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Inhibition of 6-hydroxydopamine-induced oxidative damage by 4,5-dihydro-3*H*-2-benzazepine *N*-oxides

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ABSTRACT

A number of new analogs of 3,3-dimethyl-4,5-dihydro-3*H*-2-benzazepine 2-oxide, structurally related to the nitrone spin trap α-phenyl-*N*-*tert*-butylnitrone (PBN), were synthesized and evaluated for their activity in vitro as protectants against oxidative stress induced in rat brain mitochondria by 6-hydroxydopamine (6-OHDA), a neurotoxin producing experimental model of Parkinson's disease (PD). As assessed by a fluorimetric assay, all 2-benzazepine-based nitrones were shown to decrease hydroxyl radicals (•OH) generated during 6-OHDA autoxidation. The inhibition effects on the •OH formation shown by the 5-*gem*-dimethyl derivatives, 2–4 times higher than those of the corresponding 5-methyl derivatives, were attributed to the flattening effect of the 5-*gem*-dimethyl group on the azepine ring, which should enhance nitrone reactivity and/or increase stability of the radical adducts. In contrast, owing to steric hindrance, a methyl group to C-1 diminishes the •OH-scavenging activity of the nitrone group. All the assayed compounds were more potent than PBN as inhibitors of 6-OHDA-induced lipid peroxidation (LPO) and protein carbonylation (PCO), taken as an indicator of mitochondrial protein oxidative damage. The most promising antioxidant (compound **11**), bearing 5-*gem*-dimethyl and spiro C-3 cyclohexyl groups, highlighted in this study as the best features, inhibited LPO and PCO with IC_{50} values of 20 and 48 μM, respectively, showing a potency improvement over PBN of two order magnitude. Both LPO and PCO inhibition potency data were found primarily related to the •OH-scavenging activities, whereas lipophilicity plays a role in improving the LPO (but not PCO) inhibition, as a statistically valuable two-parameter equation proved.

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Abbreviations: PD, Parkinson's disease; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; 6-OHDA, 6-hydroxydopamine; MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PBN, α-phenyl-*N*-*tert*-butylnitrone; ROS, reactive oxygen species; •OH, hydroxyl radical; LPO, lipid peroxidation; RCS, reactive carbonyl species; PCO, protein carbonylation; THA, terephthalic acid; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; MDA, malondialdehyde; MAO, monoamine oxidase; BSA, bovine serum albumin; SDS, sodium dodecylsulfate.

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1. Introduction

Free radical-induced oxidation of cell macromolecules (lipids, proteins, DNA, etc.) is implicated in several human pathogenic processes, including cardiovascular diseases (e.g., stroke, atherosclerosis) [1] and inflammatory (e.g., rheumatoid arthritis) [2]. Over the last two decades, significant accumulating evidence has also shown that generation of reactive oxygen species (ROS) and oxidative damage in the central nervous system (CNS) are major events occurring in Parkinson's disease (PD) [3] and other neurodegenerative disorders, such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS), as well as in aging processes [4]. Despite a large body of biochemical data gathered from human brain autopsy studies, the cause of neurodegeneration in PD has not yet been completely established. Nevertheless, animal models, based on the toxic damage induced by the neurotoxins 6-hydroxydopamine (6-OHDA) [5], *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [6] and related compounds [7,8], have shown that iron-dependent oxidative stress, increased levels of iron and monoamine oxidase (MAO)-B activity and depletion of antioxidants (e.g., glutathione, GSH) play major roles in PD. Indeed, antioxidants, monoamine oxidase (MAO)-B inhibitors and iron chelators, have shown neuroprotective effects in animal models either in vitro and in vivo [9–13], but unfortunately they failed in the PD human therapy [3] when administered as single drugs.

