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Targeting Mitochondria

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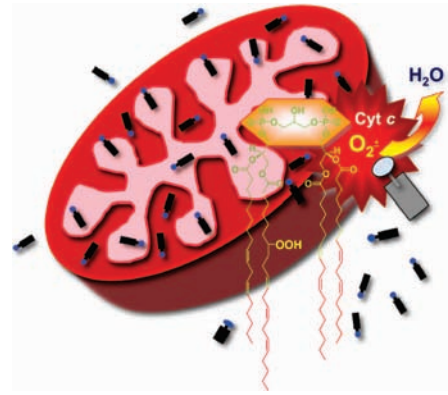
CONSPECTUS



Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are closely linked to degenerative diseases such as Alzheimer's disease, Parkinson's, neuronal death including ischemic and hemorrhagic stroke, acute and chronic degenerative cardiac myocyte death, and cancer. As a byproduct of oxidative phosphorylation, a steady stream of reactive species emerge from our cellular energy plants, the mitochondria. ROS and RNS potentially cause damage to all cellular components. Structure alteration, biomolecule fragmentation, and oxidation of side chains are trade-offs of cellular energy production. ROS and RNS escape results in the activation of cytosolic stress pathways, DNA damage, and the upregulation of JNK, p38, and p53. Incomplete scavenging of ROS and RNS particularly affects the mitochondrial lipid cardiolipin (CL), triggers the release of mitochondrial cytochrome *c*, and activates the intrinsic death pathway.

Due to the active redox environment and the excess of NADH and ATP at the inner mitochondrial membrane, a broad range of agents including electron acceptors, electron donors, and hydride acceptors can be used to influence the biochemical pathways. The key to therapeutic value is to enrich selective redox modulators at the target sites.

Our approach is based on conjugating nitroxides to segments of natural products with relatively high affinity for mitochondrial membranes. For example, a modified gramicidin S segment was successfully used for this purpose and proven to be effective in preventing superoxide production in cells and CL oxidation in mitochondria and in protecting cells against a range of pro-apoptotic triggers such as actinomycin D, radiation, and staurosporine. More importantly, these mitochondria-targeted nitroxide/gramicidin conjugates were able to protect against apoptosis in vivo by preventing CL oxidation induced by intestinal hemorrhagic shock. Optimization of nitroxide carriers could lead to a new generation of effective antiapoptotic agents acting at an early mitochondrial stage.

Alternative chemistry-based approaches to targeting mitochondria include the use of proteins and peptides, as well as the attachment of payloads to lipophilic cationic compounds, sulfonylureas, anthracyclines, and other agents with proven or hypothetical affinities for mitochondria. Manganese superoxide dismutase (MnSOD), SS tetrapeptides with 2′,6′-dimethyltyrosine (Dmt) residues, rhodamine, triphenylphosphonium salts, nonopioid analgesics, adriamycin, and diverse electron-rich aromatics and stilbenes were used to influence mitochondrial biochemistry and the biology of aging.

Some general structural principles for effective therapeutic agents are now emerging. Among these are the presence of basic or positively charged functional groups, hydrophobic substructures, and, most promising for future selective strategies, classes of compounds that are actively shuttled into mitochondria, bind to mitochondria-specific proteins, or show preferential affinity to mitochondria-specific lipids.

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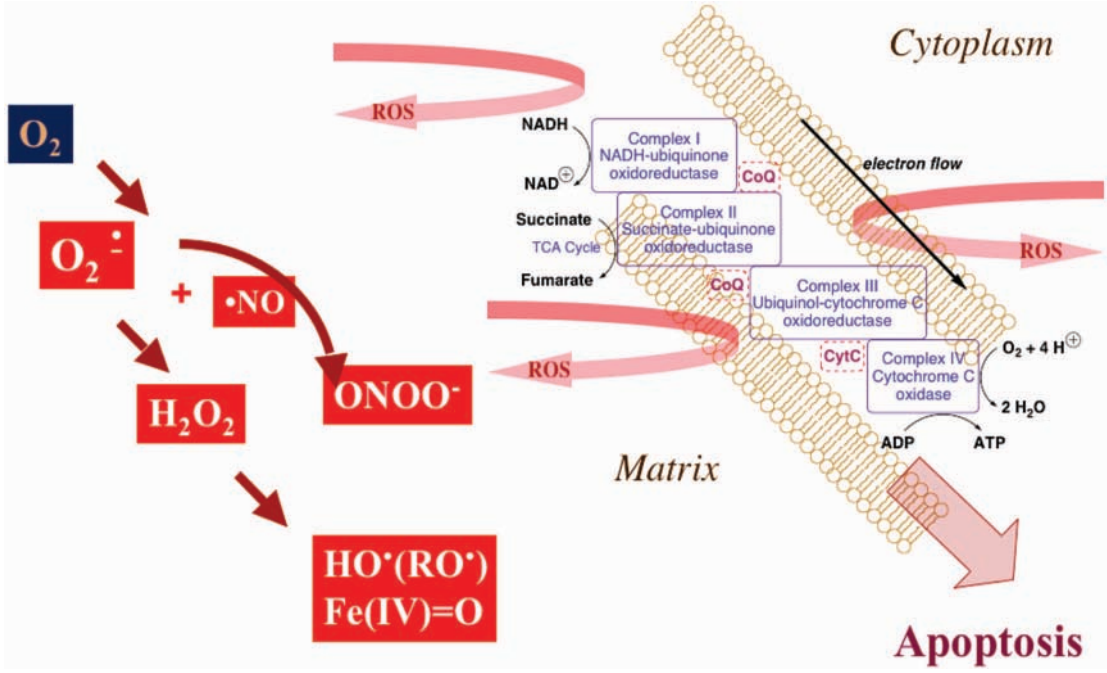

FIGURE 1. Schematic model for generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during oxidative phosphorylation in the mitochondrial membrane and matrix. The formation of superoxide radical anion initiates a cascade process that can induce programmed cell death (apoptosis).

