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Para-Substituted α-Phenyl-N-tert-butyl Nitrones: Spin-Trapping, Redox and Neuroprotective Properties

Anaïs Deletraz, Béatrice Tuccio, Julien Roussel, Maud Combes, Catherine Cohen-Solal, Paul-Louis Fabre, Patrick Trouillas, Michel Vignes, Noelle Callizot, and Grégory Durand*

ABSTRACT: In this work, a series of para-substituted α-phenyl-N-tert-butyl nitrones (PBN) were studied. Their radical-trapping properties were evaluated by electron paramagnetic resonance, with 4-CF₃-PBN being the fastest derivative to trap the hydroxymethyl radical (CH₂OH). The redox properties of the nitrones were further investigated by cyclic voltammetry, and 4-CF₃-PBN was the easiest to reduce and the hardest to oxidize. This is due to the presence of the electron-withdrawing CF₃ group. Very good correlations between the Hammett constants (σ) of the substituents and both spin-trapping rates and redox potentials were observed. These correlations were further supported by computationally determined ionization potentials and atom charge densities. Finally, the neuroprotective effect of these derivatives was studied using two different in vitro models of cell death on primary cortical neurons injured by glutamate exposure or on glial cells exposed to BuOOH. Trends between the protection afforded by the nitrones and their lipophilicity were observed. 4-CF₃-PBN was the most potent agent against BuOOH-induced oxidative stress on glial cells, while 4-Me₂N-PBN showed potency in both models.

INTRODUCTION

Oxidative stress results from an exaggerated production of reactive oxygen species (ROS). These include free radicals such as the superoxide ion O₂⁻⁻ or the hydroxyl radical HO⁻ to name two. However, reactive nitrogen species (RNS) and reactive sulfur species (RSS) must also be considered. Some of these reactive species are beneficial to living organisms as modulators of cellular function, signaling, and immune response; however, when they are present in high levels, they lead to cellular injury.¹,² Oxidative stress is often associated with several pathologies such as cancer and cardiovascular and neurodegenerative diseases to name a few.³,⁴ To prevent oxidative damage, synthetic antioxidants have been developed and nitrones are promising agents with considerable potential as therapeutics.⁴–⁶ Nitrones have also been widely used as spin traps for the detection and characterization of free radicals by electron paramagnetic resonance (EPR) spectroscopy. In the spin-trapping technique, free radicals react with the nitronyl function to form stable and EPR-identifiable aminoxyl radicals.⁷,⁸ Two families of nitrones have been developed: the cyclic ones derived from S,S-dimethyl-1-pyrroline N-oxide (DMPO) and the linear ones derived from α-phenyl-N-tert-butyl nitrone (PBN).

Over the past several decades, several analogues of DMPO and PBN have been designed to improve their spin-trapping properties and their biological activities.⁵,⁹ When used as spin traps, it is important to design nitrones with a high rate constant of free radical trapping as well as with great stability of the corresponding aminoxyl spin adduct to ensure efficient detection. In general, cyclic nitrones lead to more persistent adducts than linear ones, and the analogues of DMPO with high potency have been designed. One can cite the phosphorylated analogue S-diethoxyphosphoryl-S-methyl-1-pyrroline N-oxide (DEPMPO),¹⁰ the ester derivative S-ethoxycarbonyl-S-methyl-1-pyrroline N-oxide (EMPO),¹¹ and the amido analogue S-carbamoyl-S-methyl-1-pyrroline N-oxide (AMPO).¹²,¹³ AMPO was reported to have the highest rate constant of superoxide trapping, followed by EMPO and then DEPMPO. Linear nitrones have also been employed successfully in spin-trapping experiments but are generally less efficient than cyclic ones.¹⁴,¹⁵ However, due to their better distribution within tissues and cells, linear nitrones have been widely used for radical detection in ex vivo and in...
vivo studies. Linear nitrones are usually easier to synthesize and purify as they are often solid at room temperature and can be recrystallized to afford paramagnetic impurity-free samples. Moreover, linear PBN-type nitrones allow the possibility of rather easy functionalization both on the aromatic ring and on the N-tert-butyl group and therefore provide more chemical versatility. Several studies showed that PBN-type nitrones combine anti-inflammatory, antioxidant, and neuroprotective properties and are able to cross the blood–brain barrier. These nitrones could therefore be used for the treatment of stroke, visual loss, neuronal damage, and other age-related diseases. The PBN derivative called 2,4-disulfophenyl-tert-butyl nitrone (NXY-059) was the first neuroprotective agent to reach phase III clinical trials in the United States.

