.; Hughes, G.; Porteous, W. K.; Ledgerwood, E. C.; Smith, R. A. J.; Murphy, M. P. Selective Targeting of a Redox-Active Ubiquinone to Mitochondria within Cells: Antioxidant and Antiapoptotic Properties. *J. Biol. Chem.* **2001**, *276* (7), 4588–4596. https://doi.org/10.1074/jbc.M009093200.

(144) Dashdorj, A.; KR, J.; Lim, S.; Jo, A.; Nguyen, M. N.; Ha, J.; Yoon, K. S.; Kim, H. J.; Park, J. H.; Murphy, M. P.; Kim, S. S. Mitochondria-Targeted Antioxidant MitoQ Ameliorates Experimental Mouse Colitis by Suppressing NLRP3 Inflammasome-Mediated Inflammatory Cytokines. *BMC Med.* **2013**, *11*, 1–13. https://doi.org/10.1186/1741-7015-11-178.

(145) Gottwald, E. M.; Duss, M.; Bugarski, M.; Haenni, D.; Schuh, C. D.; Landau, E. M.; Hall, A. M.

The Targeted Anti-Oxidant MitoQ Causes Mitochondrial Swelling and Depolarization in Kidney Tissue. *Physiol. Rep.* **2018**, *6* (7), 1–9. https://doi.org/10.14814/phy2.13667.

(146) Du, K.; Farhood, A.; Jaeschke, H. Mitochondria-Targeted Antioxidant Mito-Tempo Protects against Acetaminophen Hepatotoxicity. *Arch. Toxicol.* **2017**, *91* (2), 761–773. https://doi.org/10.1007/s00204-016-1692-0.

(147) Trnka, J.; Blaikie, F. H.; Smith, R. A. J.; Murphy, M. P. A Mitochondria-Targeted Nitroxide Is Reduced to Its Hydroxylamine by Ubiquinol in Mitochondria. *Free Radic. Biol. Med.* **2008**, *44* (7), 1406–1419. https://doi.org/10.1016/j.freeradbiomed.2007.12.036.

(148) Brand, M. D.; Goncalves, R. L. S.; 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.; Ainscow, E. K. Suppressors of Superoxide-H2O2 Production at Site IQ of Mitochondrial Complex I Protect against Stem Cell Hyperplasia and Ischemia-Reperfusion Injury. *Cell Metab.* **2016**, *24* (4), 582–592. https://doi.org/10.1016/j.cmet.2016.08.012.

(149) Wong, H. S.; Monternier, P. A.; Brand, M. D. S1QELs Suppress Mitochondrial Superoxide/Hydrogen Peroxide Production from Site IQ without Inhibiting Reverse Electron Flow through Complex I. *Free Radic. Biol. Med.* **2019**, *143*, 545–559. https://doi.org/10.1016/j.freeradbiomed.2019.09.006.

(150) Banba, A.; Tsuji, A.; Kimura, H.; Murai, M.; Miyoshi, H. Defining the Mechanism of Action of S1QELs, Specific Suppressors of Superoxide Production in the Quinone-Reaction Site in Mitochondrial Complex I. *J. Biol. Chem.* **2019**, *294* (16), 6550–6561. https://doi.org/10.1074/jbc.RA119.007687.

(151) 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.; Brand, M. D. Suppressors of Superoxide Production from Mitochondrial Complex III. *Nat. Chem. Biol.* **2015**, *11* (11), 834–836. https://doi.org/10.1038/nchembio.1910.

(152) Manzoor, S.; Hoda, N. A Comprehensive Review of Monoamine Oxidase Inhibitors as Anti-Alzheimer's Disease Agents: A Review. *Eur. J. Med. Chem.* **2020**, *206*, 112787. https://doi.org/10.1016/j.ejmech.2020.112787.

(153) Youdim, M. B. H. Monoamine Oxidase Inhibitors, and Iron Chelators in Depressive Illness and Neurodegenerative Diseases. *J. Neural Transm.* **2018**, *125* (11), 1719–1733. https://doi.org/10.1007/s00702-018-1942-9.