Introduction

In 1956, Harman proposed the "free radical theory" of aging and associated degenerative diseases.[1] According to this hypothesis, "...the reaction of active free radicals, normally produced in the organisms, with cellular constituents initiates the changes associated with aging." Consequences of these biochemical alterations are an up-regulation of p53 and the activation of cytosolic stress signaling pathways. Ultimately, both life span and life quality are severely negatively affected. While some controversy regarding the generality of the Harman theory continues, experimental evidence for the link between reactive oxygen species (ROS) and the biology of aging is steadily solidifying.[2] In particular, mitochondrial metabolism and the oxidative phosphorylation cascade are emerging as key factors in the generation of ROS associated with a large number of disease states, including atherosclerosis, Alzheimer's disease, Parkinson's, neuronal death including ischemic and hemorrhagic stroke, acute and chronic degenerative cardiac myocyte death, and cancer.[3,4]

With a notable exception of NADPH oxidase in activated inflammatory cells, the lion's share, possibly up to 90%,[2] of intracellular ROS are generated in mitochondria during the controlled oxidation of NADH and FADH with molecular oxygen coupled with the phosphorylation of ADP to give ATP in a cascade of multienzyme complexes embedded in the mitochondrial membrane (Figure 1). In addition to superoxide radical anion ($O_2^{-\bullet}$), other major reactive species generated in this process include H_2O_2 and HO•. Furthermore, ROS collectively include both oxygen radicals and nonradical oxidizing species such as $^1O_2$, O_3, and ROOR; a large variety of these reactive molecules are produced *in vivo*, even though the specific reaction mechanisms involved in their generation remain still unclear.[5,6] Upon reaction with nitric oxide (NO•), $O_2^{-\bullet}$ also participates in the formation of reactive nitrogen species (RNS), such as $ONOO^-$. Several efficient enzymatic processes are continuously operational to quench ROS and RNS, including superoxide dismutase (SOD) and superoxide reductase (SOR),[7] catalase,[8] peroxiredoxin (Prx),[9] glutathione peroxidase (GP), and thioredoxin/thioredoxin reductase (Trx/TrxR) (Figure 2).[10] However, if, as a result of aging or pathological processes, the production of ROS and RNS exceeds the scavenging capacity of endogenous systems, then both mitochondrial and extramitochondrial cellular components, including proteins, nucleic acids, and lipids, can be damaged. Under these circumstances, an external chemical intervention might be beneficial. Phar-

FIGURE 2. Antioxidant scavenging reactions that eliminate ROS. SOD = superoxide dismutase; Prx = peroxiredoxin; GP = glutathione peroxidase; Trx = thioredoxin; GSH = glutathione.

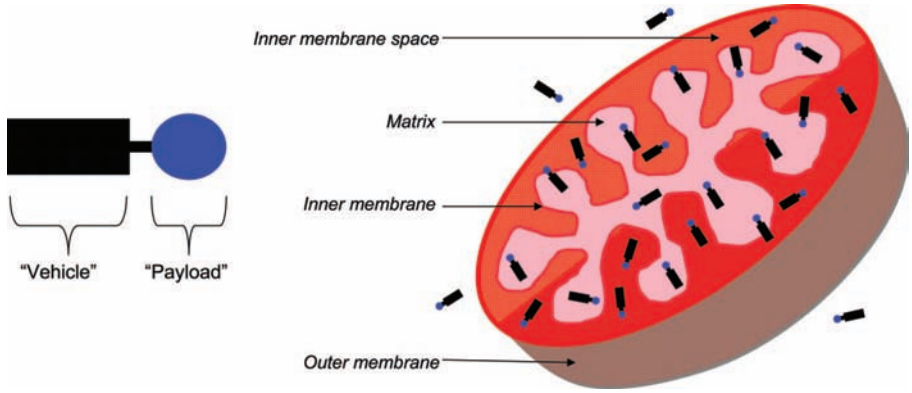

FIGURE 3. Concept of dual function agents that use a vehicle to deliver an ROS scavenging payload into mitochondria.

maceuticals that provide ROS and RNS scavenging systems are most effective if they address the problem at its source, in the inner mitochondrial membrane.[11] This Account summarizes some of the current strategies used to target antioxidants to mitochondria, including peptide and nonpeptide delivery systems.

In the past ten years, the search for new protective remedies against damage caused by excessive free radical formation in mitochondria has accelerated. Similar to our body's own natural defenses against ROS, research has been primarily focused on molecules combining antioxidant utilities with recycling capacities.[12] Large doses of antioxidants proved ineffective at preventing oxidative damage in animal disease models, presumably because the antioxidants and proteins such as manganese superoxide dismutase (MnSOD) cannot penetrate cell membranes effectively and therefore do not reach the relevant sites of ROS and RNS generation.

One solution to this general problem is to attach a molecule with antioxidant properties onto a vehicle that can penetrate both the cellular and outer mitochondrial membranes and thereby deliver the "payload" to a site where it can scavenge ROS and ameliorate oxidative damage (Figure 3). Since the mitochondrial membrane spans across a negative potential, most agents have a positively charged moiety that takes advantage of electrostatic forces in locating its target.