Nitrones have a very broad activity that depends on the nature and the position of the substituents on the nitronyl function. Therefore, the choice of substituents is very important and depends on the properties to be improved such as water solubility, lipophilicity, rate constant of radical trapping, adduct stability, bioactivity, and the possibility of cellular or tissue targeting thanks to the ligation to specific molecular targets. Over the past several years, the reactivity of the para-substituted derivatives of PBN has been explored to identify the most promising substituent for improved and optimal reactivity toward free radicals. It has been shown that the electronic nature of the substituent influences the rate of radical trapping on the nitronyl function. The presence of an electron-withdrawing group on the para position of the phenyl ring increased the reactivity toward nucleophilic addition reactions. Contrarily, nitrones with an electron-donating group exhibited high reactivity toward electrophilic radicals. The polar effect of the substituents has also been correlated with the electrochemical properties of the nitronyl function, with the derivatives bearing substituents has also been correlated with the electrochemical properties. The electronic effects of the substituents were computationally rationalized by density functional theory (DFT), and correlations between the experimental and theoretical data were established. Finally, the in vitro protection of the nitrones including previously synthesized 4-X-PBN derivatives, namely, 4-Me,N-PBN (1), 4-MeO-PBN (2), 4-AcNHCH₂-PBN (4), 4-MeNHCO-PBN (10), 4-HOOC-PBN (11), and 4-NC-PBN (13) was investigated in two paradigms of cell death on neurons and glial cells.

### RESULTS AND DISCUSSION

#### Synthesis

The derivatives 4-iPr-PBN (3), 4-Ph-PBN (5), 4-MeS-PBN (6), 4-F-PBN (8), 4-CF₂O-PBN (9), and 4-CF₃-PBN (12) were obtained by the “one-pot” reduction/condensation of 2-methyl-2-nitropropane onto the appropriate benzoic acid in the presence of zinc powder and AcOH, (method A in Scheme 1). 4-MeCONH-PBN (7) was obtained by direct condensation of 4-acetamidobenzaldehyde with N-(tert-butyl)hydroxylamine acetate using pyridoline as a catalyst according to the methodology developed by Morales et al. with a few modifications (method B in Scheme 1). The final compounds were purified by flash chromatography and two successive crystallizations, to ensure high purity. For 4-Me,N-PBN (1), 4-MeO-PBN (2), 4-AcNHCH₂-PBN (4), 4-MeNHCO-PBN (10), 4-HOOC-PBN (11), and 4-NC-PBN (13), the samples already synthesized by our team were used.

#### EPR Study of Hydroxymethyl Radical Trapping

To evaluate their ability to scavenge carbon-centered radicals, the relative rate constant of trapping of the hydroxymethyl radical (\(^{\cdot}\)CH₂OH) by 4-iPr-PBN, 4-Ph-PBN, 4-MeS-PBN, 4-MeCONH-PBN, 4-F-PBN, 4-CF₂O-PBN, and 4-CF₃-PBN was measured. Scheme 2 depicts the general mechanism of spin trapping by nitrones.

**α-Hydroxycarbon-centered radicals can be produced during oxidative stress by an attack of HO\(^{\cdot}\) on alcohols and are therefore of interest when evaluating nitrone antioxidant activities.** The Fenton reaction was used to produce in situ \(^{\cdot}\)CH₂OH radicals in the presence of methanol. Under these conditions, all of the generated spin adducts of the \(^{\cdot}\)CH₂OH radical (noted N-CH₂OH) gave rise to a triplet of doublets (noted TN) characteristic of a nitroxide adduct, as shown in Figure 2. The EPR hyperfine splitting constants (hfsc’s) \(\alpha_N\) and \(\alpha_H\) of the simulated \(^{\cdot}\)CH₂OH adducts are reported in Table 1. All of the derivatives present similar nitrogen hfsc (~15.3 G) except 4-Ph-PBN (14.0 G), probably due to the higher resonance of the phenyl substituent. We next used a kinetic competition method described elsewhere to determine the trapping efficiency of the compounds 4-iPr-PBN, 4-Ph-PBN, 4-MeS-PBN, 4-MeCONH-PBN, 4-F-PBN, 4-CF₂O-PBN, and 4-CF₃-PBN. The nitrone of interest, denoted as N, was tested in competition with 1,3,5-tri[(N-(1-diethylphosphono)-1-methylethyl)] N-oxyl-aldimine [TN] for \(^{\cdot}\)CH₂OH trapping. A series of experiments were performed in the presence of various amounts of N and TN. The analysis of the experimental EPR spectra led to the relative trapping rate of the nitrones noted \(k_{N/\text{TN}}\) as depicted in Figure 2C. To compare the new nitrones to PBN, the same experiment was performed using PBN versus TN, and from this, the \(k_{N/\text{PBN}}\) ratio was calculated for the whole series with values ranging from 0.25 to 3.18, as reported in Table 1. The details are given in the Experimental Section.

