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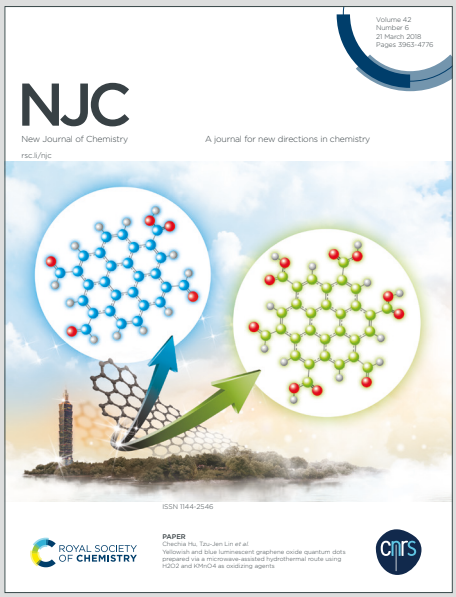

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ARTICLE

Reactivities of MeO-substituted PBN-type Nitrones

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In this work, *α*-Phenyl-*N*-tert-butylnitrone (PBN), *N*-benzylidene-1-diethoxyphosphoryl-1-methylethylamine *N*-oxide (PPN) and *N*-benzylidene-1-ethoxycarbonyl-1-methylethylamine *N*-oxide (EPPN) derivatives bearing methoxy groups (MeO-) on the phenyl ring were synthesized. Their electrochemical properties were studied by cyclic voltammetry and showed that the position and the number of methoxy substituents influence the redox potential of the nitronyl group. The spin-trapping ability of the derivatives was next investigated by EPR spectroscopy for hydroxymethyl radical ($^{\bullet}CH_2OH$). The *ortho*, *meta* and *para* mono-substituted-PBN derivatives exhibited similar trapping rates than the parent PBN while surprisingly, nitrones with two MeO-substituents on the *ortho*-position failed to trap $^{\bullet}CH_2OH$. The trapping rates of the parent compounds were ranked as follows: PPN > EPPN > PBN, indicating that the presence of diethoxyphosphoryl and ethoxycarbonyl electron withdrawing groups on the *N*-*tert*-butyl group significantly enhanced the reactivity towards hydroxymethyl radicals. The effect of the substitutions on the atomic partial charges of the nitronyl-moieties and on their ionization potentials was computationally rationalized and showed a strong correlation between the ionization potential and the experimentally measured oxidation potential.

Keywords : Nitrones, Antioxidants, Electrochemistry, Electron Paramagnetic Resonance (EPR) Spectroscopy, Spin-Trapping.

Introduction

Nitrones have been initially designed as probes for the indirect detection of free radical species in chemical and biological systems.[1] The addition of a free radical onto the nitronyl-carbon atom yields a persistent aminoxyl spin adduct, which can be detected and characterized in spin-trapping experiments by electron paramagnetic resonance (EPR) spectroscopy. This has allowed the identification of several carbon-, oxygen- or sulfur-centered biologically relevant free radicals.[2] The cyclic 5,5-dimethyl-1-pyrrolidine *N*-oxide (DMPO) and the linear α-phenyl-*N*-tert-butyl nitrone (PBN) have been the most commonly used spin traps. DMPO-type nitrones have shown better spin-trapping properties with more distinctive EPR spectra, faster kinetics of trapping and longer half-lives of their spin adducts whereas PBN-type nitrones exhibited a better distribution within tissues and cells and have been largely developed as very promising therapeutic agents.[3,4] Indeed, PBN-type nitrones have shown protective properties in animal models of neurodegenerative diseases,[5] stroke,[6] cardiovascular diseases[7] and cancer.[8] The PBN derivative disodium [(*tert*-butylimino)methyl]-benzene-1,3-disulfonate *N*-oxide (NXY-059) was the first neuroprotective agent to reach phase III clinical trials in the USA for the treatment of acute stroke.[9,10]

In this context, several PBN derivatives have been synthesized and studied over the past decades with the aim to improve their biological and/or spin-trapping activities.[11–19] With regards to the spin-trapping properties, excellent results have been obtained with *N*-benzylidene-1-diethoxyphosphoryl-1-methylethylamine *N*-oxide (PPN),[20–22] a phosphorylated analogue of PBN and with *N*-benzylidene-1-ethoxycarbonyl-1-methylethylamine *N*-oxide (EPPN),[13,23] an ester analogue of PBN. These derivatives, bearing an electron-withdrawing group in *α*-position of the nitronyl-function have been shown to efficiently trap superoxide in aqueous media and to significantly increase stability of the superoxide adduct. Our previous work on *para*-substituted PBNs has identified 4-MeO-PBN as a potent nitrone derivative with low oxidation potential and efficient trapping of phenyl radical.[24]

