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# Mitochondrial drugs

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Mitochondria are cellular organelles that perform pivotal functions essential for ATP production, homeostasis, and metabolism. Moreover, mitochondria are integral to a variety of cell death and survival pathways. These roles identify mitochondria as a potential target for drugs to treat metabolic and hyperproliferative diseases. Differences in the redox state of pathogenic versus non-pathogenic cells may be exploited to achieve selective anti-proliferative and cytotoxic activity against target cell populations. Pro-oxidant drugs, such as Trisenox™ and Elesclomol™, are demonstrating clinical utility in the treatment of cancer. Results obtained with Bz-423 in mice demonstrate the potential for mitochondria-targeted drugs to control disorders of immune function. Research associating an elevated oxidant state with mitochondrial damage, degenerative disease, and aging dictates the need for a better understanding of when and how pharmacological manipulation of mitochondrial function provides most therapeutic benefit.

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## Introduction

Mitochondria perform pivotal biochemical functions necessary for homeostasis and are arbiters of cell death and survival, in addition to being a source of ATP [1]. They represent a convergence point for death signals triggered by both extracellular and intracellular cues [2,3]. Not surprisingly, therefore, mitochondria also offer targets for xenobiotics to exert either therapeutic or detrimental effects on cell function and survival [4,5]. Efforts to harness mitochondrial targets for therapeutic benefit have focused largely on cancer, although treatments for neurodegenerative diseases, metabolic diseases, and ischemia also are being explored [6,7–11]. These approaches follow two general strategies that may be broadly categorized as either pro-oxidant or antioxidant.

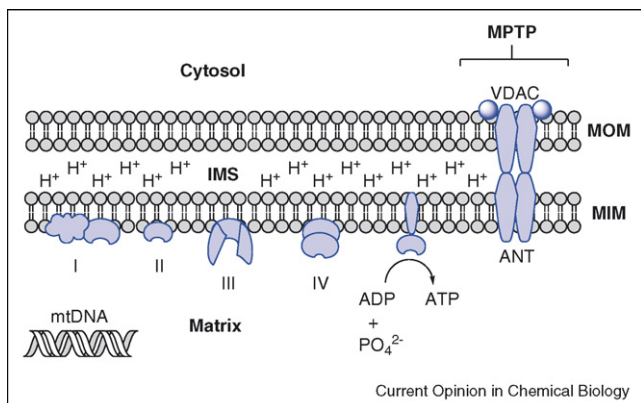
Hyperproliferating cells, such as neoplastic cells that survive under conditions of elevated oxidant stress, are susceptible to pro-oxidant induced cell death as the further increase in oxidant levels overwhelms the anti-oxidant capacity of the cell. Conversely, under conditions where persistently high oxidant levels are proposed to lead to macromolecular damage and degenerative disease, antioxidants are expected to exert a mitigating effect. This review will describe current thinking and recent advances in the discovery of small molecule drugs acting on targets in the mitochondrion, with an emphasis on the pro-oxidant approach.

## Targets in the electron transport chain

Mitochondria are discrete organelles found in eukaryotic cells that bear ancestral and architectural similarities to prokaryotes. The mitochondrion comprises a matrix surrounded by two membranes, the mitochondrial inner membrane (MIM) and the mitochondrial outer membrane (MOM; Figure 1). The MIM includes multiple invaginations called cristae and is highly impermeable to small molecules and ions, which require specific transport proteins to enter or exit the mitochondrial matrix. Under aerobic conditions, the proteins of the electron transport chain (ETC), located in the MIM, reduce oxygen to water through a series of steps along the electron transport chain that employ NADH and FADH<sub>2</sub> derived from the tricarboxylic acid cycle and glycolysis. These reductions effectively pass protons (H<sup>+</sup>) across the MIM such that they accumulate in the inter-membrane space (IMS) creating a pH gradient across the MIM that contributes to an overall electrochemical gradient ( $\Delta\Psi$ ). This gradient is used by the mitochondrial F<sub>1</sub>F<sub>0</sub>-ATPase as a source of energy to drive the synthesis of ATP from ADP and phosphate. This sequence of chemical steps is collectively known as oxidative phosphorylation (OXPHOS).