Proteins, Peptides, and Peptide Mimetics as Mitochondrial Targeting Systems

Proteins. For polypeptide strands to be properly recognized and imported into mitochondria, precursor proteins that are synthesized in the cytosol often require a specific amino acid sequence that is recognized by an import pathway.[13] While precursor proteins are prone to misfolding and aggregation, cytosolic chaperone proteins maintain them in an import-competent form.[14] The processed protein is then bound by translocases of the outer and inner membranes (TOM and TIM), which transport the target across the lipid bilayers.[15]

Protein import and recognition is generally directed by an N-terminal or, less frequently, a C-terminal signal sequence consisting of about 20−30 amino acid residues, which are cleaved by mitochondrial processing peptidase (MPP) either during import or once inside the mitochondrial matrix. Comparison of known presequences reveals that they do not share a common primary structure. In these cases, however, a common secondary structure as well as certain basic (arginine), hydrophobic (alanine, leucine), and polar residues (serine) might be present. Alternatively, proteins such as cytochrome *c* and superoxide dismutases are imported with minimal processing since they contain the necessary recognition elements as part of their primary sequence.[16,17] The N-terminal regions are postulated to fold into amphiphilic helices.[18] It is proposed that this amphiphilicity in combination with localized positive charges emanating from basic residues are the two main features required for successful protein import. Electrostatic interactions are thought to occur between the positive charges found on the helix and the negative charges of the TOM receptors.[19] Consequently, in an electrophoretic event, the presequence protein is pulled across the inner membrane by the very large membrane potential (typically 150−180 mV).[20]

Manganese Superoxide Dismutase (MnSOD). Superoxide dismutases (SOD) are responsible for catalyzing the dismutation of superoxide into oxygen and hydrogen peroxide and are therefore an important part of the antioxidant defense in most cells exposed to oxygen.[21] Several families of SOD are known, and the activities of each family depend on a redox-active metal cofactor such as manganese, iron, copper, or nickel. A single metal cofactor will catalyze a single electron oxidation and reduction of two separate superoxide anions to give oxygen and hydrogen peroxide, respectively.

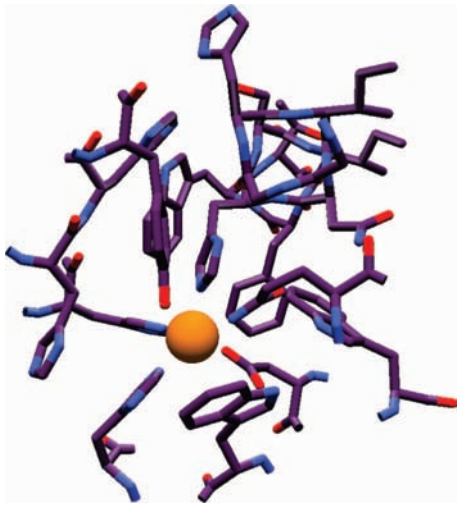

FIGURE 4. Structure of the active site of human superoxide dismutase 2. His74, His26, His163, and Asp159 provide a tight complex for manganese(III).

These reactions are self-contained and do not require an external source of redox equivalents.

A generic mechanism for the catalytic breakdown of superoxide anion is:

$$M^{(n+1)+}\text{-SOD} + O_2^{\bullet -} \rightleftharpoons M^{n+}\text{-SOD} + O_2$$
$$M^{n+}\text{-SOD} + O_2^{\bullet -} + 2H^+ \rightleftharpoons M^{(n+1)+}\text{-SOD} + H_2O_2$$
where M = Cu (n=1) ; Mn (n=2) ; Fe (n=2) ; Ni (n=2).

The family of manganese superoxide dismutases (MnSOD)[22] consists of dimers or tetramers of approximately 21 kDa subunits and contains considerable sequence homology and well conserved protein folds across various phyla.[23] Dimeric forms of MnSOD are typical of bacteria, while in eukaryotes the tetramer is most commonly observed. At the active site of the enzyme, a single manganese atom catalyzes the disproportionation of superoxide and is coordinated in a trigonal bipyramidal geometry to three histidine residues, one aspartate, and a solvent water molecule (Figure 4).[24]

In the case of eukaryotic MnSOD (SOD2), the polypeptide is initially encoded by a nuclear gene as a precursor polypeptide containing the requisite mitochondrial targeting sequence at its N-terminus. The mechanism by which SOD obtain their manganese cofactors is still unknown; however, the manganese-containing form of SOD typically resides in the matrix of the mitochondrion. Enzymatically inactive mutant forms of *Saccharomyces cerevisiae* SOD2 lacking the mitochondrial targeting sequence [denoted as SOD2P] were found to accumulate in the cytosol rather than the mitochondria.[25] Their inactivity was attributed to a manganese deficiency, and further investigations suggested that SOD2P required mitochondrial localization in order to efficiently acquire manganese. Therefore it is proposed that as the SOD2 polypeptide precursor enters mitochondria, manganese is inserted into the polypeptide, and the complex then assembles into the quaternary enzyme structure.[21]

MnSOD plays an essential role in oxidative stress protection, and the assembly of this tetrameric peptide into the active manganese-containing enzyme is critical for survival. For example, in neonatal mice, the loss of MnSOD is lethal,[26] while its modification in fruit flies leads to a severely reduced adult life span.[27] In contrast, due to its role as a negative modulator of apoptosis, inhibition of MnSOD in cancer cells is considered to be a promising target for anticancer therapies.[28] The MnSOD gene is known to be induced by tumor necrosis factor alpha (TNFα) and provides protection against TNF-induced apoptosis.[29] As such, even small amounts of this enzyme are considered crucial for tumor cell resistance to inflammatory stimuli, ionizing radiations, and commonly employed anticancer drugs.[28]

Peptides and Peptide Mimetics. Several cell-permeable mitochondrial targeting peptides with attached antioxidants have been conceived and tested, including SS peptides,[30] which feature a 2′,6′-dimethyltyrosine (Dmt) payload, as well as the XJB peptide mimetics,[31] which deliver 4-amino-TEMPO (4-AT), a stable nitroxide radical.

