Targeting mitochondria with novel, differently vectorized linear phosphorylated nitrones:

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ABSTRACT

The free radicals (FR) occurrence in biological signalling or as pathologies triggers is well-documented and the design of specific scavengers to limit oxidative stress is enthralling. Because FR become inactivated upon addition to nitrones, efforts have focused on developing alpha-phenyl-*N*-tert-butylnitrone derivatives (PBNs) with promising pre-clinical data. To improve efficacy, a new generation of nitrones derived from 2-(diethoxyphosphoryl)-*N*-(benzylidene)propan-2-amine-*N*-oxide (PPNs) incorporating an antioxidant framework, gave rise to multipotent drugs exhibiting EPR-spin-trapping properties. As mitochondrial dysfunction is involved in many disorders, efforts have been concentrated on developing tools (pH probes) or pharmacological agents targeting-mitochondria. A series of mitochondria-targeted PPNs was synthesized based on the improved biocompatibility of P-containing nitrones and the expected benefits of introducing a cationic vector. Spin-adducts EPR-parameters toward biologically-relevant FR were established. Cytotoxicity was determined on cells and mitochondria-permeation assessed in isolated rat organs. New mitochondria-targeting nitrones appeared as lower cytotoxic alternative to the commonly used mito-PBN and were safely applied for investigating the anticancer-mechanism of new drugs impacting mitochondria.

Keywords

Mitochondria-targeted nitrones, ^{31}P NMR, EPR spin trapping, mitochondrial permeability, H_2O_2-induced apoptosis

Introduction

Mitochondria are complex, metabolically active and dynamic organelles that regulate critical cellular processes from ATP-production to intrinsic cell death. They are major oxygen-consumers with roughly 95% of the total amount of breathed O_2 undergoing, in desirable conditions, reduction to water by cytochrome *c* oxidase (complex IV) in four consecutive one-electron steps. Activation of O_2 by monovalent reduction leads to superoxide anion radical ($O_2^{\bullet-}$), a reactive oxygen species (ROS) participating in many key cellular pathways [1]. Since the 1970s, popular estimates of the steady state amount of mitochondrial $O_2^{\bullet-}$ ranged 1−2% of inhaled oxygen (i.e., 215−430 mmol of $O_2^{\bullet-}$ daily for an 80 kg human) [1a], yet recent knowledge proposed it could actually be one order of magnitude less under non pathological conditions [1b]. Nethertheless, still the mitochondrial respiratory chain is regarded, together with cytosolic NADPH oxidases (Nox), as a major cellular source for $O_2^{\bullet-}$. In mitochondria electron transport chain, $O_2^{\bullet-}$ formation involves complexes I (NADH:ubiquinone oxidoreductase) and III (cytochrome bc_1 complex), releasing this free radical within the mitochondrial matrix and, mostly, into the intermembrane space, respectively [1c].

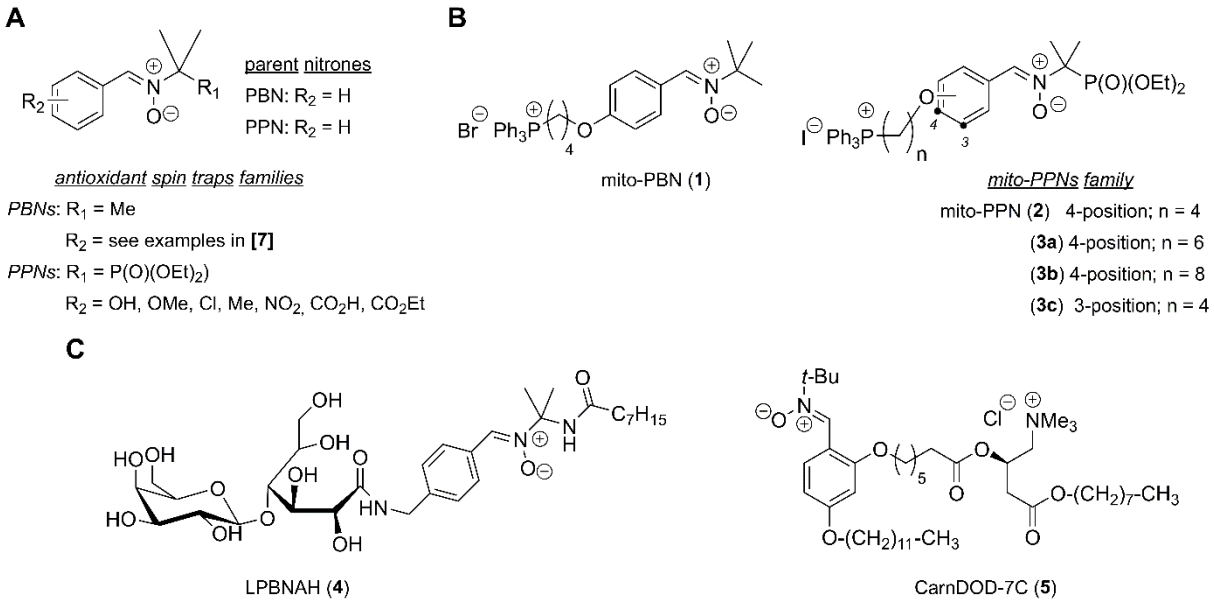

Figure 1. General structures of noncyclic nitrones and related families exhibiting antioxidant and/or pharmacological activity (A) deriving from PBN and PPN [7b,8], or targeted to the mitochondrion (B) by a TPP cation [6a,8] or (C) using alternative vectors [15].

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