During normal OXPHOS, small amounts of reactive oxygen species (ROS) are generated as a result of incomplete oxygen reduction. ROS include superoxide, the result of partial oxygen reduction, and the subsequently formed hydrogen peroxide, and hydroxyl radicals, each of which displays different chemistry. A high NADH:NAD<sup>+</sup> ratio (as may arise owing to high rates of glycolysis) can increase ROS production, as does state 4 respiration in which electron transport occurs in the absence of ATP synthesis, for example, when ADP levels are low [12]. Inhibitors of the electron transport chain and of the F<sub>1</sub>F<sub>0</sub>-ATPase can also increase mitochondrial ROS production. ROS contribute to oxidative damage of cellular macromolecules, but in addition act as secondary messengers with important signaling roles [13]. It is also noteworthy

Figure 1



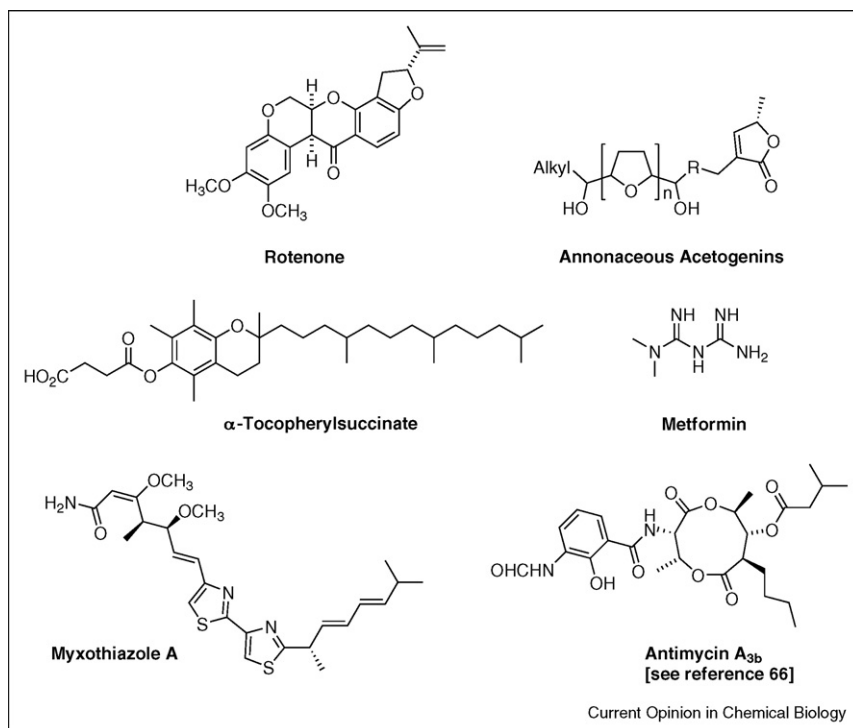
A schematic view of the mitochondrial membranes and key respiratory proteins. MOM, mitochondrial outer membrane; MIM, mitochondrial inner membrane; IMS, inter-membrane space; MPTP, mitochondrial permeability transition pore.

that the production of ROS has been identified as a common mechanism for the bactericidal effect of many widely used antibiotics including drugs targeting protein synthesis, DNA, and the cell wall [14]. Thus, ROS perform both a necessary role and a destructive role in cells.