#### Synthesis

4-iPr-PBN, 4-MeS-PBN, 4-MeCONH-PBN, and 4-Ph-PBN trapped \(^{\cdot}\)CH₂OH slower than PBN, while 4-F-PBN and 4-CF₂O-PBN trapped \(^{\cdot}\)CH₂OH 1.7 and 1.8 times faster than PBN, respectively. With a spin-trapping rate 3.2 times higher...
than that observed for PBN, 4-CF3-PBN, which bears a strong electron-withdrawing substituent, was the most potent of the series even including the para-PBN derivatives previously studied.33,45 The two very potent PBN-type nitrones that have been previously used as spin traps PPN and EPPN exhibited $k_N/k_{PBN}$ values of 6.4 and 1.5, respectively.45 This indicates the high efficiency of the fluorinated derivatives 4-F-PBN, 4-CF3O-PBN, and 4-CF3-PBN.

The plot of the rate constants by the different nitrones versus the Hammett values of their substituents showed a very good correlation (Figure 3A, $R^2 = 0.86$). A positive value of the slope $\rho$, as observed, is indicative of the nucleophilic nature of the addition of the radical on the nitronyl function, which agrees with the work of De Vleeschouwer et al., who classified hydroxymethyl radicals as strong nucleophiles.52 Moreover, it has been already shown that an electron-withdrawing substituent in PBN-type derivatives increases the reactivity of the nitronyl function for nucleophilic radical addition,13,53 further supporting our observation.

The atomic partial charges of the nitronyl atoms (H, C, N, and O) and the atomic total charge of the nitronyl moiety were calculated for all of the derivatives using natural population analysis (NPA) within the natural bond orbital (NBO) framework and are reported in Table S2.54,55 A positive correlation between the rate constants of $\cdot$CH2OH addition to nitrones with the atomic total charge of the nitronyl moiety was observed (Figure 3B) as well as a good correlation between the atomic total charge of the nitronyl atoms of all of the series and the Hammett constants ($\sigma_p$)51 of the substituents (Figure S1). This confirms again that $\cdot$CH2OH free radical scavenging occurs through nucleophilic radical addition requiring electron-withdrawing groups in the nitrones, which directly impact on the partial charges of the nitronyl moiety, where the nucleophilic addition is expected to occur.42

**Table 1. Physicochemical and Spin-Trapping Properties of PBN Derivatives**

| nitrones         | $\sigma_p$ | $\alpha_H$ (G) | $\alpha_O$ (G) | $k_N/k_{PBN}$ b (± 0.05) |
|------------------|------------|----------------|----------------|--------------------------|
| 4-iPr-PBN (3)    | −0.15      | 15.4           | 3.8            | 0.25                     |
| 4-Ph-PBN (5)     | −0.01      | 14.0           | 3.0            | 0.78                     |
| 4-MeS-PBN (6)    | 0          | 15.4           | 3.5            | 0.33                     |
| PBN              | 0          | 15.3           | 3.8            | 1.00                     |
| 4-MeCONH-PBN (7) | 0          | 15.3           | 3.6            | 0.58                     |
| 4-F-PBN (8)      | 0.06       | 15.4           | 3.4            | 1.72                     |
| 4-CF3O-PBN (9)   | 0.35       | 15.3           | 3.3            | 1.84                     |
| 4-CF3-PBN (12)   | 0.54       | 15.2           | 3.3            | 3.18                     |

aData from Hansch et al.51 bRatio of the second-order rate constants for the hydroxymethyl radical trapping by various nitrones ($k_N$) and by PBN ($k_{PBN}$) in methanol, calculated with the ratio $k_{PBN}/k_{TN} = 0.057$.

**Scheme 1. Methods Used for the Synthesis of 4-X-PBN Nitrones**

**Scheme 2. General Spin-Trapping Mechanism by Nitrones**

**Figure 2. EPR signals of TN and N hydroxymethyl radical adducts, respectively. (A) N = PBN and (B) N = 4-CF3-PBN. The hydroxymethyl radical was generated by a Fenton system and the concentration ratio [N]/[TN] = 4. The peaks topped by a cross (×) correspond to the hydroxymethyl radical adduct of N. (C) Determination of the relative rate constants $k_N/k_{TN}$ of $\cdot$CH2OH trapping by PBN, 4-CF3-PBN, and 4-iPr-PBN.**
Cyclic Voltammetry. The electrochemical properties of 4-iPr-PBN, 4-Ph-PBN, 4-MeS-PBN, 4-MeCONH-PBN, 4-F-PBN, 4-CF,0-PBN, and 4-CF,3-PBN were investigated using cyclic voltammetry in acetonitrile containing tetra-butylammonium perchlorate (TBAP) as an electrolyte and the redox potentials are reported in Table 2. Reduction and oxidation potentials of all of the nitrones have been observed in the electroactivity domain of the solvent (Figures 4, S2, and S3). As expected, the peak currents are linearly related to the square root of the potential scan rate (Figures S4-S6), demonstrating that the process is diffusion-controlled.56,57 By calibrating the current versus ferrocene, the number of electrons involved in the reduction or oxidation processes can be deduced.58 PBN, 4-iPr-PBN, 4-Ph-PBN, 4-F-PBN, and 4-CF,0-PBN were reduced through two successive two-electron transfers in agreement with previous observations on other PBN-type nitrones.33,59,60 For the derivatives 4-MeCONH-PBN and 4-CF,3-PBN, an additional electron transfer was also observed, as shown in Figure 4. This is likely due to the reduction of the trifluoromethyl substituent of 4-CF,3-PBN, while this corresponds to the reduction of the carbonyl group for 4-MeCONH-PBN. 4-MeS-PBN, which possesses a thiomethyl substituent, underwent a one-step two-electron reduction. For all of the derivatives except 4-Ph-PBN, the reduction appeared irreversible, as no associated backward peaks were observed. The reduction of the nitronyl group into a radical nitroso anion corresponds to the first peak obtained, with values ranging from −2.21 to −1.83 V.