SS Tetrapeptides. The SS tetrapeptides represent a series of mitochondria-targeting antioxidant peptides that feature a common structural motif of alternating aromatic and basic residues (Figure 5).[30] Prior to the discovery of their antioxidant properties, these tetrapeptides were already extensively studied due to their high affinity and selectivity to the μ-opioid receptor, and they were found to be surprisingly potent and long-acting analgesics.[32]

The antioxidant properties of **SS-02** and **SS-31** are likely to originate from their dimethyltyrosine (Dmt) residues.[33] More specifically, tetrapeptides **SS-31** and **SS-02** were found to be equally effective in scavenging H_2O_2 and inhibiting linoleic acid oxidation *in vitro*. This result indicates that the specific location of the Dmt residue in the sequence of the antioxidant peptide is inconsequential. The basic residues provide for localization in the inner mitochondrial membrane, and the Dmt phenol moieties of **SS-02** and **SS-31** are likely responsible for chemically reducing reactive oxygen species and peroxide bonds. Tetrapeptide **SS-20**, where Dmt is substituted with a phenylalanine residue, was devised as a control and, in agreement with the hypothesis, demonstrated no ROS scavenging ability.

Despite the net positive charge of these small peptides at physiological pH values, their amino acid sequence allows them

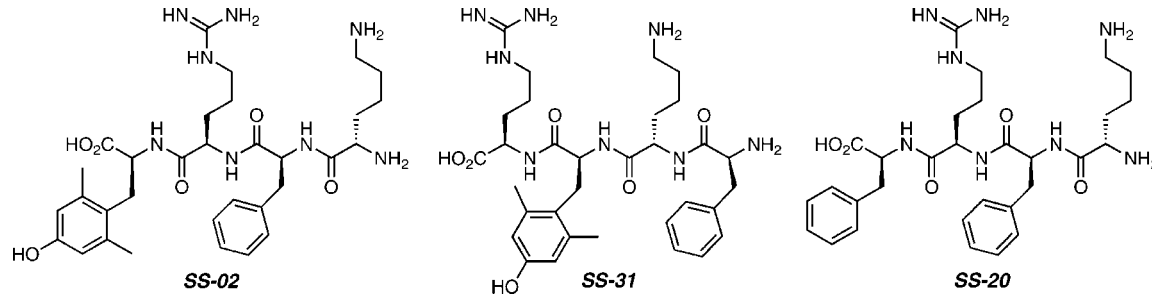

FIGURE 5. Structures of active and control SS peptides.

to freely penetrate cells in an energy-independent nonsaturable passive manner.[34] A fluorescent SS-peptide analogue (Dmt-D-Arg-PheantDap-NH_2; ant = β-anthraniloyl-L-α,β-diaminopropionic acid), **SS-19**, was prepared to study mitochondrial and cellular uptake in living cells. Confocal images showed that the pattern of localization of the fluorescent analogue was similar to that of Mitotracker TMRM, a fluorescent dye that is readily taken up by mitochondria. To ensure that uptake was not an artifact caused by the presence of the fluorophore, these aromatic cationic peptides were further studied by incubating [^{3}H]**SS-03** with mouse liver mitochondria. The uptake of [^{3}H]**SS-03** reached maximal levels within 2 min and reflected a 100-fold concentration in mitochondria.

In consideration of their positive net charge, these peptides were expected to pass through the inner membrane of the mitochondria (IMM) into the matrix; however, pretreatment with the mitochondrial uncoupler carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) only decreased **SS-19** or [^{3}H]**SS-03** uptake by about 20%. This finding suggests that the bulk of the peptide was localized in the IMM, while only 20% was delivered to the matrix in a potential-driven manner.

tert-Butyl hydroperoxide (*t*-BHP) is a membrane-permeable oxidant known to induce apoptosis in treated cells.[35] Apoptosis induced by *t*-BHP is triggered by a phenomenon called the mitochondrial permeability transition (MPT),[36] which delivers an increase in the permeability of the mitochondrial membranes to small molecules of less than 1500 Da molecular weight. In a series of *in vitro* studies, the peptides **SS-02** and **SS-31** were demonstrated to be effective antioxidants, which inhibited triggering of the MTP and partially ameliorated apoptosis of *t*-BHP-treated cells.[37]

Reperfusion injury following ischemia or hemorrhagic shock is thought to include ROS production and mitochondrial permeability transition.[38] Once blood loss occurs, if intravascular volume expansion successfully restores arterial blood pressure before hemostasis has been achieved, then paradoxically, resuscitation can actually promote bleeding and reduce the chances of survival.[39] In animal models, the overexpression of SOD was associated with protection against reperfusion injury, while SOD knockout animals were more susceptible.

In an *ex vivo* reperfusion study of guinea pig heart, both **SS-02** and **SS-31** were able to prevent myocardial stunning and significantly improve contractile force, albeit at drastically different doses (100 μM and 1 nM, respectively).[30,40] The discrepancy in activity must be attributed to the amino acid sequence alone, since both **SS-02** and **SS-31** were of similar efficiency as antioxidants in the nonenzymatic *in vitro* assay. **SS-20**, which lacks the scavenging Dmt moiety, was unable to prevent myocardial stunning when administered upon reperfusion. Furthermore *in vivo* testing of **SS-02** was found to protect against myocardial stunning in rats,[40] thus supporting the theory that ROS play a major role in reperfusion-induced myocardial stunning. Since mitochondria contain μ-type opiod receptors, further study on these SS tetrapeptides is required to determine whether it is the opiod receptor association of these peptides that mediates mitochondrial targeting and whether this event is the cause of cardiac protection at reperfusion.[41]

XJB Gramicidin S Analogs. XJB peptides and peptide mimetics are based on the sequence of the membrane-active gramicidin S (GS) antibiotics (Figure 6); their antioxidant properties stem from the attachment to the stable free radical, 4-amino-TEMPO (4-AT).[42] Among the advantages of TEMPO is the ability to use electron spin resonance (ESR) to measure distribution of the spin label and detect oxidative stress in the local cellular environment.[43]