6-OHDA, used in this and previous studies [14] for inducing oxidative stress associated with the PD-related dopaminergic neuron loss, is readily autoxidized and oxidatively deaminated by MAO, yielding hydrogen peroxide (H_2O_2) and the corresponding *p*-quinone. H_2O_2 may generate the most reactive ROS, that is hydroxyl radicals ($^{\bullet}OH$) [15], and, ultimately, lipid-derived carbon- and oxygen-centered radicals, as lipid peroxidation (LPO) products. In turn, 6-OHDA quinone triggers a cascade of oxidative reactions finally resulting in the formation of an insoluble polymeric pigment related to neuromelanin [16,17]. It has also been reported that 6-OHDA inhibits complexes I and IV of the mitochondrial respiratory chain [18,19]. The oxidative stress caused by $^{\bullet}OH$ generated during 6-OHDA autoxidation is suggested as a major causal factor of its neurotoxicity [20].

Proteins are major targets for ROS and secondary by-products of oxidative stress, and ROS-induced protein modifications can lead to unfolding or alteration of protein structure. Protein carbonylation (PCO) is an irreversible oxidative damage, that often leads to formation of high-molecular-weight aggregates, which are resistant to degradation and accumulated as damaged or unfolded proteins [21]. PCO can take place through different oxidative pathways. ROS can react (i) directly with the protein, or (ii) with molecules, such as sugars and lipids, generating in turn reactive carbonyl species (RCS), which then react with protein. RCS, generated by peroxidation of polyunsaturated fatty acids, induce PCO, playing a major role in the etiology and/or progression of several human illnesses, including neurodegenerative disorders associated with the deposition of protein aggregates in tissues [22,23]. Some proteins are more susceptible than others to oxidative stress. For example, human brain copper–zinc superoxide dismutase (SOD1) has been proven to be a major target of oxidative damage in PD and AD [24].

In the last decade, nitrone spin traps, α-phenyl-*N*-*tert*-butylnitrone (PBN) and structurally related compounds (Fig. 1), which react covalently with short-lived free radicals, such as $^{\bullet}OH$, have shown efficacy in a variety of animal models of CNS injury [25–27], proving to be effective in ischemic stroke models (especially the PBN disodium 1,3-disulfonate salt derivative, NXY-059) [28] and aging [29,30]. Incorporating the nitrone functionality in a ring (cyclic PBN analogs) did strongly increase potency relative to PBN against oxidative injury and cell toxicity, as a result of favorable changes in the HOMO/LUMO energy levels and greater accessibility of the nitrone double bond [26,31–34]. A number of 3,3-dimethyl-3,4-dihydroisoquinoline 2-oxides (**1**, Fig. 1), 3,3-dimethyl-4,5-dihydro-3*H*-2-benzazepine 2-oxides (**2**), and their spiro C-3 cycloalkyl analogs, have been found out as inhibitors of LPO. Electron spin resonance (ESR) spectroscopy has clearly demonstrated that cyclic nitrones **1** and **2** are able to trap radicals, forming more stable spin adducts than PBN [33,34]. In particular, chloro-substituted 2-benzazepine nitrones (**2**, X = 8-Cl or 7,9-$(Cl)_2$) proved to be potent radical scavengers in both lipid and apoprotein fractions of low density lipoproteins [33]. Moreover, structure–activity relationship (SAR) studies have highlighted the following main factors increasing their in vitro inhibitory potency: (i) the presence of a second antioxidant functionality, such as *o*,*o*′-dimethylphenol (e.g., X = 6,8-$(CH_3)_2$-7-OH in **2**); (ii) lipophilicity increments, as resulted from expansion of the nitrone-containing ring (2-benzazepine versus isoquinoline), replacement of the *gem*-dimethyl group

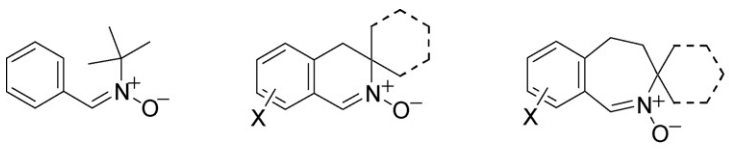

Fig. 1 – Structures of PBN spin trap and structurally related cyclic nitrones. The structures of 3,3-dimethyl-3,4-dihydroisoquinoline 2-oxides (1), 3,3-dimethyl-4,5-dihydro-3*H*-2-benzazepine 2-oxides (2), and their spiro C-3 cycloalkyl analogs, are shown, along with benzene ring X-substitution which proved to achieve significant improvements in activity over the parent compounds (X = H).