Considering the anodic behavior, PBN and the derivatives 4-iPr-PBN, 4-Ph-PBN, 4-CF,0-PBN were oxidized through an irreversible one-electron transfer, whereas 4-MeS-PBN, 4-MeCONH-PBN, 4-F-PBN, and 4-CF,0-PBN possess two oxidation peaks. The first peak observed corresponds to the oxidation of the nitronyl function with values ranging from 1.41 to 1.89 V, except for 4-MeS-PBN where the first peak would correspond to the oxidation of the thiomethyl group. 4-CF,3-PBN bearing an electron-withdrawing group is the easiest to reduce, with the lowest cathodic peak potential in the series and the hardest to oxidize. On the contrary, the derivative with the highest reduction potential was 4-iPr-PBN, which has an electron-donating group and very good correlations between the Hammett constants (σp),51 and the redox potentials were observed (Figure S7). The ionization potentials (IPs) were also computationally determined (Table 2), and a good correlation between IP and the oxidation potential (R2 = 0.71, Figure 4) was also observed.

In connection with the spin-trapping applications, the potential window where the spin-trapping technique can be safely applied with electrochemically generated radicals, refer to as the stability domain, was calculated and is reported in Table 2. It represents the potential window where there is no risk of an inverted spin-trapping process.61,62 4-iPr-PBN, 4-Ph-PBN, 4-F-PBN, 4-CF,0-PBN, and 4-CF,3-PBN have a similar stability domain to PBN (∼3.70 V), whereas 4-Ph-PBN, 4-MeS-PBN, and 4-MeCONH-PBN present a slightly slower stability domain (∼3.40 V). Therefore, all of the spin traps exhibit

Table 2. Electrochemical Properties and Calculated Ionization Potentials of 4-X-PBN Derivatives

|   | First peak | Second peak | Third peak | First peak | Second peak | Stability domain | IP (eV) |
|---|------------|-------------|------------|------------|-------------|-----------------|---------|
| 4-iPr-PBN (3) | −2.21 | −2.30 | | 1.52 | | 3.73 | 5.9 |
| 4-Ph-PBN (5) | −1.96 | −2.56 | | 1.52 | | 3.48 | 5.9 |
| 4-MeS-PBN (6) | −2.06 | | | 1.32 | 1.62 | 3.38 | 7.0 |
| PBN | −2.12 | −2.27 | | 1.60 | | 3.72 | 6.0 |
| 4-MeCONH-PBN (7) | −1.93 | −2.35 | −2.56 | 1.41 | 1.80 | 3.34 | 5.7 |
| 4-F-PBN (8) | −2.10 | −2.19 | | 1.57 | 1.78 | 3.67 | 6.0 |
| 4-CF,0-PBN (9) | −2.02 | −2.13 | | 1.64 | 1.86 | 3.66 | 6.1 |
| 4-CF,3-PBN (12) | −1.83 | −2.13 | −2.27 | 1.89 | | 3.72 | 6.2 |

The peak potentials are given versus a silver wire electrode for a potential scan rate of 0.1 V/s; in general, the electron transfers appeared irreversible (no backward peak observed) except for nitrone 4-Ph-PBN noted (p). The IPs were calculated at the (CPCM)/M06-2X/6-31+g(d,p) level of theory. Containing 0.1 M TBAP with reduction E(d)(c) and oxidation E(a)(c) at a glassy carbon (GC) electrode. The stability domain is E(a)(c) − E(d)(c).
potential windows broad enough to be used in electrochemical investigations.60,63