In addition to **XJB-5-131**, initial proof-of-principle experiments also used two different hemi-GS segments (Leu-D-Phe-Pro-Val-Orn and D-Phe-Pro-Val-Orn-Leu) in conjugation with 4-AT (**XJB-5-125** and **XJB-7-75**, respectively, Figure 7). Incubation of mouse embryonic cells with either **XJB-5-125** or **XJB-7-75** attenuated ActD-induced phosphatidylserine (PS) externalization in a dose-dependent manner at a concentration nearly 1000 times lower than untargeted 4-AT. Hemi-GS-TEMPO derivatives almost completely inhibited superoxide production in ActD-treated mouse embryonic cells. The shortened peptide isostere

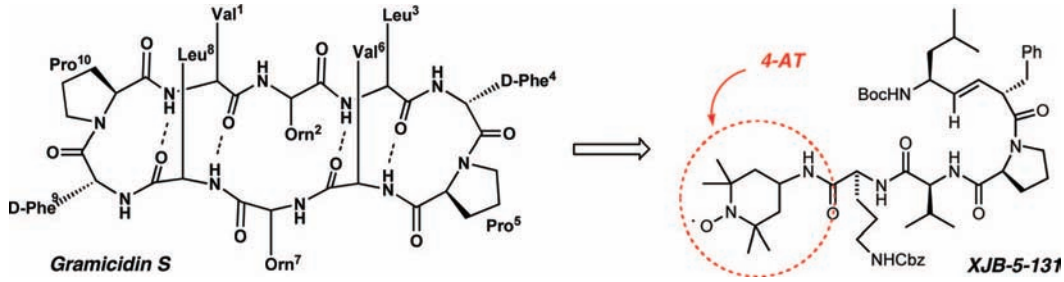

FIGURE 6. Structures of the cyclodecapeptide antibiotic, microbial lipid targeting gramicidin S, and the designed **XJB-5-131**, which delivers the ROS scavenging unit 4-AT to mitochondrial membranes.

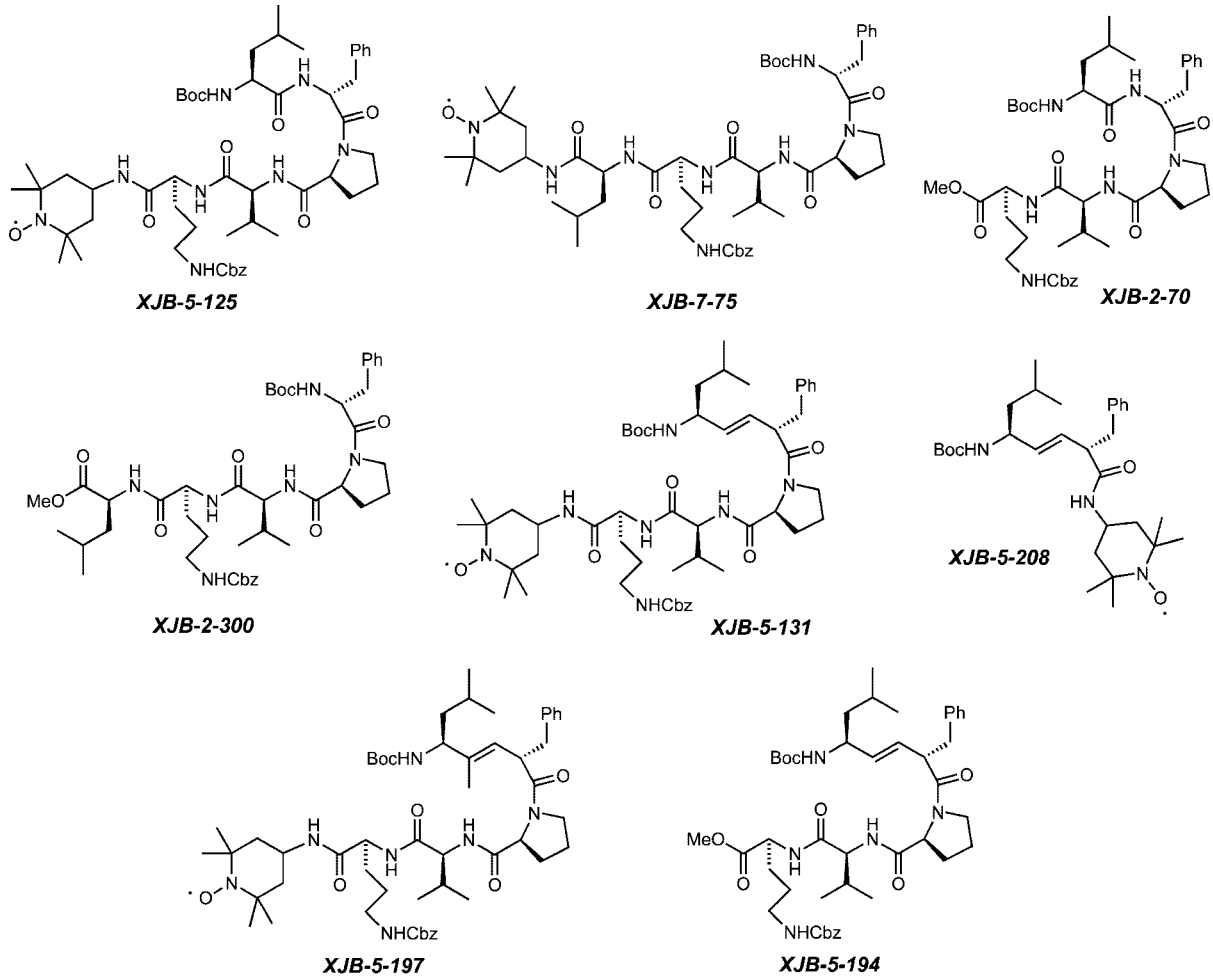

FIGURE 7. Structures of **XJB-5-131** analogs.