The current work aims at studying the influence of the electron-donating methoxy (MeO-) substituent on the reactivity of the nitronyl group of three classical nitrones, that is, *α*-phenyl-*N*-*tert*-butylnitrone (PBN), *N*-benzylidene-1-diethoxyphosphoryl-1-methylethylamine *N*-oxide (PPN), and *N*-benzylidene-1-ethoxycarbonyl-1-methylethylamine *N*-oxide (EPPN) (Figure 1). A series of methoxy-substituted phenyl nitrones were thus

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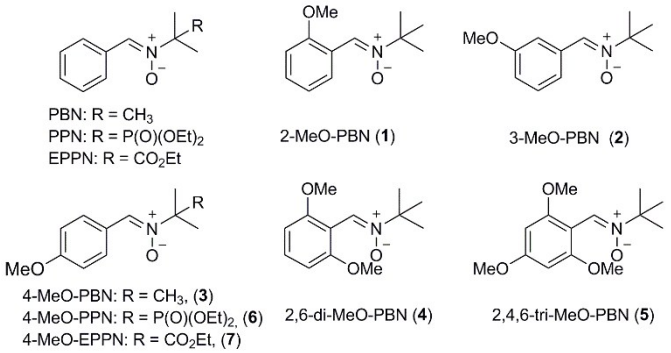
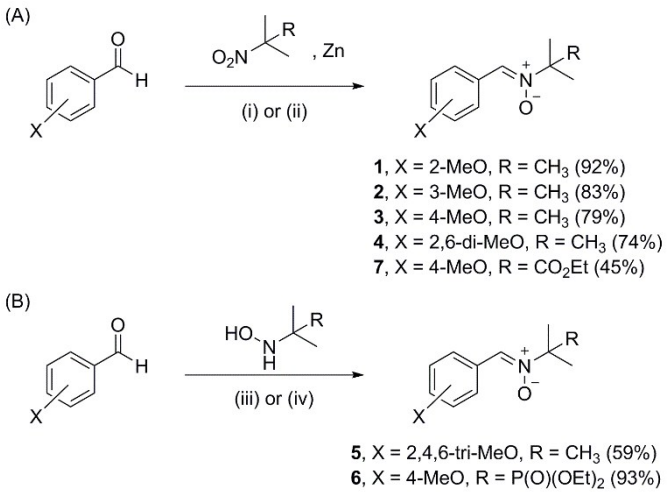


Figure 1. Chemical structures of nitrones used in this study.

synthesized and their electrochemical properties were first studied. Using EPR competition kinetic experiments, the relative rate constant of hydroxymethyl radical trapping was determined. Finally, the effect of methoxy substituents on the nitronyl atomic charge densities and on the Ionization Potential (IP) were described, as supported by Density Functional Theory (DFT) calculations.

Results and Discussion

Synthesis. The MeO-PBN derivatives **1**-**4** were obtained by a one-pot reduction/condensation of 2-methyl-2-nitropropane onto the appropriate benzaldehyde in the presence of zinc powder,[25] as shown in Scheme 1. The 2,4,6-tri-MeO-PBN compound (**5**) was synthesized through direct condensation between 2,4,6-trimethoxybenzaldehyde and *N*-(*tert*butyl)hydroxylamine acetate in CH_2Cl_2, using pyrrolidine as catalyst, following the procedure described by Morales et al.[26] PPN[21] and EPPN[23] were synthesized as previously described and the methoxy derivatives 4-MeO-PPN and 4-MeO-EPPN were obtained using similar synthetic routes. The 4-MeO-PPN compound (**6**) was prepared by condensing



Scheme 1. Synthesis of nitrones by (A) a one-pot reduction/condensation of nitro-derivatives onto the appropriate benzaldehyde or by (B) direct condensation of *N*-substituted hydroxylamines with aromatic aldehydes.[a]

[a]Reagents and conditions: (i) AcOH, EtOH, 60 °C for compounds **1**-**4**; (ii) NH_4Cl, H_2O/MeOH (6/4, v/v), 60 °C for compound **7**; (iii) pyrrolidine, DCM, rt for compound **5**; (iv) EtOH, 60 °C for compound **6**.

diethyl [1-(hydroxyamino)-1-methylethyl]phosphonate, which was obtained by the method described by Petrov et al.[24] with *p*-anisaldehyde in EtOH. For 4-MeO-EPPN (**7**), ethyl 2-methyl-2-nitropropanoate was first synthesized as previously reported[28] and was reduced *in situ* in hydroxylamine and condensed with *p*-anisaldehyde and NH_4Cl, following the procedure described by Stolze et al.[23]