Inhibitors of the electron transport chain are useful tools for furthering our understanding of this essential bioenergetic process (Figure 2) [10]. Inhibitors of complex I (NADH ubiquinone oxidoreductase) include rotenone (used as a rodenticide), and the phytochemical *Annonaceous* acetogenins that have been attributed with antimicrobial and anticancer properties. The widely used diabetes drug metformin inhibits complex I and has been shown to induce a p53- and AMP-activated protein kinase-dependent increase in glycolysis to compensate for modulation of the respiratory chain, which effectively increases glucose consumption [15]. Complex II (succinate-ubiquinone oxidoreductase) is one proposed target of redox-silent vitamin E analogs such as  $\alpha$ -tocopheryl succinate [16–19]. Complex III (cytochrome *c* oxidoreductase) is inhibited by antimycin A (the active constituent of the piscicide Fintrol) and by the natural product myxothiazole. Complex IV (cytochrome *c* oxidase) is a target of cyanide. Complex I and complex III are the major sources of mitochondria-derived ROS *in vitro*, although the synthesis of superoxide by complex III is considered to be more physiologically relevant [12]. The electron transport chain supplies the  $H^+$  gradient that is necessary for the mitochondrial  $F_1F_0$ -ATPase to function.

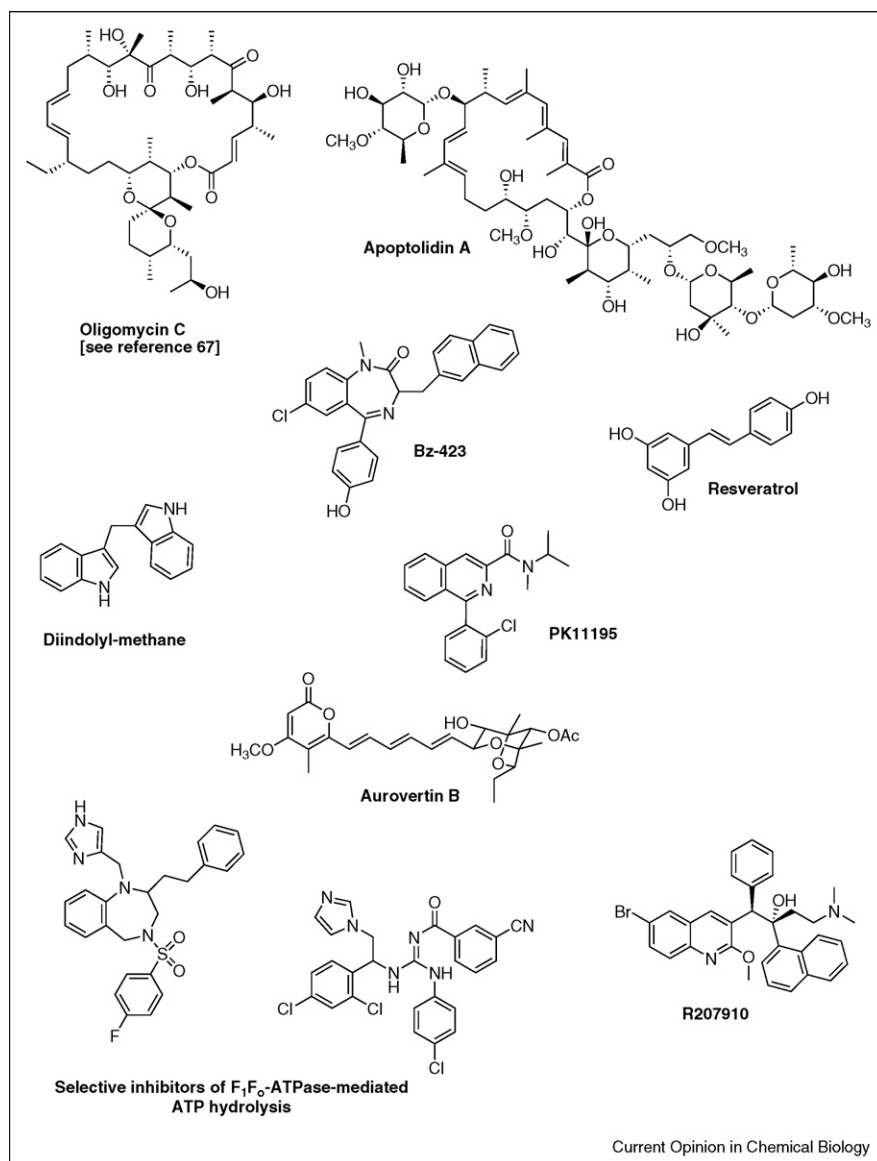
Oligomycin, a natural product that blocks the proton channel, and the related macrolide apoptolidin, are both

Figure 2



Inhibitors of complexes I, II, and III in the mitochondrial respiratory chain. Further information about these and other mitochondrial inhibitors may be found in review references [5,6,7–10], and [15,16–19,66].