sequence **XJB-5-208** showed no antiapoptotic effects, and, in accordance with the functional hypothesis, control fragments with slightly altered sequences such as **XJB-5-197** and **XJB-5-194** also provided no protection.[44] Most subsequent follow up studies used the peptide isostere **XJB-5-131**,[42] since the replacement of an amide bond with an alkene group often leads to an extended bioavailability, possibly due to increased resistance against protease action.[45]

The levels of internalization of **XJB-5-125**, **XJB-5-131**, and **XJB-5-208**, as well as untethered 4-AT, after their incubation with mouse embryonic cells were tested using ESR spectroscopy and ESI-MS. **XJB-5-125**, **XJB-5-131**, and **XJB-5-208** were readily detected in mitochondria, whereas the presence of free 4-AT was not observed. The biodistribution profile of non-antiapoptotic **XJB-5-208** indicates that the simple accumulation of nitroxides in the mitochondria is not sufficient for preventing ActD-induced apoptosis. It was speculated that both the nitroxide functionality and the reverse turn (β-turn) of the targeting sequence are essential for the antiapoptotic properties of the hemi-GS-TEMPO derivatives.[44]

Since **XJB-5-131** proved to be a novel and effective mitochondrial ROS and electron scavenger,[46] a follow up study was performed to assess its ability to prolong the survival of rats with lethal hemorrhagic shock.[39] Thirteen rats were bled over 60 min with a total blood loss of 33.5 mL/kg or approximately 55% of total blood volume. Six were randomly assigned to receive **XJB-5-131**, and seven received only its vehicle, a 1:2 (vol/vol) mixture of DMSO and normal saline. All seven of the vehicle-treated (control) group died within 125 min, whereas animals treated with **XJB-5-131** survived significantly longer. Three survived longer than 3 h and one survived for the whole postbleeding observation period. This study showed for the first time that acute administration of a single dose of a mitochondria-targeting ROS scavenger, **XJB-5-131**, can have a dramatic physiological effect in a whole animal model of critical illness. Furthermore, these studies suggest that treatment with **XJB-5-131** might prolong the period of time that patients can survive after losing large quantities of blood, thereby allowing transport of otherwise mortally wounded individuals to locations where additional care can be provided. Further assays of these compounds to determine toxicity, half-life, distribution, metabolism, and physiological elimination are currently being pursued.

Non-Peptidic Mitochondrial Targeting Species

Lipophilic Cationic Compounds. Mitochondria use ion channel pumps and oxidation pathways to maintain a constant membrane potential of ca. −180 mV across their lipid bilayer. The magnitude of this potental is unprecedented in any other organelle; it is twice that of the plasma membrane of excitable cells and roughly six times higher than the plasma membrane of nonexcitable cells.[47] The unique nature of the mitochondrial membrane distinguishes it from its intracellular counterparts and offers a unique chemical opportunity for selectively targeting the mitochondrion.

The use of lipophilic cations as selective targeting agents has been explored to capitalize on this physiological phenomenon. Rhodamine 123 (**1**) and similar compounds containing cationic functionality in an otherwise nonpolar framework have the ability to traverse the mitochondrial lipid membrane by using the negative potential gradient of the organelle as an electrostatic driving force. According to the Nernst equation, this can result in a 100−500-fold increase in accumulation. Rhodamine 123 and related analogs have been used to assess the accumulation of this class of fluorescent dyes in mitochondria, and as a result of the success and reproducibility of their selective incorporation into mitochondria, practical rhodamine-based stains for mitochondrial assays have been developed and are routinely employed.

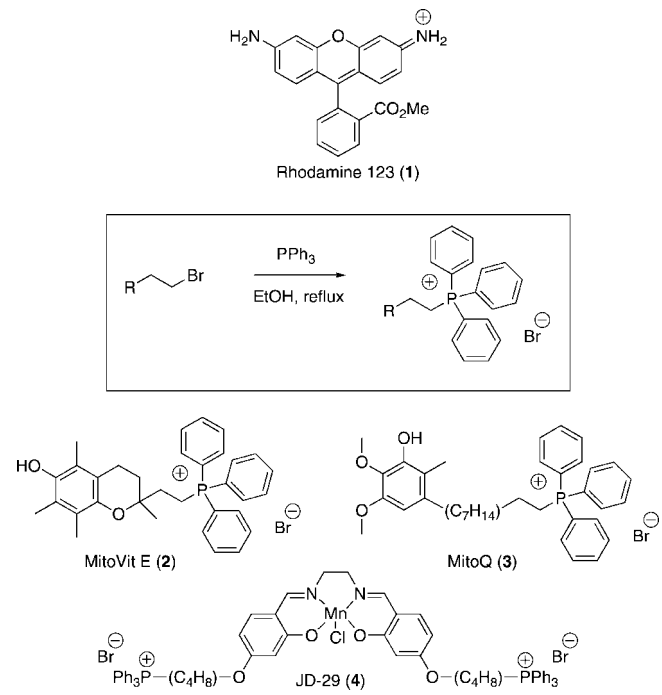

FIGURE 8. Lipophilic cationic targeting agents.