(154) Hauser, R. A.; Li, R.; Pérez, A.; Ren, X.; Weintraub, D.; Elm, J.; Goudreau, J. L.; Morgan, J. C.; Fang, J. Y.; Aminoff, Michael J. Christine, Chadwick W. Dhall, R.; Umeh, C. C.; Boyd, J. T.; Stover, N.; Leehey, M.; Zweig, R. M.; Nicholas, A. P.; Bodis-Wollner, Ivan Willis, A.; Kieburtz, K.; Tilley, B. C. Longer Duration of MAO-b Inhibitor Exposure Is Associated with Less Clinical Decline in Parkinson's Disease: An Analysis of NETPD LS1. *J. Park. Dis.* **2017**, *7* (1), 117–127. https://doi.org/doi.org/10.3233/ jpd-16096 5.

(155) Binde, C. D.; Tvete, I. F.; Gåsemyr, J.; Natvig, B.; Klemp, M. A Multiple Treatment Comparison Meta-Analysis of Monoamine Oxidase Type B Inhibitors for Parkinson's Disease. *Br. J. Clin. Pharmacol.* **2018**, *84* (9), 1917–1927. https://doi.org/10.1111/bcp.13651.

(156) Birkmayer, W. Deprenyl (Selegiline) in the Treatment of Parkinson ' s Disease. *Acta Neurol. Scand.* **1983**, *68* (Suppl 95), 103–105. https://doi.org/doi.org/10.1111/j.1600-0404.1983.tb01522.x.

(157) Guay, D. R. P. Rasagiline (TVP-1012): A New Selective Monoamine Oxidase Inhibitor for Parkinson's Disease. *Am. J. Geriatr. Pharmacother.* **2006**, *4* (4), 330–346. https://doi.org/10.1016/j.amjopharm.2006.12.001.

(158) Filip, V.; Kolibás, E. Selegiline in the Treatment of Alzheimer's Disease: A Long-Term Randomized Placebo-Controlled Trial. *J. Psychiatry Neurosci.* **1999**, *24* (3), 234–243.

(159) Ryu, I.; Ryu, M. J.; Han, J.; Kim, S. J.; Lee, M. J.; Ju, X.; Yoo, B. H.; Lee, Y. L.; Jang, Y.; Song, I. C.; Chung, W.; Oh, E.; Heo, J. Y.; Kweon, G. R. L-Deprenyl Exerts Cytotoxicity towards Acute Myeloid Leukemia through Inhibition of Mitochondrial Respiration. *Oncology Reports*. 2018, pp 3869–3878. https://doi.org/10.3892/or.2018.6753.

(160) Lighezan, R.; Sturza, A.; Duicu, O. M.; Ceausu, R. A.; Vaduva, A.; Gaspar, M.; Feier, H.; Vaida, M.; Ivan, V.; Lighezan, D.; Muntean, D. M.; Mornos, C. Monoamine Oxidase Inhibition Improves Vascular Function in Mammary Arteries from Nondiabetic and Diabetic Patients with Coronary Heart Disease. *Can. J. Physiol. Pharmacol.* **2016**, *94* (10), 1040–1047. https://doi.org/10.1139/cjpp-2015-0580.

(161) Tábi, T.; Vécsei, L.; Youdim, M. B.; Riederer, P.; Szökő, É. Selegiline: A Molecule with Innovative Potential. *J. Neural Transm.* **2020**, *127* (5), 831–842. https://doi.org/10.1007/s00702-019-02082-0.

(162) Lecht, S.; Haroutiunian, S.; Hoffman, A.; Lazarovici, P. Rasagiline - A Novel MAO B Inhibitor in Parkinson's Disease Therapy. *Ther. Clin. Risk Manag.* **2007**, *3* (3), 467–474.

(163) Sieradzan, K.; Channon, S.; Ramponi, C.; Stern, G. M.; Lees, A. J.; Youdim, M. B. The Therapeutic Potential of Moclobemide, a Reversible Selective Monoamine Oxidase A Inhibitor in Parkinson's Disease. *J. Clin. Psychopharmacol.* **1995**, *15* (4 Suppl 2), 51S—59S. https://doi.org/10.1097/00004714-199508001-00010.

(164) Vuohelainen, V.; Hämäläinen, M.; Paavonen, T.; Karlsson, S.; Moilanen, E.; Mennander, A. Inhibition of Monoamine Oxidase A Increases Recovery after Experimental Cardiac Arrest. *Interact. Cardiovasc. Thorac. Surg.* **2015**, *21* (4), 441–449. https://doi.org/10.1093/icvts/ivv175.