**Cell Culture and Viability Studies.** Excitotoxicity and oxidative stress trigger different mechanisms of cell death but there is strong evidence that both mechanisms may cooperate in inducing neuron cell death. Therefore, the in vitro cytoprotective effect of the derivatives was next evaluated on two types of assays. The first one relies on 1BuOOH-induced oxidative stress and the second one relies on glutamate-induced excitotoxicity. We added to the evaluation of other 4-X-PBN nitrones previously synthesized in our laboratory. This includes 4-Me2N-PBN, 4-MeO-PBN, 4-AcNHCH2-PBN, 4-MeNHCO-PBN, 4-HOOC-PBN, and 4-NC-PBN (Figure 1).33,42,45 In the first test, nitrones at 10 μM were preincubated with glial cells for 24 h before the 1BuOOH exposure (300 μM), and the cell survival was evaluated by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Figure 5). We checked the effect of 4-X-PBN derivatives at 10 μM after 24 h of incubation (Figure S8). None of the nitrones tested showed any toxicity at this concentration with a survival rate being comprised between 98.4 ± 4.2 and 103.4 ± 10.9%. Exposure of 300 μM 1BuOOH resulted in a significant drop (∼2.5 times) of the cell viability indicating high toxicity of 1BuOOH toward glial cells. Preincubation with nitrones led to different levels of protection depending on the nature of the substituents. 4-MeO-PBN, 4-NC-PBN, 4-MeNHCO, and 4-Ph-PBN did not afford any protection with cellular viability very similar to that obtained with 1BuOOH alone. PBN led to slight protection with a cellular viability of 45.3 ± 1.4% while the control was 41.3 ± 2.0%. 4-AcNHCH2-PBN, 4-HOOC-PBN, 4-F-PBN, 4-MeS-PBN, 4-MeCONH-PBN, and 4-iPr-PBN led to moderate protection, which was however not statistically significant when compared to the 1BuOOH control condition. In contrast, 4-CF3O-PBN, 4-Me2N-PBN, and 4-CF3-PBN exhibited statistically significant protection with viability of 55.6 ± 4.1, 56.9 ± 4.5, and 57.8 ± 4.5%, respectively. No correlation between the neuroprotection afforded by the nitrones and their spin trapping (Figure S9) or redox properties was observed; however, it has to be underlined that the most efficient derivative in this model, 4-CF3-PBN, exhibited the highest spin-trapping rate as well as the lowest reduction and the highest oxidation potentials. This indicates the peculiar properties of this derivative. Previous work showed that 4-CF3-PBN efficiently protected rat retina from light damage when administered intraperitoneal.23 Our data therefore confirm the potency of 4-CF3-PBN to reduce oxidative stress and cell death.

In the second assay, the nitrones were preincubated with primary cortical neurons (consisting of 80% neurons and 20% glial cells; mainly astrocytes) for 1 h at different concentr-
Glutamate concentrations in the extracellular space are low (nanomolar concentrations) and tightly controlled by a large number of mechanisms operating at the synapse. Disturbances in this regulatory system can have deleterious effects such as the excessive release of glutamate (micromolar concentration), which can induce hyperexcitability of post-synaptic neurons to the point of excitotoxicity, followed by cell death (cytotoxicity). Glutamate excitotoxicity is one of the well-accepted pathophysiological mechanisms behind many neurodegenerative diseases like amyotrophic lateral sclerosis (ALS), Alzheimer’s, and Huntington’s disease. Glutamate, the major excitatory neurotransmitter of the central nervous system, acts as a neurotoxin at high concentrations. High glutamate concentration causes an overstimulation of glutamate receptors and an increase in the intracellular calcium level, which can further activate various enzymes such as proteases, endonucleases, phospholipases, and nitric oxide synthase (NOS). This results in enhanced structural degradation, mitochondrial damage, ROS/RNS production, DNA damage, and increased expression of inflammatory mediators, which eventually lead to neuronal death.

Forty-eight hours after the glutamate washout, cell survival was evaluated by an MTT assay (Figure 6). In the absence of nitrore, the survival was dropped by about 30%, demonstrating the toxicity of glutamate exposure. Brain-derived neurotrophic factor (BDNF), a well-known neurotrophin acting via its TrkB receptor, was used as a positive control. The stimulation of the signaling cascades includes the phosphatidylinositol-3-kinase (PI3K), PLCγ, and MAPK pathways. All these pathways promote cell survival and aid in cell growth and differentiation. 4-MeO-PBN, 4-iPr-PBN, 4-MeS-PBN, 4-MeCONH-PBN, and the three fluorinated derivatives 4-F-PBN, 4-CF3-PBN, and 4-CF3O-PBN failed to show any protection against glutamate, and the cellular viability was in certain conditions even lower than the control glutamate, suggesting slight toxicity. In contrast, a dose-dependence effect was noted for PBN, 4-MeNHCO-PBN, 4-Me2N-PBN, 4-AcNHCH2-PBN, 4-Ph-PBN, and 4-NC-PBN, but only 4-Me2N-PBN, 4-MeNHCO-PBN, and 4-NC-PBN led to statistically significant protection at 10 μM with cellular viability of 78.8 ± 2.4, 79.9 ± 3.0, and 81.7 ± 3.7%, respectively. Irrespective of the concentration (0.1, 1, or 10 μM), treatment with 4-HOOC-PBN led to a significant protection higher than 80%, indicating a plateau effect.