Rhodamine 123 has also been successfully used as a chaperone to direct tethered compounds into mitochondria. The anticancer drug cisplatin was selectively incorporated into the mitochondria of cancer cells using this method,[48] and similar approaches have been demonstrated with other small molecules. Arguably the most beneficial outcome of the rhodamine trials has been the discovery of the utility of the chaperone effect, which was further extended toward the development of lipophilic triphenylphosphonium (TPP) salts. The latter compound class includes the majority of the nonpeptidic mitochondrial targeting agents synthesized to date (Figure 8).[47−50]

Given the similar uptake and selectivity profile of alkyl TPP compounds to that of the more structurally complex rhodamine analogues, a number of antioxidant-tethered lipophilic TPP cations have been synthesized.[50,51] The common synthetic strategy for constructing this class of compounds proceeds via the displacement of the corresponding primary bromide using triphenylphosphine as outlined in Figure 8. Vitamin E has been shown to diminish the amount of ROS-associated mitochondrial damage by itself, but because the compound cannot effectively accumulate within mitochondria and due to health concerns related to higher dosages, only a limited effectiveness was observed without the use of the lipophilic cation transport tether. In contrast, MitoVit E (**2**), named in reference to the tethered ben-

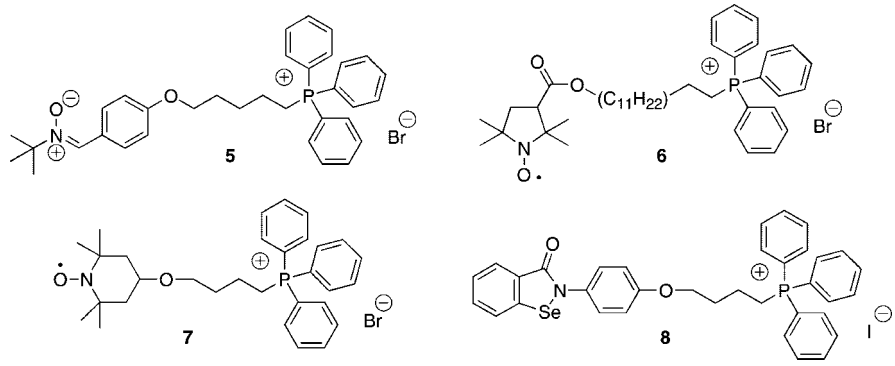

FIGURE 9. Natural ROS scavenger mimics with TPP-based lipophilic cations.

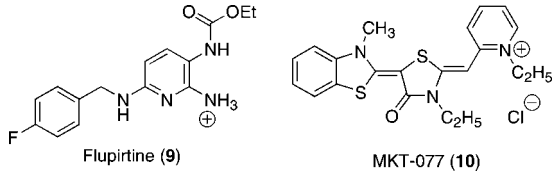

FIGURE 10. Other lipophilic cation targeting agents.

zopyran moiety of vitamin E, is localized in energized mitochondria and protects isolated rat liver mitochondria from iron/ascorbate- and *tert*-butylhydroperoxide-induced oxidative damage.[52] MitoQ (**3**) is a related redox-active compound using ubiquinol as the active moiety, which selectively protects mitochondria against cardiac ischemia-reperfusion oxidative injury.[53] Both **3** and the salen-bound manganese compound JD-29 (**4**) were shown to exhibit MnSOD-like behavior and prevent mitochondrial oxidative damage.[54] MitoPBN, MitoCP, and Tempol-TPP (**5**−**7**) contain N-oxide moieties that scavenge ROS by mimicking SOD (Figure 9).[47,50,55] MitoPeroxidase (**8**) contains the glutathione peroxidase mimic ebselen to achieve its protective effects against Fe^{2+}/H_2O_2-induced lipid peroxidation; it is part of a larger class of biologically significant organoselenium antioxidants.[50,56]

Other lipophilic cations also show selectivity for mitochondria and are attractive candidates for potential therapeutic development. Flupirtine (**9**) is a nonopioid analgesic localized within mitochondria and protects against cell injury induced by *N*-methyl-D-aspartate and against ischemic injury and prevents glutamate-induced increase of intracellular Ca^{2+} levels leading to apoptosis (Figure 10).[57,58] MKT-077 (**10**) accumulates in mitochondria due to its cationic amino function and relatively nonpolar scaffold. However, instead of acting in a protective fashion, **10** displays selective toxicity to carcinoma mitochondria due to the increased mitochondrial membrane potential in cancer cells compared to normal cells.[59,60]

While lipophilic cations elicit protective effects toward mitochondrial injury, their dependence on the membrane potential in mitochondria also constitutes a major drawback. As

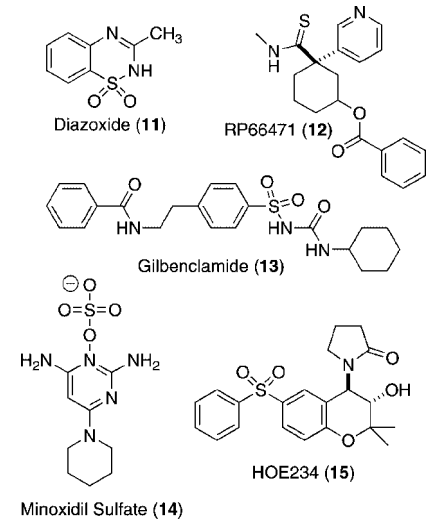

FIGURE 11. Sulfonyl-urea and related mitochondrial targeting agents.

increasing numbers of lipophilic cations enter the organelle, the potential gradient diminishes to the point where a rapid efflux of the inhibitor from the mitochondrion results in loss of activity until entry is regained. Therefore, unless inhibition is irreversible, uninterrupted activity of the lipophilic cation cannot occur. This deficiency poses a potential problem depending on the rates of influx and efflux of cationic species.[48]

Sulfonylurea and Related Compounds. Compounds **11**−**15** were observed to both activate and inhibit mitochondrial potassium ATP-regulated ion channels (mitoK_{ATP}) (Figure 11). They bind with high affinity to sulfonylurea receptors (SURs) in the plasma membrane across a number of cell types. A potassium channel structurally related to the SUR was detected in the inner membrane of mitochondria and identified as the target of these compounds, although the exact mechanism of action and specificity have not been elucidated.[58,61] Potassium channel openers and inhibitors exhibit diverse activity ranging from vasodilation to hair growth, and recent evidence indicates that K_{ATP} channel open-