Calculated octanol-water partition coefficients (Clog*P*) of the nitrones were determined using ALOGPS 2.1 software and are listed in Table 1, as well as experimental values found in the literature. The introduction of a methoxy substituent on the phenyl ring decreases very slightly the lipophilicity compared to the parent nitrones (~ 0.10), with no influence of the position of the substituent. This is in good agreement with the experimental values found in the literature where PBN, 3-MeO-PBN (**2**) and 4-MeO-PBN (**3**) exhibited similar Log*P* values.[29] The polar effect of the methoxy groups on the lipophilicity seems to be additive with nitrone **4** and **5**, being slightly less lipophilic than the monosubstituted derivatives **1-3**. This does not perfectly fit with the experimental value for nitrone **5** that was found to be similar to PBN.[29] With regards to PPN and EPPN, more pronounced differences were observed between the calculated and

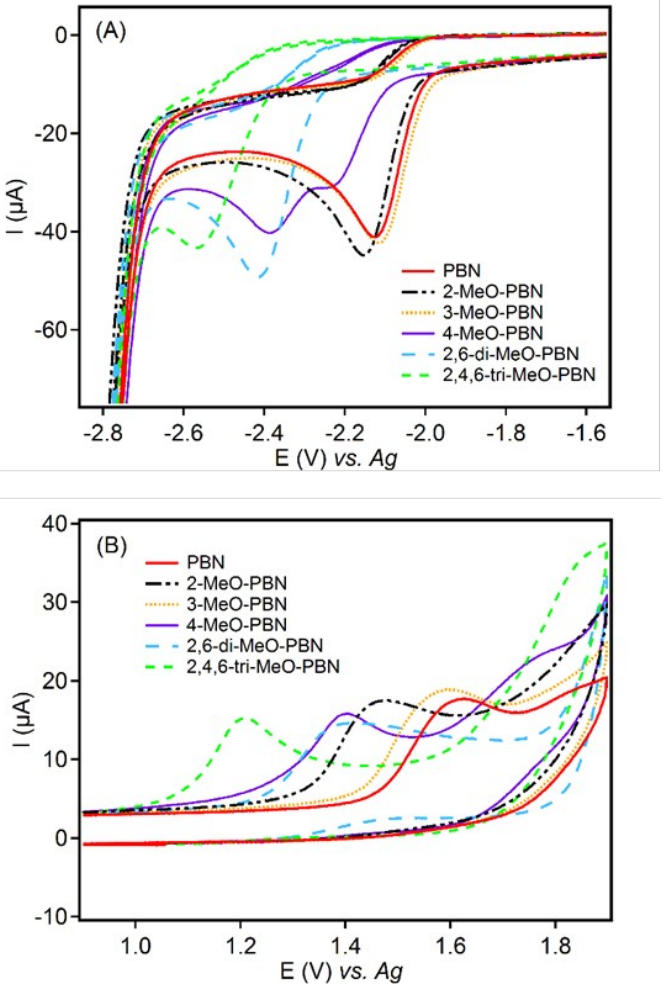


Figure 2. Cyclic voltammograms of PBN-derivatives in acetonitrile containing 0.1 M of TBAP at a Cv electrode, potential scan rate υ = 0.1 V/s vs. Ag: (A) reduction and (B) oxidation.

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Table 1. Physicochemical, Electrochemical and Spin-Trapping Properties of MeO-Derivatives.

			Voltammetry[f]			EPR Spin Trapping			
	Lipophilicity		in CH_3CN[g], compound (1 mM)			$^{\bullet}CH_2OH$			
Compounds	ClogP[a]	LogP	$E_p(c)$ (V)	$E_p(a)$ (V)	Stability domain (V)[h]	a_N (G)	a_H (G)	a_P (G)	k_N/k_{PBN}[j]
PBN	2.67	1.2[b]	-2.12 (-2.12)[i]	1.60 (1.53)[i]	3.72	14.3	2.7	-	1
2-MeO-PBN (1)	2.57	nd[c]	-2.15	1.46	3.61	14.1	3.2	-	1.026
3-MeO-PBN (2)	2.57	1.4[b]	-2.11	1.57	3.68	14.0	3.2	-	0.935
4-MeO-PBN (3)	2.57	1.2	-2.22 (-2.23)[i]	1.40 (1.26)[i]	3.62	14.1	3.1	-	1.128 (0.832)[g]
2,6-di-MeO-PBN (4)	2.46	nd[c]	-2.41	1.39	3.80	nd[c,k]	nd[c,k]	-	nd[c,k]
2,4,6-tri-MeO-PBN (5)	2.36	1.2[b]	-2.56	1.21	3.77	nd[c,k]	nd[c,k]	-	nd[c,k]
PPN	3.01	1.0[d]	-1.96	1.64	3.60	13.5	2.7	38.5	6.369
4-MeO-PPN (6)	2.90	nd[c]	-2.08	1.44	3.52	13.5	2.9	38.8	5.882
EPPN	2.20	1.5[e]	-1.95	1.68	3.63	14.2	3.0	-	1.498
4-MeO-EPPN (7)	2.10	nd[c]	-2.11	1.46	3.57	13.7	3.0	-	0.891