Previous studies showed that, in vitro, PBN-protected rat cerebellar neurons against excitotoxic glutamate exposure (100 μM) with a half-maximal effective concentration (EC50) of 2.7 mM. The ability of PBN to alleviate glutamate neurotoxicity in vivo was also demonstrated but was attributed to the free radical scavenging mechanism of PBN. The absence of correlation between the neuroprotection and the spin trapping (Figure S9) or redox properties was also noted in this assay. Taken together, these data show that 4-Me2N-PBN is able to protect neurons and glial cells from a glutamate injury and is also able to reduce glial cell death induced by massive oxidative stress. Although 4-Me2N-PBN was found to be a poor spin trap, it exhibits here the lowest ionization potential of the series. Moreover, its strong antioxidant potency was noted on the oxygen radical absorbance capacity (ORAC) test, the activity being superior to curcumin, while none of the other nitrones presented here showed any activity (data not shown). In contrast, 4-CF3-PBN, which was the most potent spin trap, was also the most potent agent against the BuOOH astrocyte toxicity but failed to show any significant protection of neurons. Altogether, these results indicate that the cell protection afforded by nitrones depends on the type of toxicity (oxidative stress or excitotoxic event) as well as on the type of cells (neurons or glial cells). However, the mechanisms leading to cell death triggered in neurons and glial cells are rather similar in these cell types, as they both involve oxidative stress. Some evidence suggest that antioxidants may act directly by scavenging free radicals or indirectly by increasing endogenous cellular antioxidant defenses such as activation of the nuclear factor erythroid 2 (Nrf2). The potential for Nrf2-mediated transcription to protect from neurodegeneration resulting from oxidative stress mechanisms is a well-known neuroprotective mechanism. The Nrf2 signaling pathway is closely associated with the protective effect on neurons exposed to oxidative stress insults. In addition, Nrf2 has been reported to inhibit apoptosis based on its effect on mitochondria-related apoptotic proteins such as Bcl-2 and Bax. The activation of this pathway is also protective against glial cell death. In addition, reductions in nitric oxide (NO) production modulate the expression of proteins, such as iNOS,
or regulators of the genes encoding proinflammatory mediators, such as nuclear factor-xB. Particularly in cell survival, NF-xB is well known to promote the transcription of bcl-2 and inhibition of apoptosis proteins.\textsuperscript{73} The difference in the protection by nitrones on the two models may originate from their intrinsic properties highlighted in a cell-specific manner. The lipophilicity of the nitrones could be a key factor in their protective action in either model. Indeed, as shown in Figure 7, a positive trend is noted when plotting the protection of para substituents was correlated with the atomic total charge of the nitronyl function; the higher the charge, the faster the reaction. The electrochemical determinations, EPR measurements, hydroxymethyl spin-trapping kinetics, computational calculations, cyclic voltammetric measurements, and in vitro neuroprotective measurements are described in the Supporting Information. They were described by Deleraz et al.\textsuperscript{74} except for the glial cell model.

**General Procedure for the Synthesis of 4-X-PBNs (Except 4-MeCONH-PBN).** Under an argon atmosphere and under stirring, the corresponding benzaldehyde (1.0 equiv), 2-methyl-2-nitropropane (2.0 equiv), and AcOH (6.0 equiv) were dissolved in dry EtOH. The mixture was cooled down to 0 °C, and then zinc powder (4.0 equiv) was slowly added to keep the temperature below 15 °C. The mixture was stirred at room temperature for 30 min and then heated overnight at 60 °C in the dark in the presence of molecular sieves (4 Å). The reaction mixture was filtered through a pad of Celite, and the solvent was removed under vacuum. The crude mixture was purified by flash chromatography (EtOAc/cyclohexane), followed by two successive crystallizations from EtOAc/n-hexane.

**N-tert-Butyl-α-(4-isopropyl)phenylnitrotrone (3).** Following the general procedure, the reaction of 4-isopropylbenzaldehyde (319 mg, 2.16 mmol), 2-methyl-2-nitropropane (444 mg, 4.31 mmol), AcOH (0.74 mL, 12.96 mmol), and Zn (562 mg, 8.64 mmol) in dry EtOH for 16 h gave, after flash chromatography (EtOAc/cyclohexane, 2.8 v/v), nitron 3 (284 mg, 60%) as a white powder. R\textsubscript{f} (EtOAc/cyclohexane, 2.8 v/v) = 0.18. Elemental analysis calculated for C\textsubscript{14}H\textsubscript{21}NO: C, 76.67; H, 9.65; N, 5.88. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 8.22 (2H, d, \(J = 8.4\) Hz), 7.51 (1H, s), 7.27 (4H, d, \(J = 8.4\) Hz); \textsuperscript{13}C{1H} NMR (100 MHz, CDCl\textsubscript{3}) \(\delta\) 151.5, 129.9, 129.1, 128.9, 126.6, 70.6, 34.3, 28.5, 23.9. HPLC (XTerra, Waters, RP18; 280 nm) \(t\textsubscript{R}\) = 11.6 min, 98.7% purity. HR-MS (ESI/Q-TOF) m/z: [M + H]\textsuperscript{+} calcd for C\textsubscript{14}H\textsubscript{22}NO, 220.1701; found, 220.1704. The spectral data were in agreement with the literature.\textsuperscript{75}