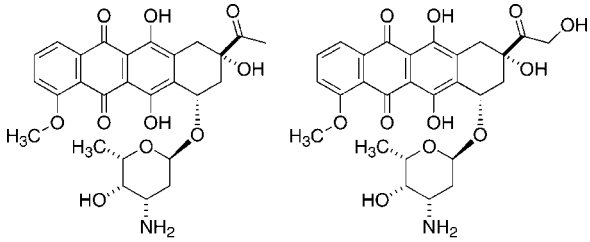

FIGURE 12. The mitochondria-targeting anthracyclines adriamycin and daunomycin.

ers could prove to be effective as pharmacological lead structures for the cardioprotection of tissues against ischemic necrosis and ultimately coronary artery disease, the main cause for fatalities in the industrialized world.[62]

Anthracyclines. Adriamycin (**16**) and daunomycin (**17**) belong to a class of anthracyclines that exhibit potent antitumor activity, presumably through direct binding to DNA in an intercalative fashion, that is, through insertion between stacked bases and charge interaction on the outer regions of the DNA helix (Figure 12). However, data has emerged that suggests these compounds interact, perhaps exclusively, with mitochondria by the disruption of major mitochondrial functions.[63] The cytotoxic side effects of anthracyclines have been attributed to the accumulation of these compounds in the mitochondrial lipid membrane and subsequent redox activity of the quinone moiety, resulting in damage of membrane-bound proteins and enzymes. The mitochondrial specificity of these compounds is noteworthy and has been correlated to their high affinity toward binding an inner mitochondrial membrane-specific phospholipid, cardiolipin. The glycosidic amino function in **16** and **17** was critical for the observed activity and differentiates them from related anthracycline analogues.[63]

Other Structural Classes. Several other structural classes have been shown to target mitochondria selectively, but more detailed investigations are required before their utility can be established. For example, resveratrol (**18**) and its analogues **19** and **20** selectively initiate the apoptotic mitochondrial pathway in cancer cell lines while leaving normal cells untouched; however, their precise mechanism of action is not fully understood (Figure 13).[64]

The mitochondrial targeting ability of porphyrin-based antitumor compounds such as verteporfin (**21**, Visudyne, Novartis AG) was postulated to derive from the enrichment of these compounds within mitochondria. Apparently, this accumulation is due to an interaction with mitochondrial-specific benzodiazepine receptors. The porphyrin moiety serves as a photosensitizer and induces the apoptotic pathways in tumor

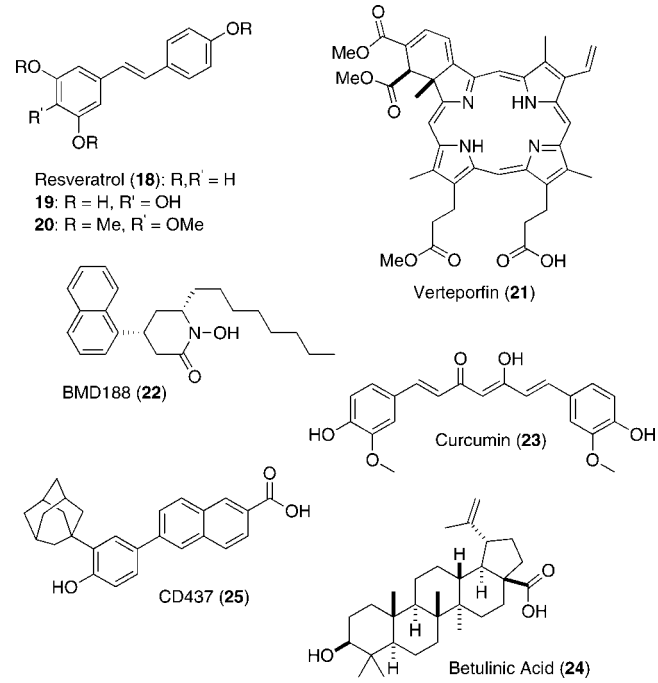

FIGURE 13. Diverse mitochondria-targeting small molecules.

cell lines.[65] BMD188 (**22**) is a cyclic hydroxamic acid derivative that has shown potent activity against prostate cancer, an effect that is hypothesized to be the result of an interaction with mitochondria. A number of other structurally diverse compounds, including curcumin (**23**), the steroid betulinic acid (**24**), and the retinoid CD437 (**25**) have also been shown to induce mitochondrial apoptosis through an opening of the mitochondrial transition permeability pore.[65]

Conclusions

Due to the high concentration of mitochondria in heart tissue, it is not surprising that a major goal in cardioprotection is a decrease in the burst of mitochondrial ROS formation that characterizes postischemic reperfusion. Other important areas for therapeutic intervention based on controlling the mitochondrial pathway for apoptosis include neurodegeneration, diabetes, cancer, and antiviral diseases.[66]

Accordingly, mitochondrial targeting of ROS scavengers or compounds that interfere with the unique biochemistry in mitochondria is emerging as a novel and highly relevant approach in drug discovery for the treatment of degenerative diseases and acute conditions derived from surging ROS and RNS. No pharmaceutical agents specifically designed to deliver a therapeutic compound to mitochondria have yet reached the market, but it is likely that a significant number of essential nutrients, including ascorbic acid, selenium, vitamins E and Q10, carotenoids, etc., fulfill at least part of their function by controlling the surge of reactive byproducts of the

oxidative phosphorylation process and ATP generation in mitochondria. Since age-related conditions are rapidly becoming a major source of a declining quality of life in a graying population, we can only hope that the near future will show the emergence of a new class of effective therapies that involve mitochondrial survival strategies. This is an exciting development for synthetic chemists who are being challenged with the discovery of innovative approaches to deliver functional small organic compounds as well as larger biomolecules across cell membranes to specific intracellular targets.[67]

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FOOTNOTES

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