[a] Calculated octanol/water partition coefficient values obtained using ALOGPS 2.1 software (http://www.vcclab.org/lab/alogps/).
[b] Data from Janzen et al.[29]
[c] Not determined.
[d] Data from Roubaud et al.[21]
[e] Data from Roubaud et al.[13]
[f] The peak potentials are given versus a silver wire electrode for a potential scan rate 0.1 V/s.
[g] Containing 0.1 M of TBAP with reduction $E_p(c)$ and oxidation $E_p(a)$ at vitreous carbon Cv electrode.
[h] The stability domain of potentials is given as: $E_p(a)$-$E_p(c)$.
[i] Data from Rosselin et al.[24]
[j] Ratio 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 a ratio k_{PBN}/k_{TN} = 0.069.
[k] The EPR signal of the adduct was too weak to allow a reliable determination of the ratio.

experimental values, this, however, remains within the same range.[13,21]

Cyclic Voltammetry. The electrochemical properties of the seven MeO-derivatives were investigated using cyclic voltammetry, and redox potentials are reported in Table 1. The experiments were performed in acetonitrile containing *tetra*-butylammonium perchlorate (TBAP) as electrolyte. For the sake of comparison, the parent compounds were also analysed. PBN was oxidized through one-electron irreversible transfer and was reduced through a one-step two-electron reduction as previously reported,[30–32] the same behaviour being observed for the other derivatives.

Oxidation and reduction of nitrones were clearly observed, with values ranging from 1.21 to 1.68 V and from -2.56 to -1.95 V, respectively (Figure 2). The oxidation potentials measured for the MeO-derivatives were always lower than that of their parent compounds, showing that the presence of an electron-donating substituent facilitates the oxidation of the nitronyl group in agreement with the literature.[24,30–32] An opposite effect was noted for the reduction, with reduction potentials being higher than that of the parent compound, indicating harder reduction.

For the mono-substituted-PBNs, the *para* derivative (**3**) exhibited the lowest oxidation potential and the highest reduction potential while the *meta* derivative (**2**) exhibited the highest oxidation potential and the lowest reduction potential, however the differences remained below 0.2 V. This indicates that the position of the substituent slightly influences the electrochemical properties of nitrones. The effect was more pronounced when increasing the number of substituents. The oxidation and reduction potentials were respectively decreased and increased, suggesting an additive effect of the substituents. When comparing to the parent compounds, the presence of the diethoxyphosphoryl or the ethoxycarbonyl electron-withdrawing groups facilitates reduction (~ 0.2 V) while slightly impairing oxidation (~ 0.05 V).

Table 1 shows the values of the stability domains of the derivatives, which corresponds to the differences between the reduction and oxidation potentials, namely $E_p(a)$-$E_p(c)$. These values represent the windows of electrochemical potentials over which spin-trapping can be employed. This is of importance when detecting electro-chemically generated radical species. Indeed, inverted spin–trapping may occur and lead to incorrect conclusions. In the inverted spin–trapping process,[33,34] the nitronyl group is oxidized to a radical cation or reduced to a radical anion, which can then react with a nucleophile or an electrophile, respectively, both yielding an aminoxyl radical which does not come from the radical addition onto the nitronyl group. For PBN, the stability domain value was 3.72 V, as previously reported.[24] The mono-substituted derivatives (**1**-**3**) exhibited a slightly smaller potential window whereas the stability domain of the di- and tri-substituted derivatives (**4** and **5**) was slightly larger. For both PPN and EPPN derivatives, the stability domain remains in the same range.

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Hydroxymethyl radical trapping by Electron Paramagnetic Resonance. The spin-trapping properties of the derivatives were studied by evaluating their capacity of trapping of the hydroxymethyl radical ($^{\bullet}CH_2OH$), which is representative of the formation of alcohol-derived radicals during oxidative stress. Oxidative stress leads to overproduction of several reactive oxygen species ($O_2^{\bullet-}$, H_2O_2, $HO^{\bullet}$) which further react with sugars, proteins, lipids and DNA thereby yielding carbon-centered radicals, like hydroxyalkyl radicals.[35] The $^{\bullet}CH_2OH$ radical was generated *in situ* by a standard Fenton system in the presence of the nitrone derivatives noted **N** and the competitive scavenger 1,3,5-tri[(*N*-(1-diethylphosphono)-1-methylethyl) *N*-oxy-aldimine] noted **TN**.[16] For 2,6-di-MeO-PBN (**4**) and 2,4,6-tri-MeO-PBN (**5**) only background noise was observed indicating that these nitrones did not efficiently trap hydroxymethyl radicals. Although we have no strong evidence to discard side-reactions between $^{\bullet}OH$ or $^{\bullet}CH_2OH$ radicals with the methoxy groups of these nitrones, the steric hindrance due to the *ortho* substituents very likely explains the inefficiency of (**4**) and (**5**) to act as spin traps. The five mono-substituted derivatives gave rise to a standard six-line EPR spectrum due to hyperfine couplings of the unpaired electron with nitrogen and β-hydrogen nuclei. For PPN and 4-MeO-PPN (**6**), additional lines due to the coupling with the phosphorous atom were observed. The hyperfine coupling constants (hfcc) are reported in Table 1 and are consistent with PBN-type spin adducts.[36]