**N-tert-Butyl-α-(4-phenyl)phenylnitrotrone (5).** Following the general procedure, the reaction of 4-biphenylcarboxaldehyde (910 mg, 5.00 mmol), 2-methyl-2-nitropropane (1.03 g, 10.00 mmol), AcOH (1.72 mL, 30.00 mmol), and Zn (1.30 g, 20.00 mmol) in dry EtOH for 20 h gave, after flash chromatography (EtOAc/cyclohexane, 2.8 v/v), nitron 5 (718 mg, 57%) as a white powder. R\textsubscript{f} (EtOAc/cyclohexane, 3.7 v/v) = 0.32.

**EXPERIMENTAL SECTION**

The general methods and procedures for the synthesis of the derivatives, \(\log P\) determination, EPR measurements, hydroxymethyl spin-trapping kinetics, computational calculations, cyclic voltammetric measurements, and in vitro neuroprotective measurements are described in the Supporting Information. They were described by Deletraz et al.\textsuperscript{74} except for the glial cell model.

**CONCLUSIONS**

A series of para-substituted PBN nitrones were studied to investigate the influence of the nature of the substituent on the spin-trapping, redox, and cytoprotective activities of the nitronyl function. The spin-trapping ability toward hydroxymethyl radicals showed that the presence of an electron-withdrawing group at the para position significantly increased the trapping rate. The derivative 4-CF\textsubscript{3}-PBN gave the best spin-trapping activity with a trapping rate 3.2 times higher than PBN, and it is therefore a potential candidate for spin-trapping experiments. The spin-trapping rate was positively correlated with the atomic total charge of the nitronyl function; the higher the charge, the faster the reaction. The electrochemical properties of the derivatives were studied and the polar effect of para substituents was confirmed. With a strong electron-withdrawing CF\textsubscript{3} group, 4-CF\textsubscript{3}-PBN was the easiest to reduce and the hardest to oxidize. 4-CF\textsubscript{3}-PBN also showed good protection against BuOOH toxicity on glial cells but no protection of neurons challenged by glutamate exposure was observed. In contrast, 4-Me\textsubscript{2}N-PBN showed good protection of neurons challenged by glutamate. This work therefore provides insight into the design of nitrone derivatives that could be further used as spin traps and therapeutics.
Elemental analysis calculated for C_{12}H_{20}N_{2}O: C, 66.64; H, 7.74; N, 11.12%. \(^{1}H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.24 (2H, d, \(J = 8.8\) Hz), 7.58 (2H, d, \(J = 8.8\) Hz), 7.50 (1H, s), 1.59 (9H, s); \(^{13}C\{1H\} NMR (100 MHz, CDCl\(_3\)) \(\delta\) 168.7, 139.8, 130.0, 129.8, 70.9, 28.5. HPLC (XTerra, Waters, RP18; 280 nm) \(t_{R}\): 3.6 min, 98.1% purity. HR-MS (ESI-Q/TOF) \(m/z\): [M + H\(^{+}\)]\(^{+}\) calculated for C\(_{13}\)H\(_{16}\)F\(_{3}\)NO: C, 58.77; H, 5.75; N, 5.71%; found: C, 58.94; H, 5.53; N, 5.57%. \(^{1}H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.35 (2H, d, \(J = 8.4\) Hz), 7.62 (1H, s), 1.61 (9H, s). \(^{13}C\{1H\} NMR (100 MHz, CDCl\(_3\)) \(\delta\) 131.3, 131.3 (d, \(J = 32\) Hz), 128.8, 128.6, 125.5–125.3 (m), 124.0 (d, \(J = 269\) Hz), 71.8, 28.5. HPLC (XTerra, Waters, RP18; 280 nm) \(t_{R}\): 11.6 min, 99.8% purity. HR-MS (ESI-Q/TOF) \(m/z\): [M + H\(^{+}\)]\(^{+}\) calculated for C\(_{13}\)H\(_{16}\)F\(_{3}\)NO, 246.1106; found, 246.1111. The spectral data were in agreement with the literature.\(^{78}\)