(165) Xu, Y. xiang; Wang, H.; Li, X. kang; Dong, S. nan; Liu, W. wen; Gong, Q.; Wang, T. duan yi; Tang, Y.; Zhu, J.; Li, J.; Zhang, H. yan; Mao, F. Discovery of Novel Propargylamine-Modified 4-Aminoalkyl Imidazole Substituted Pyrimidinylthiourea Derivatives as Multifunctional Agents for the Treatment of Alzheimer's Disease. *Eur. J. Med. Chem.* **2018**, *143*, 33–47. https://doi.org/10.1016/j.ejmech.2017.08.025.

(166) Youdim, M. B. H.; Weinstock, M. Molecular Basis of Neuroprotective Activities of Rasagiline and the Anti-Alzheimer Drug TV3326 [(N-Propargyl-(3R)Aminoindan-5-YL)-Ethyl Methyl Carbamate]. *Cell. Mol. Neurobiol.* **2001**, *21* (6), 555–573. https://doi.org/10.1023/A:1015131516649.

(167) Bautista-Aguilera, Ó. M.; Hagenow, S.; Palomino-Antolin, A.; Farré-Alins, V.; Ismaili, L.; Joffrin, P. L.; Jimeno, M. L.; Soukup, O.; Janočková, J.; Kalinowsky, L.; Proschak, E.; Iriepa, I.; Moraleda, I.; Schwed, J. S.; Romero Martínez, A.; López-Muñoz, F.; Chioua, M.; Egea, J.; Ramsay, R. R.; Marco-Contelles, J.; Stark, H. Multitarget-Directed Ligands Combining Cholinesterase and Monoamine Oxidase Inhibition with Histamine H3R Antagonism for Neurodegenerative Diseases. *Angew. Chemie - Int. Ed.* **2017**, *56* (41), 12765–12769. https://doi.org/10.1002/anie.201706072.

(168) Bautista-Aguilera, Ó. M.; Budni, J.; Mina, F.; Medeiros, E. B.; Deuther-Conrad, W.; Entrena, J. M.; Moraleda, I.; Iriepa, I.; López-Muñoz, F.; Marco-Contelles, J. Contilisant, a Tetratarget Small

Molecule for Alzheimer's Disease Therapy Combining Cholinesterase, Monoamine Oxidase Inhibition, and H3R Antagonism with S1R Agonism Profile. *J. Med. Chem.* **2018**, *61* (15), 6937–6943. https://doi.org/10.1021/acs.jmedchem.8b00848.

(169) Blair, H. A.; Dhillon, S. Safinamide: A Review in Parkinson's Disease. *CNS Drugs* **2017**, *31* (2), 169–176. https://doi.org/10.1007/s40263-017-0408-1.

(170) Binda, C.; Wang, J.; Pisani, L.; Caccia, C.; Carotti, A.; Salvati, P.; Edmondson, D. E.; Mattevi, A. Structures of Human Monoamine Oxidase B Complexes with Selective Noncovalent Inhibitors: Safinamide and Coumarin Analogs. *J. Med. Chem.* **2007**, *50* (23), 5848–5852. https://doi.org/10.1021/jm070677y.

(171) Cattaneo, C.; Caccia, C.; Marzo, A.; Maj, R.; Fariello, R. G. Pressor Response to Intravenous Tyramine in Healthy Subjects after Safinamide, a Novel Neuroprotectant with Selective, Reversible Monoamine Oxidase B Inhibition. *Clin. Neuropharmacol.* **2003**, *26* (4), 213–217. https://doi.org/10.1097/00002826-200307000-00012.

(172) Reis, J.; Manzella, N.; Cagide, F.; Mialet-Perez, J.; Uriarte, E.; Parini, A.; Borges, F.; Binda, C. Tight-Binding Inhibition of Human Monoamine Oxidase B by Chromone Analogs: A Kinetic, Crystallographic, and Biological Analysis. *J. Med. Chem.* **2018**, *61* (9), 4203–4212. https://doi.org/10.1021/acs.jmedchem.8b00357.

(173) Reis, J.; Cagide, F.; Chavarria, D.; Silva, T.; Fernandes, C.; Gaspar, A.; Uriarte, E.; Remião, F.; Alcaro, S.; Ortuso, F.; Borges, F. Discovery of New Chemical Entities for Old Targets: Insights on the Lead Optimization of Chromone-Based Monoamine Oxidase B (MAO-B) Inhibitors. *J. Med. Chem.* **2016**, *59* (12), 5879–5893. https://doi.org/10.1021/acs.jmedchem.6b00527.