The $^{\bullet}CH_2OH$ spin trapping rate was assessed by measuring the relative intensity (as the signal area) of the EPR signals of the **N**-CH_2OH and **TN**-CH_2OH adducts (Figure 3). PPN and 4-MeO-PPN (**6**) were directly tested versus PBN instead of **TN**. The standard kinetic competition model described by Roubaud et al.[16] was employed (equation 1):

$$R/r = 1 + k_N[N]/k_{TN}[TN] \quad (1)$$

Where, k_N and k_{TN} correspond to the second-order rate constants for $^{\bullet}CH_2OH$ trapping by the nitrones **N** and **TN** respectively, while *R* and *r* represent the trapping rate by both **TN** and **N**, and by **TN** only, respectively.

By plotting the *R*/*r* ratio as a function of [**N**]/[**TN**] ratio, a straight line was obtained for each derivative (Figure 3) and five different [**N**]/[**TN**] ratios kept between 2 and 5 were used. In order to compare the spin trapping of MeO derivatives with PBN for nitrones **1**-**3**, EPPN and nitrone **7**, a k_{PBN}/k_{TN} ratio of 0.069 was determined using commercially available PBN instead **N**. From these results, the k_N/k_{PBN} ratio was calculated and the values obtained are reported in Table 1.

All the derivatives tested exhibited lower $^{\bullet}CH_2OH$ radical trapping efficiency than **TN**, which bears three nitronyl groups. For derivatives **1**-**3**, k_N/k_{PBN} values ranged from 0.935 to 1.128, indicating similar rates of trapping than PBN. This suggests that for a given nitrone, the position of the MeO-substituent on the phenyl ring has no significant effect on the trapping of $^{\bullet}CH_2OH$. With regards to the α-substituted derivatives, EPPN trapped $^{\bullet}CH_2OH$ radicals 1.5 times faster than PBN, while PPN exhibited even more improved reactivity, that is, 6.4 times faster trapping. This demonstrates that the introduction of an electron-withdrawing group in α-position of the nitronyl-function improves the spin-trapping properties.

Calculated Nitronyl Atomic Partial Charge and Ionization Potentials. The electronic population analysis within the natural bond orbitals (NBO) framework[37,38] on the nitronyl atoms (H, C, N and O) as well as the IPs of PBN derivatives were calculated in water using the DFT formalism (Table 2). The electronic densities on the nitronyl atoms depend on the position and on the number of MeO substituents as well as on the presence of an electron-withdrawing group in α-position of the nitronyl function. Therefore, two electronic effects are operatively affecting the nitronyl atomic partial charges of the derivatives, the inductive effect of the diethoxyphosphoryl and carbonyl groups at the α position and the mesomeric effect of the methoxy substituents. Using ^{1}H NMR spectroscopy, the chemical shifts of the nitronyl-H in $CDCl_3$ were measured and showed a good correlation with the nitronyl H-atom partial atomic charges (Figure 4A). It is worth mentioning that only 2,6-di-MeO-PBN (**4**) and 2,4,6-tri-MeO-PBN (**5**) were out of the correlation. This correlation confirms the polar effect of the substituents both on the aromatic ring and in α-position of the nitronyl function, however with subtler effects in case of poly-