**N-tert-Butyl-α-(4-fluoro)phenylnitrone (8).** Following the general procedure, the reaction of 4-fluorobenzaldehyde (410 mg, 3.94 mmol), 2-methyl-2-nitropropane (575 mg, 5.58 mmol), AcOH (1.03 mL, 17.94 mmol), and Zn (777 mg, 11.96 mmol) in dry EtOH for 18 h gave, after flash chromatography (EtOAc/cyclohexane, 2:8 v/v), \nitrone 8 (395 mg, 54%) as a white powder. \(R_{f}\) (EtOAc/cyclohexane, 3.7 v/v) = 0.20. Elemental analysis calculated for C\(_{11}\)H\(_{15}\)F\(_{2}\)NO: C, 64.54; H, 7.67; N, 7.23; found: C, 64.65; H, 7.79; N, 7.57%. \(^{1}H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.35–8.32 (2H, m), 7.53 (1H, s), 7.10 (2H, \(J = 8.8\) Hz), 1.61 (9H, s). \(^{13}C\{1H\} NMR (100 MHz, CDCl\(_3\)) \(\delta\) 163.4 (d, \(J = 21\) Hz), 131.1 (d, \(J = 8\) Hz), 128.9, 127.6 (d, \(J = 3\) Hz), 115.6 (d, \(J = 21\) Hz), 70.9, 28.5. HPLC (XTerra, Waters, RP18; 280 nm) \(t_{R}\): 3.6 min, 98.1% purity. HR-MS (ESI-Q/TOF) \(m/z\): [M + H\(^{+}\)]\(^{+}\) calculated for C\(_{11}\)H\(_{15}\)F\(_{2}\)NO, 235.1447; found, 235.1449. The spectral data were in agreement with the literature.\(^{78}\)

**N-tert-Butyl-α-(4-trifluoromethoxy)phenylnitrone (9).** Following the general procedure, the reaction of 4-trifluoromethoxy)benzaldehyde (531 mg, 2.79 mmol), 2-methyl-2-nitropropane (575 mg, 5.58 mmol), AcOH (0.96 mL, 16.74 mmol), and Zn (725 mg, 11.16 mmol) in dry EtOH for 16 h gave, after flash chromatography (EtOAc/cyclohexane, 2.8 v/v), \nitrone 9 (395 mg, 54%) as a white powder. \(R_{f}\) (EtOAc/cyclohexane, 2.8 v/v) = 0.19. Elemental analysis calculated for C\(_{12}\)H\(_{21}\)F\(_{3}\)NO\(_{2}\): C, 55.17; H, 5.50; N, 5.36%; found: C, 55.52; H, 5.25; N, 5.08%. \(^{1}H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.36 (2H, d, \(J = 8.8\) Hz), 7.56 (1H, s), 7.25 (2H, d, \(J = 8.8\) Hz), 1.62 (9H, s). \(^{13}C\{1H\} NMR (100 MHz, CDCl\(_3\)) \(\delta\) 149.8, 130.4, 129.8, 128.5, 120.7, 120.5 (d, \(J = 256\) Hz), 71.3, 28.5. HPLC (XTerra, Waters, RP18; 280 nm) \(t_{R}\): 11.7 min, 99.9% purity. HR-MS (ESI-Q/TOF) \(m/z\): [M + H\(^{+}\)]\(^{+}\) calculated for C\(_{12}\)H\(_{21}\)F\(_{3}\)NO\(_{2}\), 262.1055; found, 262.1060. The spectral data were in agreement with the literature.\(^{77}\)

**N-tert-Butyl-α-(4-trifluoromethyl)phenylnitrone (12).** Following the general procedure, the reaction of 4-(trifluoromethyl)benzaldehyde (433 mg, 2.49 mmol), 2-methyl-2-nitropropane (513 mg, 4.98 mmol), AcOH (0.85 mL, 14.94 mmol) and Zn (647 mg, 9.96 mmol) in dry EtOH for 21 h gave, after flash chromatography (EtOAc/cyclohexane, 2.8 v/v), \nitrone 12 (274 mg, 60%) as a white powder. \(R_{f}\) (EtOAc/cyclohexane, 1.9 v/v) = 0.13. Elemental analysis calculated for C\(_{13}\)H\(_{17}\)F\(_{3}\)NO\(_{2}\): C, 55.77; H, 5.75; N, 5.71%. \(^{1}H\) NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.39 (2H, d, \(J = 8.4\) Hz), 7.65 (2H, d, \(J = 8.4\) Hz), 7.62 (1H, s), 1.63 (9H, s). \(^{13}C\{1H\} NMR (100 MHz, CDCl\(_3\)) \(\delta\) 142.7, 140.4, 130.2, 129.7, 129.4, 129.0, 127.9, 127.2, 127.1, 70.9, 28.5. The spectral data were in agreement with the literature.\(^{75}\)
EPR measurements, hydroxymethyl spin-trapping kinetics, computational calculations, cyclic voltammetric measurements, and in vitro neuroprotective measurements; and $^1$H and $^{13}$C NMR spectra, reverse-phase high-performance liquid chromatography (HPLC) chromatograms, and mass spectrometry spectra of 4-iPr-PBN, 4-Ph-PBN, 4-MeS-PBN, 4-MeCONH-PBN, 4-F-PBN, 4-CF$_3$O-PBN, and 4-CF$_3$-PBN (PDF).

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