(174) Tzvetkov, N. T.; Hinz, S.; Küppers, P.; Gastreich, M.; Müller, C. E. Indazole-and Indole-5-Carboxamides: Selective and Reversible Monoamine Oxidase B Inhibitors with Subnanomolar Potency. *J. Med. Chem.* **2014**, *57* (15), 6679–6703. https://doi.org/10.1021/jm500729a.

(175) Gealageas, R.; Devineau, A.; So, P. P. L.; Kim, C. M. J.; Surendradoss, J.; Buchwalder, C.; Heller, M.; Goebeler, V.; Dullaghan, E. M.; Grierson, D. S.; Putnins, E. E. Development of Novel Monoamine Oxidase-B (MAO-B) Inhibitors with Reduced Blood-Brain Barrier Permeability for the Potential Management of Noncentral Nervous System (CNS) Diseases. *J. Med. Chem.* **2018**, *61* (16), 7043–7064. https://doi.org/10.1021/acs.jmedchem.7b01588.

(176) Bredemeier, M.; Lopes, L. M.; Eisenreich, M. A.; Hickmann, S.; Bongiorno, G. K.; d'Avila, R.; Morsch, A. L. B.; da Silva Stein, F.; Campos, G. G. D. Xanthine Oxidase Inhibitors for Prevention of Cardiovascular Events: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *BMC Cardiovasc. Disord.* **2018**, *18* (1), 1–11. https://doi.org/10.1186/s12872-018-0757-9.

(177) Pacher, P.; Nivorozhkin, A.; Szabó, C. Therapeutic Effects of Xanthine Oxidase Inhibitors: Renaissance Half a Century after the Discovery of Allopurinol. *Pharmacol. Rev.* **2006**, *58* (1), 87–114. https://doi.org/10.1124/pr.58.1.6.

(178) Robins, R. K. Potential Purine Antagonists. I. Synthesis of Some 4,6-Substituted Pyrazolo [3,4-d] Pyrimidines. *J. Am. Chem. Soc.* **1956**, *78* (4), 784–790. https://doi.org/10.1021/ja01585a023.

(179) Hosoya, T.; Sasaki, T.; Ohashi, T. Clinical Efficacy and Safety of Topiroxostat in Japanese Hyperuricemic Patients with or without Gout: A Randomized, Double-Blinded, Controlled Phase 2b Study. *Clin. Rheumatol.* **2017**, *36* (3), 649–656. https://doi.org/10.1007/s10067-016-3474-8.

(180) Becker, M. A.; Schumacher, H. R.; Wortmann, R. L.; MacDonald, P. A.; Palo, W. A.; Eustace, D.; Vernillet, L.; Joseph-Ridge, N. Febuxostat, a Novel Nonpurine Selective Inhibitor of Xanthine

Oxidase: A Twenty-Eight-Day, Multicenter, Phase II, Randomized, Double-Blind, Placebo-Controlled, Dose-Response Clinical Trial Examining Safety and Efficacy in Patients with Gout. *Arthritis Rheum.* **2005**, *52* (3), 916–923. https://doi.org/10.1002/art.20935.

(181) Kelley, W. N.; Beardmore, T. D. Allopurinol : Alteration in Pyrimidine Metabolism in Man. *Am. Assoc. Adv. Sci.* **1970**, *169* (3943), 388–390. https://doi.org/10.1126/science.169.3943.388.

(182) Fox, R. M.; Royse-Smith, D.; O'Sullivan, W. J. Orotidinuria Induced by Allopurinol. *Am. Assoc. Adv. Sci. Stable* **1970**, *168* (3933), 861–862. https://doi.org/10.1126/science.168.3933.861.

(183) Hasegawa, M. A Facile One-Pot Synthesis Of 4-Alkoxy-1,3. Benzenedicarbonitrile. *Reactions* **1998**, *47* (2), 857–864. https://doi.org/10.3987/COM-97-S(N)89.

(184) Takano, Y.; Hase-Aoki, K.; Horiuchi, H.; Zhao, L.; Kasahara, Y.; Kondo, S.; Becker, M. A. Selectivity of Febuxostat, a Novel Non-Purine Inhibitor of Xanthine Oxidase/Xanthine Dehydrogenase. *Life Sci.* **2005**, *76* (16), 1835–1847. https://doi.org/10.1016/j.lfs.2004.10.031.