Figure 3



Inhibitors of the mitochondrial  $F_1F_0$ -ATPase and bacterial ATP synthase. These compounds are described in references [20–42,67].

inhibitors of the  $F_1F_0$ -ATPase (Figure 3) [20–22]. Apoptolidins display remarkably selective cytotoxicity toward a subset of tumor cell lines *in vitro*, suggesting that inhibition of the ATPase is not indiscriminately cytotoxic. Other compounds reported to bind to the  $F_1F_0$ -ATPase include Bz-423 [23], resveratrol [24], diindolyl methane (DIM) [25], aurovertin [26], and PK11195 [27]. The benzodiazepine derivative Bz-423 was identified as a lead for the treatment of autoimmune diseases [28–31]. It reduces disease in murine models of lupus, arthritis, and psoriasis and has anti-proliferative and cytotoxic effects on tumor cells *in vitro* [32••]. Bz-423 is an uncompetitive inhibitor of the  $F_1F_0$ -ATPase, slowing the ATPase without causing a significant drop in cellular

ATP levels ([33••], TB Sundberg *et al.*, unpublished results). The therapeutic effects of this compound are mediated by the induction of superoxide. Resveratrol, a constituent of grape skins, increases longevity in rodents [34] and has been attributed with beneficial effects against cancer, heart disease, and inflammation [35]. Despite the existence of a crystal structure of resveratrol bound to the  $F_1F_0$ -ATPase [24], this protein is one of several reported targets for resveratrol and related compounds [34–36], including the protein deacetylase, sirtuin [37,38].

The  $F_1F_0$ -ATPase and the electron transport chain proteins can be decoupled by uncoupling proteins that

promote the leakage of protons back across the MIM. This is a natural process that results in thermogenesis. The resulting drop in membrane potential reduces ROS production and represents a natural protective mechanism against inhibition of respiration [12].  $F_1F_0$ -ATPase inhibitors that specifically block ATP hydrolysis without affecting ATP synthesis have been described: such compounds should be effective under ischemic conditions when the ATPase can operate in the reverse of its normal direction leading to a catastrophic drop in ATP levels that causes cell death [39–41]. This premise has not been tested clinically. The mycobacterial ATP synthase inhibitor, R207910, is currently in Phase III trials for the treatment of tuberculosis [42]. Bacterial and mammalian ATP synthases exhibit substantial differences in structure and intracellular location presenting the opportunity for species selective ATP synthase modulation.

### Targets in the mitochondrial permeability transition pore

In contrast to the MIM, the mitochondrial outer membrane is more permeable to small molecules so that the inter-membrane space (IMS) resembles cytosol in its small molecule composition. In addition, however, the IMS sequesters proteins such as cytochrome *c*, smac/Diablo (second mitochondria derived activator of caspases/direct inhibitor of apoptosis binding protein with low isoelectric point), and apoptosis inducing factor (AIF) that when released into the cytosol activate caspases and induce apoptosis. One process for the release of these death inducing protein factors involves swelling of the mitochondrion so that the outer membrane ruptures producing mitochondrial outer membrane permeability (MOMP). These events are mediated by the mitochondrial permeability transition pore (MPTP), a megachannel that comprises several proteins including the adenine nucleotide transporter (ANT) located in the MIM, a voltage-dependent anion channel (VDAC) located in the MOM, as well as the peripheral benzodiazepine receptor (PBR: also known as the translocator protein, TSPO), hexokinase, cyclophilin D, and possibly also Bcl-2 and Bax [43]. Inhibitors of the MPTP have been reviewed elsewhere as have inhibitors of bcl-family proteins [2,6,8]. High affinity ligands of the PBR have been associated with anticancer and immunotherapeutic properties [44]. The relationship of these effects to physiological functions of the PBR requires further study [45]. Recently, VDAC ligands identified in cell-based screens were shown to be selectively cytotoxic toward cells bearing oncogenic Ras protein [46].