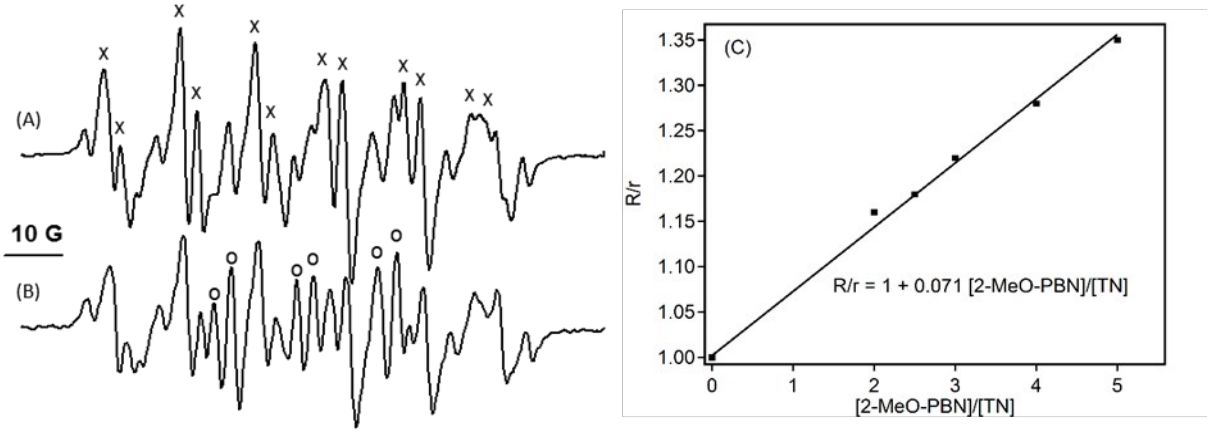

Figure 3. (A)-(B) EPR signals of TN and 2-MeO-PBN (**1**) hydroxymethyl radical adducts, respectively. Hydroxymethyl radical was generated by a Fenton system and the ratio [**1**]/[TN] was: (A) [**1**]/[TN] = 2; (B) [**1**]/[TN] = 5. The peaks topped by a cross (×) correspond to the hydroxymethyl radical adduct of TN and those topped by a round (○) to the hydroxymethyl radical adduct of 1. (C) Determination of the relative rate constant k_N/k_{TN} of $^{\bullet}CH_2OH$ trapping by nitrone **1** (R^2 = 0.999).

substitution likely due to the compensation of each electronic effect over the whole charge delocalization.

Table 2. Calculated Atomic Partial Charges of the Nitronyl Atoms and Ionization Potentials of MeO-Derivatives at the (CPCM)/M06-2X/6-31+g(d,p) Level of Theory.

Compounds	Atomic partial charges of the nitronyl atoms (*e*)				IP (eV)
	H	C	N	O	
PBN	0.254	0.019	0.080	-0.636	6.0
2-MeO-PBN (1)	0.262	0.009	0.084	-0.633	5.6
3-MeO-PBN (2)	0.255	0.021	0.081	-0.635	6.0
4-MeO-PBN (3)	0.253	0.028	0.069	-0.651	5.7
2,6-di-MeO-PBN (4)	0.261	0.018	0.085	-0.638	5.7
2,4,6-tri-MeO-PBN (5)	0.259	0.036	0.075	-0.644	5.4
PPN	0.258	0.033	0.073	-0.628	6.0
4-MeO-PPN (6)	0.254	0.056	0.056	-0.644	5.6
EPPN	0.250	0.020	0.084	-0.610	6.0
4-MeO-EPPN (7)	0.248	0.031	0.072	-0.626	5.7

Since nitrones have a similar reactivity than carbonyl groups allowing nucleophilic addition reactions, the electronic population of the nitronyl-carbon provides hints about the reactivity of the nitronyl function. For the *ortho*-derivative (**1**), the nitronyl C-atomic partial charge is close to zero, being the lowest over the series. This suggests that this position would be mildly reactive toward a nucleophilic attack. Conversely, for 4-MeO-PPN (**6**), both *para* electron-donating MeO substituent and *α*-position electron-withdrawing diethoxyphosphoryl group lead to synergic electronic effects. This suggests that it would be highly reactive toward a nucleophilic attack, as shown by the nitronyl-C atomic partial charge which was the most positive of the series. While plotting the relative rate constants of $^{\bullet}CH_2OH$ addition to nitrones versus the nitronyl-C atomic partial charge (Figure 4B), a good correlation for PBN, PPN and EPPN was observed (R^2 = 0.9996), showing that the more positive the nitronyl-C atomic partial charge, the faster the addition reaction. Our experimental values therefore indicate that electron withdrawing inductive effect on the *N-tert* butyl group increases the reactivity of the nitronyl group towards $^{\bullet}CH_2OH$ radical. This is supported by the nucleophilic nature of the $^{\bullet}CH_2OH$ radical.[39] However when adding the MeO-derivatives, the positive mesomeric effects on the aromatic ring tend to decrease the reactivity and no correlation with the nitronyl-C atomic partial charge was observed, suggesting that not only electronic factors are involved in the trapping reaction of $^{\bullet}CH_2OH$ with the nitronyl group. This points out the need for further studies to determine how these two effects alter the reactivity of the nitronyl group and which of the two is more prominent.

Given the chemical accuracy of IP calculations at the DFT level of theory (ca. 0.1 eV), the following ranking was obtained (See Table 3): 2,4,6-tri-MeO-PBN (**5**) < 4-MeO-PPN (**6**) ≈ 2-MeO-PBN (**1**) ≈ 4-MeO-PBN (**3**) ≈ 2,6-di-MeO-PBN (**3**) ≈ 4-MeO-EPPN (**7**) < PBN ≈ EPPN ≈ PPN ≈ 3-MeO-PBN (**2**). This ranking is in excellent agreement with experimentally observed oxidation potentials