(185) Okamoto, K.; Matsumoto, K.; Hille, R.; Eger, B. T.; Pai, E. F.; Nishino, T. The Crystal Structure of Xanthine Oxidoreductase during Catalysis: Implications for Reaction Mechanism and Enzyme Inhibition. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (21), 7931–7936. https://doi.org/10.1073/pnas.0400973101.

(186) Sato, T.; Ashizawa, N.; Matsumoto, K.; Iwanaga, T.; Nakamura, H.; Inoue, T.; Nagata, O. Discovery of 3-(3-Cyano-4-Pyridyl)-5-(4-Pyridyl)-1,2,4-Triazole, FYX-051-a Xanthine Oxidoreductase Inhibitor for the Treatment of Hyperuricemia. *Bioorganic Med. Chem. Lett.* **2009**, *19* (21), 6225–6229. https://doi.org/10.1016/j.bmcl.2009.08.091.

(187) Matsumoto, K.; Okamoto, K.; Ashizawa, N.; Nishino, T. FYX-051: A Novel and Potent Hybrid-Type Inhibitor of Xanthine Oxidoreductase. *J. Pharmacol. Exp. Ther.* **2011**, *336* (1), 95–103. https://doi.org/10.1124/jpet.110.174540.

(188) Minhas, R.; Bansal, Y.; Bansal, G. Inducible Nitric Oxide Synthase Inhibitors: A Comprehensive Update. *Med. Res. Rev.* **2020**, *40* (3), 823–855. https://doi.org/10.1002/med.21636.

(189) Alderton, W. K.; Angell, A. D. R.; Craig, C.; Dawson, J.; Garvey, E.; Moncada, S.; Monkhouse, J.; Rees, D.; Russell, L. J.; Russell, R. J.; Schwartz, S.; Waslidge, N.; Knowles, R. G. GW274150 and GW273629 Are Potent and Highly Selective Inhibitors of Inducible Nitric Oxide Synthase in Vitro and in Vivo. *Br. J. Pharmacol.* **2005**, *145* (3), 301–312. https://doi.org/10.1038/sj.bjp.0706168.

(190) Strub, A.; Ulrich, W.-R.; Hesslinger, C.; Eltze, M.; Fuchß, T.; Strassner, J.; Strand, S.; Lehner, M. D.; Boer, R. The Novel Imidazopyridine 2- [2- (4-Methoxy-Pyridin-2-Yl) -Ethyl] - 3 H - Imidazo [4 , 5- b] Pyridine (BYK191023) Is a Highly Selective Inhibitor of the Inducible Nitric-Oxide Synthase. *Mol. Pharmacol.* **2006**, *69* (1), 328–337. https://doi.org/10.1124/mol.105.017087.

(191) Garvey, E. P.; Oplinger, J. A.; Furfine, E. S.; Kiff, R. J.; Laszlo, F.; Whittle, B. J. R.; Knowles, R. G. 1400W Is a Slow , Tight Binding , and Highly Selective Inhibitor of Inducible Nitric-Oxide Synthase in Vitro and in Vivo. *J. Biol. Chem.* **1997**, *272* (8), 4959–4963. https://doi.org/10.1074/jbc.272.8.4959.

(192) Moore, W. M.; Webber, R. K.; Jerome, G. M.; Tjoeng, F. S.; Misko, T. P.; Currie, M. G. L-N6-(1-Iminoethyl)Lysine: Selective Inhibitor of Inducible Nitric Oxide. *J. Med. Chem.* **1994**, *37*, 3886–3888. https://doi.org/10.1021/jm00049a007.

(193) Chatterjee, P. K.; Patel, N. S. A.; Kvale, E. O.; Cuzzocrea, S.; Brown, P. A. J.; Stewart, K. N.; Mota-Filipe, H.; Thiemermann, C. Inhibition of Inducible Nitric Oxide Synthase Reduces Renal

Ischemia/Reperfusion Injury. *Kidney Int.* **2002**, *61* (3), 862–871. https://doi.org/10.1046/j.1523-1755.2002.00234.x.

(194) Chatterjee, P. K.; Patel, N. S. A.; Sivarajah, A.; Kvale, E. O.; Dugo, L.; Cuzzocrea, S.; Brown, P. A. J.; Stewart, K. N.; Mota-Filipe, H.; Britti, D.; Yaqoob, M. M.; Thiemermann, C. GW274150, a Potent and Highly Selective Inhibitor of INOS Reduces Experimental Renal Ischemia/Reperfusion Injury. *Kidney Int.* **2003**, *63* (3), 853–865. https://doi.org/10.1046/j.1523-1755.2003.00802.x.