### Redox

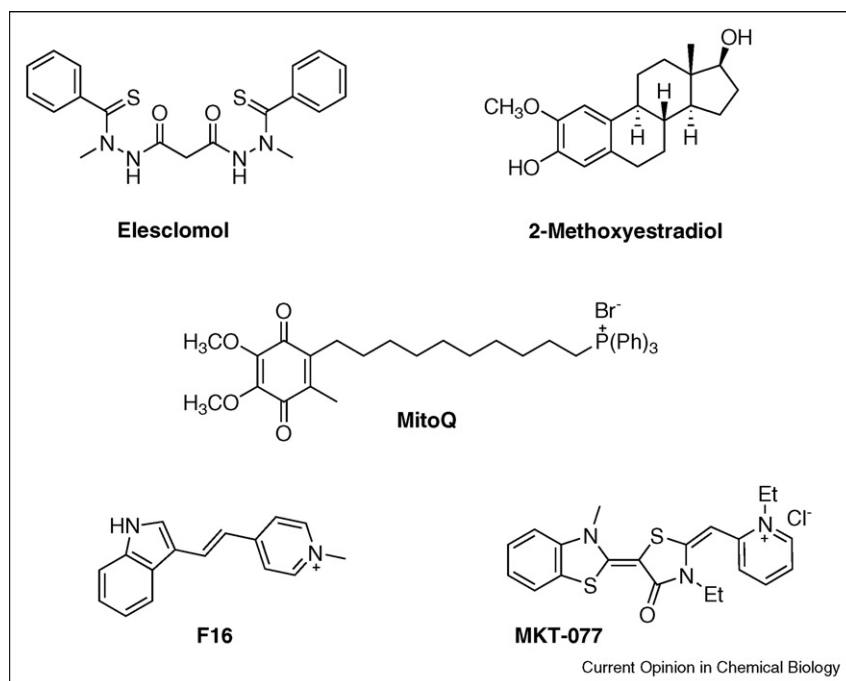
Cellular redox potential can be altered by proliferative state as well as by the function of OXPHOS proteins [12]. Elevations in intracellular oxidant potential can have

discrete chemical consequences: for example, a pair of cysteine thiols in the ANT becomes oxidized to a disulfide linkage that results in a conformational change and opening of the MPTP. Thus, manipulating cellular redox represents an approach to altering mitochondrial function. Arsenic trioxide (Trisenox™) is currently marketed for the treatment of acute promyelocytic leukemia. Its mechanism of action is undoubtedly multifactorial but is understood to involve the formation of disulfide linkages in mitochondrial proteins, including members of the MPTP (possibly mediated by thioredoxin) leading to their inhibition and the production of ROS [47]. Elesclomol™ (STA-4783, Figure 4), an injectable drug currently undergoing Phase III clinical evaluation for the treatment of metastatic melanoma, selectively kills cancer cells through apoptosis as a result of an increase in their already raised oxidant level [48]. Complementary to the use of pro-oxidant molecules is the application of inhibitors of proteins designed to maintain cellular redox by reducing ROS, for example, superoxide dismutase (SOD), catalase, and various peroxidases. 2-Methoxyestradiol magnifies the effects of cytotoxic agents and displays anti-leukemic activity as a single agent in culture stemming from accumulation of ROS, which is proposed to be due to its inhibition of SOD [49]. By contrast, persistent mitochondrial production of ROS leading to persistently increased oxidant stress and mitochondrial damage has been linked to degenerative diseases and aging [50]. This free radical theory of aging suggests that a reduction in ROS should have therapeutic benefit. Indeed insulin sensitivity and glucose homeostasis was improved in obese insulin-resistant mice treated with the antioxidants *N*-acetylcysteine or manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP) [51]. MitoQ, an analog of coenzyme Q linked to a delocalized cation for targeting the mitochondrion, is currently in trials for the treatment of Parkinson's Disease. This apparent contradiction between the beneficial and harmful effects of ROS underscores the importance of a detailed understanding of where and under what conditions ROS production is occurring, as well as the chemical identity and duration of the ROS produced.