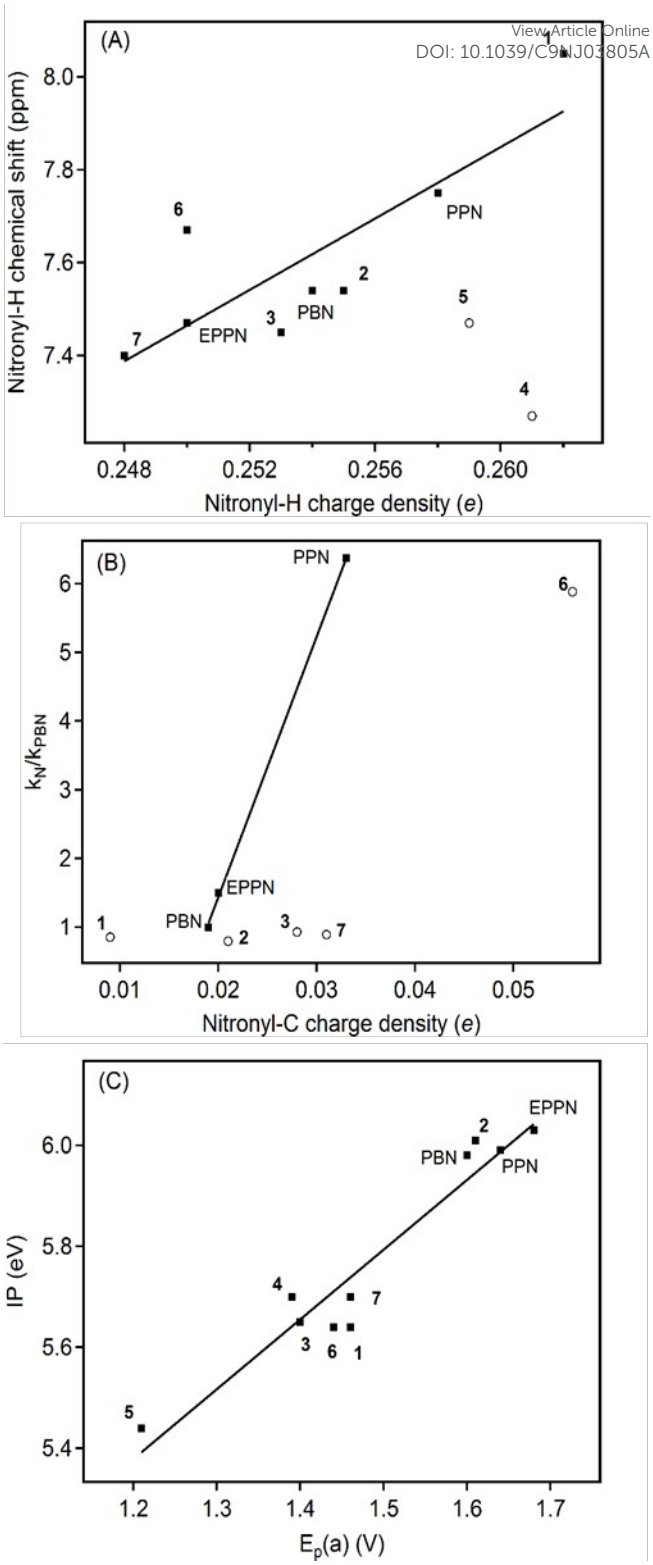

Figure 4. (A) Correlation of nitronyl-H chemical shifts with nitronyl-H charge densities (R^2 = 0.852 excluding the outliers 2,6-di-MeO-PBN and 2,4,6-tri-MeO-PBN marked as (○)); (B) Plots of experimental relative rate constants of hydroxymethyl addition to nitrones with nitronyl-C charge density; (C) Correlation of ionization potential with oxidation potential of nitrones (R^2 = 0.926).

(See Figure 4C); the lower the IP, the lower the oxidation potential. For instance, the highest calculated IP value was observed for EPPN, which also exhibits the highest oxidation

potential. Such strong correlation between IP and oxidation potential (R^2 = 0.926, Figure 4C) clearly suggests that a computational approach might (i) predict the electrochemical properties of nitrones but also (ii) provide hints in order to tune oxidation properties of nitronyl derivatives. For example, the presence of electron-withdrawing group in α-position of the nitronyl moiety does not modify the stability of the radical cation formed after electron transfer event, as depicted by similar spin density distributions (Figure 5). Likewise, *meta*-substitution by donating moieties (*e.g.*, MeO) does not stabilize radical cation forms. However, the significant increase of π-delocalization over the aromatic ring strongly stabilizes the radical cation, that in turn decreases IP and thus the oxidation potential.