(195) Cuzzocrea, S.; Chatterjee, P. K.; Mazzon, E.; McDonald, M. C.; Dugo, L.; Di Paola, R.; Serraino, I.; Britti, D.; Caputi, A. P.; Thiemermann, C. Beneficial Effects of GW274150, a Novel, Potent and Selective Inhibitor of INOS Activity, in a Rodent Model of Collagen-Induced Arthritis. *Eur. J. Pharmacol.* **2002**, *453* (1), 119–129. https://doi.org/10.1016/S0014-2999(02)02338-5.

(196) Chatterjee, P. K.; Kvale, E. O.; Patel, N. S. A.; Thiemermann, C. GW274150 Inhibits Nitric Oxide Production by Primary Cultures of Rat Proximal Tubular Cells. *Med. Sci. Monit.* **2003**, *9* (10), BR357–BR362.

(197) Broom, L.; Marinova-Mutafchieva, L.; Sadeghian, M.; Davis, J. B.; Medhurst, A. D.; Dexter, D. T. Neuroprotection by the Selective INOS Inhibitor GW274150 in a Model of Parkinson Disease. *Free Radic. Biol. Med.* **2011**, *50* (5), 633–640. https://doi.org/10.1016/j.freeradbiomed.2010.12.026.

(198) Garcin, E. D.; Arvai, A. S.; Rosenfeld, R. J.; Kroeger, M. D.; Crane, B. R.; Andersson, G.; Andrews, G.; Hamley, P. J.; Mallinder, P. R.; Nicholls, D. J.; St-Gallay, S. A.; Tinker, A. C.; Gensmantel, N. P.; Mete, A.; Cheshire, D. R.; Connolly, S.; Stuehr, D. J.; Åberg, A.; Wallace, A. V.; Tainer, J. A.; Getzoff, E. D. Anchored Plasticity Opens Doors for Selective Inhibitor Design in Nitric Oxide Synthase. *Nat. Chem. Biol.* **2008**, *4* (11), 700–707. https://doi.org/10.1038/nchembio.115.

(199) Hagmann, W. K.; Caldwell, C. G.; Chen, P.; Durette, P. L.; Esser, C. K.; Lanza, T. J.; Kopka, I. E.; Guthikonda, R.; Shah, S. K.; MacCoss, M.; Chabin, R. M.; Fletcher, D.; Grant, S. K.; Green, B. G.; Humes, J. L.; Kelly, T. M.; Luell, S.; Meurer, R.; Moore, V.; Pacholok, S. G.; Pavia, T.; Williams, H. R.; Wong, K. K. Substituted 2-Ammopyridines as Inhibitors of Nitric Oxide Synthases. *Bioorganic Med. Chem. Lett.* **2000**, *10* (17), 1975–1978. https://doi.org/10.1016/S0960-894X(00)00389-9.

(200) Mukherjee, P.; Cinelli, M. A.; Kang, S.; Silverman, R. B. Development of Nitric Oxide Synthase Inhibitors for Neurodegeneration and Neuropathic Pain. *Chem. Soc. Rev.* **2014**, *43* (19), 6814–6838. https://doi.org/10.1039/c3cs60467e.

(201) Mukherjee, P.; Li, H.; Sevrioukova, I.; Chrei, G.; Marta, P.; Roman, L. J.; Poulos, T. L.; Silverman, R. B. Novel 2,4-Disubstituted Pyrimidines as Potent, Selective, and Cell- Permeable Inhibitors of Neuronal Nitric Oxide Synthase. *J. Med. Chem.* **2015**, *59* (3), 1067–1088. https://doi.org/10.1021/jm501719e.

(202) Davey, D. D.; Adler, M.; Arnaiz, D.; Eagen, K.; Erickson, S.; Guilford, W.; Kenrick, M.; Morrissey, M. M.; Ohlmeyer, M.; Pan, G.; Paradkar, V. M.; Parkinson, J.; Polokoff, M.; Saionz, K.; Santos, C.; Subramanyam, B.; Vergona, R.; Wei, R. G.; Whitlow, M.; Ye, B.; Zhao, Z.; Devlin, J. J.; Phillips, G. Design, Synthesis, and Activity of 2-Imidazol-1-Ylpyrimidine Derived Inducible Nitric Oxide Synthase Dimerization Inhibitors. *J. Med. Chem.* **2007**, *50* (6), 1146–1157. https://doi.org/10.1021/jm061319i.