### Mitochondrial selectivity and toxicity

Given the importance of mitochondria to cellular bioenergetics and homeostasis, it should not be surprising that shutting down mitochondrial respiration can be detrimental [52]. In addition, since mitochondria possess their own DNA, compounds that target DNA can also perturb mitochondrial integrity, including intercalators (e.g. ethidium bromide), reverse transcriptase inhibitors (e.g. AZT) and topoisomerase inhibitors (e.g. ciprofloxacin and etoposide). Non-nucleoside reverse transcriptase inhibitors, used in the treatment of HIV-AIDS, affect mitochondrial function by inhibiting DNA pol  $\gamma$ , which is required for the replication of mtDNA [53]. The lactic acidosis and idiosyncratic hepatic failure associated with

Figure 4



Compounds that modulate cellular redox levels or the mitochondrial membrane potential. See references [10,48\*,49].

these drugs has been attributed to their effects on mtDNA. Delocalized lipophilic cations such as F16 and MKT-077 (Figure 4) can dissipate the mitochondrial membrane gradient by facilitating anion flux across the MIM, inhibiting ATP synthesis. A purported association between mitochondrial toxicity and either withdrawal from the market or a black-box label, has prompted calls to screen for mitochondrial toxicity early in the drug discovery process in order to reduce late-stage attrition [54]. Notwithstanding these concerns, the electron transport chain has evolved to allow for some suppression in the activity of individual complexes without a net reduction in the overall rate of respiration (the threshold effect) [55]. Moreover, neoplastic cells and immune receptor-activated lymphocytes undergo a metabolic switch to increased glycolysis and may be less sensitive to the inhibition of OXPHOS [56\*,57\*,58,59]. At the same time, cells in which electron transport and ATP synthesis are weakly coupled or that otherwise generate higher than normal oxidant levels, are more sensitive to a pharmacological increase in ROS and more prone to apoptosis [60–62]. Thus, the production of ROS can have profoundly different consequences depending on context.

## Conclusions

Clearly a greater understanding of mitochondrial biology is needed to facilitate the judicious selection and development of mitochondrial drugs. However, targeting

mitochondria for the treatment of a wide variety of ailments associated with inappropriately regulated cell proliferation appears to offer considerable promise. System-wide approaches have the potential to identify both promising new classes of mitochondrial drug and potential sources of clinical toxicity for existing medicines [63\*\*], although, the field is replete with compounds that appear to possess multiple targets at the concentrations at which they are studied. Currently, antioxidants are of interest for their anti-aging and potential anti-diabetic properties, while pro-oxidant and cytotoxic agents are being investigated primarily as treatments for cancer. Given the irreversible nature of cell death, it may transpire that pro-oxidants and inhibitors of the electron transport chain are most beneficial and safe when used periodically, or for short duration, whereas antioxidants may need to be present continually. These parameters will only be established through clinical experience. The future will probably see broader application of pro-oxidants to other therapeutic areas where pathogenesis depends on deregulated proliferation and survival, including autoimmune disorders. Additional specificity may be achieved through targeted approaches, including packaging, exploiting affinity for mitochondrial proteins such as the PBR, which is overexpressed in several tumor types, or the selective mitochondrial accumulation of delocalized lipophilic cations [64,65]. Growing interest in mitochondrial targets suggests that significant advances along these lines will be forthcoming.

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