(203) Ji, H.; Delker, S. L.; Li, H.; Martásek, P.; Roman, L. J.; Poulos, T. L.; Silverman, R. B. Exploration of the Active Site of Neuronal Nitric Oxide Synthase by the Design and Synthesis of

Pyrrolidinomethyl 2- Aminopyridine Derivatives. *J. Med. Chem.* **2010**, *53* (21), 7804–7824. https://doi.org/10.1021/jm100947x.

(204) Conrad, M.; Pratt, D. A. The Chemical Basis of Ferroptosis. *Nat. Chem. Biol.* **2019**, *15* (12), 1137–1147. https://doi.org/10.1038/s41589-019-0408-1.

(205) Miotto, G.; Rossetto, M.; Di Paolo, M. L.; Orian, L.; Venerando, R.; Roveri, A.; Vučković, A. M.; Bosello Travain, V.; Zaccarin, M.; Zennaro, L.; Maiorino, M.; Toppo, S.; Ursini, F.; Cozza, G. Insight into the Mechanism of Ferroptosis Inhibition by Ferrostatin-1. *Redox Biol.* **2020**, *28* (July 2019), 101328. https://doi.org/10.1016/j.redox.2019.101328.

(206) Devisscher, L.; Van Coillie, S.; Hofmans, S.; Van Rompaey, D.; Goossens, K.; Meul, E.; Maes, L.; De Winter, H.; Van Der Veken, P.; Vandenabeele, P.; Berghe, T. Vanden; Augustyns, K. Discovery of Novel, Drug-Like Ferroptosis Inhibitors with in Vivo Efficacy. *J. Med. Chem.* **2018**, *61* (22), 10126–10140. https://doi.org/10.1021/acs.jmedchem.8b01299.

(207) Zilka, O.; Shah, R.; Li, B.; Friedmann Angeli, J. P.; Griesser, M.; Conrad, M.; Pratt, D. A. On the Mechanism of Cytoprotection by Ferrostatin-1 and Liproxstatin-1 and the Role of Lipid Peroxidation in Ferroptotic Cell Death. *ACS Cent. Sci.* **2017**, *3* (3), 232–243. https://doi.org/10.1021/acscentsci.7b00028.

(208) Friedmann Angeli, J. P.; Schneider, M.; Proneth, B.; Tyurina, Y. Y.; Tyurin, V. A.; Hammond, V. J.; Herbach, N.; Aichler, M.; Walch, A.; Eggenhofer, E.; Basavarajappa, D.; Rådmark, O.; Kobayashi, S.; Seibt, T.; Beck, H.; Neff, F.; Esposito, I.; Wanke, R.; Förster, H.; Yefremova, O.; Heinrichmeyer, M.; Bornkamm, G. W.; Geissler, E. K.; Thomas, S. B.; Stockwell, B. R.; Odonnell, V. B.; Kagan, V. E.; Schick, J. A.; Conrad, M. Inactivation of the Ferroptosis Regulator Gpx4 Triggers Acute Renal Failure in Mice. *Nat. Cell Biol.* **2014**, *16* (12), 1180–1191. https://doi.org/10.1038/ncb3064.

(209) Shah, R.; Margison, K.; Pratt, D. A. The Potency of Diarylamine Radical-Trapping Antioxidants as Inhibitors of Ferroptosis Underscores the Role of Autoxidation in the Mechanism of Cell Death. *ACS Chem. Biol.* **2017**, *12* (10), 2538–2545. https://doi.org/10.1021/acschembio.7b00730.

(210) Poon, J. F.; Zilka, O.; Pratt, D. A. Potent Ferroptosis Inhibitors Can Catalyze the Cross-Dismutation of Phospholipid-Derived Peroxyl Radicals and Hydroperoxyl Radicals. *J. Am. Chem. Soc.* **2020**, *142* (33), 14331–14342. https://doi.org/10.1021/jacs.0c06379.

(211) Vejražka, M.; Míček, R.; Štípek, S. Apocynin Inhibits NADPH Oxidase in Phagocytes but Stimulates ROS Production in Non-Phagocytic Cells. *Biochim. Biophys. Acta - Gen. Subj.* **2005**, *1722* (2), 143–147. https://doi.org/10.1016/j.bbagen.2004.12.008.

(212) Stolk, J.; Hiltermann, T. J.; Dijkman, J. H.; Verhoeven, A. J. Characteristics of the Inhibition of NADPH Oxidase Activation in Neutrophils by Apocynin, a Methoxy-Substituted Catechol. *Am. J. Respir. Cell Mol. Biol.* **1994**, *11* (1), 95–102. https://doi.org/10.1165/ajrcmb.11.1.8018341.

(213) Heumüller, S.; Wind, S.; Barbosa-Sicard, E.; Schmidt, H. H. H. W.; Busse, R.; Schröder, K.; Brandes, R. P. Apocynin Is Not an Inhibitor of Vascular NADPH Oxidases but an Antioxidant. *Hypertension* **2008**, *51* (2), 211–217. https://doi.org/10.1161/HYPERTENSIONAHA.107.100214.

(214) Gatto, G. J.; Ao, Z.; Kearse, M. G.; Zhou, M.; Morales, C. R.; Daniels, E.; Bradley, B. T.; Goserud, M. T.; Goodman, K. B.; Douglas, S. A.; Harpel, M. R.; Johns, D. G. NADPH Oxidase-Dependent and -Independent Mechanisms of Reported Inhibitors of Reactive Oxygen Generation. *J. Enzyme Inhib. Med. Chem.* **2013**, *28* (1), 95–104. https://doi.org/10.3109/14756366.2011.636360.

(215) Solbak, S. M. Ø.; Zang, J.; Narayanan, D.; Høj, L. J.; Bucciarelli, S.; Softley, C.; Meier, S.; Langkilde, A. E.; Gotfredsen, C. H.; Sattler, M.; Bach, A. Developing Inhibitors of the P47phox−p22phox Protein−Protein Interaction by Fragment-Based Drug Discovery.Pdf. *J. Med. Chem.* **2020**, *63* (3), 1156–1177. https://doi.org/10.1021/acs.jmedchem.9b01492.

(216) Kuhn, R. Lactoflavin (Vitamin B2). *Angew. Chemie* **1936**, *49* (1), 6–10. https://doi.org/10.1002/ange.19360490103.

(217) Schramm, M.; Wiegmann, K.; Schramm, S.; Gluschko, A.; Herb, M.; Utermöhlen, O.; Krönke, M. Riboflavin (Vitamin B 2) Deficiency Impairs NADPH Oxidase 2 (Nox2) Priming and Defense against Listeria Monocytogenes. *Eur. J. Immunol.* **2014**, *44* (3), 728–741. https://doi.org/10.1002/eji.201343940.

(218) Zhao, R.; Wang, H.; Qiao, C.; Zhao, K. Vitamin B2 Blocks Development of Alzheimer ' s Disease in APP / PS1 Transgenic Mice via Anti-Oxidative Mechanism. *Trop. J. Pharm. Res. June* **2018**, *17* (6), 1049–1054. https://doi.org/10.4314/tjpr.v17i6.10.

(219) Pérez-Torres, I.; Guarner-Lans, V.; Rubio-Ruiz, M. E. Reductive Stress in Inflammation-Associated Diseases and the pro-Oxidant Effect of Antioxidant Agents. *Int. J. Mol. Sci.* **2017**, *18* (10), 1–26. https://doi.org/10.3390/ijms18102098.

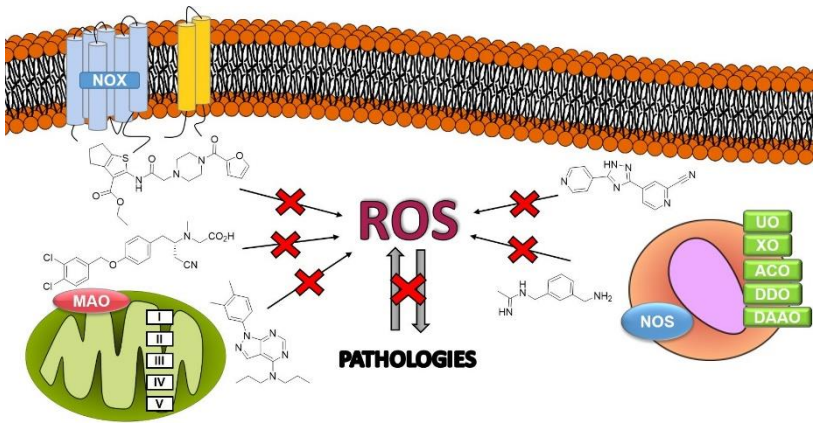

Table of Contents graphic.