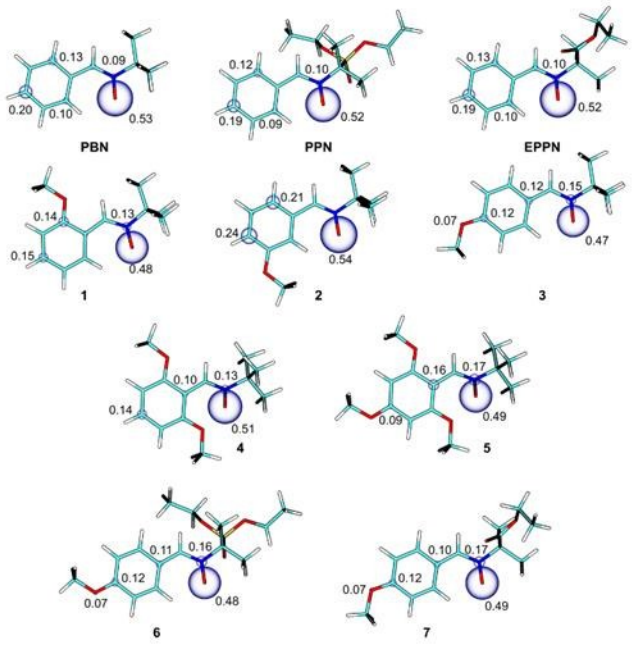

Figure 5. Mulliken spin density distribution of the radical cations formed after single electron abstractions.

Conclusion

In this work, we have studied the effect of the position and the number of the electron-donating MeO-substituents on the reactivity of phenyl nitrones. A series of mono-substituted (*ortho*-, *meta*- and *para*-) and di- and tri-substituted derivatives of PBN were synthesized as well as the 4-MeO-derivatives of PPN and EPPN. The substituent effect was also demonstrated by a computational approach, from which a correlation between ^{1}H NMR chemical shifts and nitronyl-H atomic partial charges of the derivatives was obtained. Cyclic voltammetry experiments showed that the redox potentials depend on the position and on the number of substituents. While electron-donating groups on the phenyl ring tend to favour oxidation compared to unsubstituted derivatives, electron-withdrawing groups in α-position on the nitronyl function slightly impair oxidation. An opposite trend was obviously demonstrated for the reduction of the nitronyl function. With regard to the number of substituents, the electronic effects appeared additive. The computed Ionisation Potentials were found to correlate with the experimental oxidation potentials, suggesting that a computational approach may predict the electrochemical properties of nitrones. The reactivity towards $^{\bullet}CH_2OH$ radical trapping was correlated with the electron-withdrawing nature of the substituent in α-position on the nitronyl function, with the PPN being the most effect trap, supporting the nucleophilic nature of $^{\bullet}CH_2OH$. However, when adding MeO-substituents, the rates of trapping were significantly lowered, with an absence of correlation with the nitronyl-C atomic partial charges. In addition, with the di-*ortho* substituted derivatives no trapping was observed. This suggests that not only electronic factors are involved in the spin trapping reaction and therefore this warrants further investigation.

Experimental Section

Synthesis. All reagents were from commercial sources and were used as received. All solvents were distilled and dried according to standard procedures. The TLC analysis was performed on aluminium sheets coated with silica gel (40–63 µm). Compound detection was achieved either by exposure to UV light (254 nm) and/or by spraying a 5% sulfuric acid solution in ethanol or a 2% ninhydrin solution in ethanol and then by heating up to ~150 °C. Flash column chromatography was carried out on silica gel (40–63 µm). Melting points have not been corrected. NMR spectra were recorded on a Bruker AC400 at 400, 100 and 162 MHz for ^{1}H, ^{13}C and ^{31}P experiments, respectively. Chemical shifts are given in ppm relative to the solvent residual peak as a heteronuclear reference for ^{1}H and ^{13}C. Abbreviations used for signal patterns are as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; td, triplet of doublets; q, quartet; m, multiplet. HR-MS spectra were recorded on a mass spectrometer equipped with a TOF analyzer for ESI+ experiments.

***N*-*tert*-butyl-α-(2-methoxy)phenylnitrone (1).** In an argon atmosphere and under stirring, o-anisaldehyde (1.22 g, 9.0 mmol, 1.0 equiv), 2-methyl-2-nitropropane (1.9 mL, 17.9 mmol, 2.0 equiv) and AcOH (3.1 mL, 54.0 mmol, 6.0 equiv) were dissolved in dry EtOH. The mixture was cooled down to 0 °C, then zinc powder (2.4 g, 36.0 mmol, 4.0 equiv) was slowly added in order to keep the temperature below 15 °C. The mixture was stirred at room temperature for 30 min then heated at 60 °C in the dark for 22h in the presence of molecular sieves (4Å). The reaction mixture was filtered off through a pad of Celite, and the solvent was removed under vacuum. The crude mixture was purified by flash chromatography (EtOAc/cyclohexane, 2/8) followed by two successive crystallisations from EtOAc/*n*-hexane to afford 1.70 g of the title compound (8.2 mmol, yield 92%) as a white powder. R_f (EtOAc/cyclohexane, 2/8) = 0.16. ^{1}H NMR (400 MHz, $CDCl_3$,) δ 9.36 (1H, dd, *J* = 8.0 and 1.2 Hz), 8.05 (1H, s), 7.35 (1H, td, *J* = 8.4 and 2.0 Hz), 7.02 (1H, t, *J* = 7.6 Hz), 6.88 (1H, d, *J* = 8.2 Hz), 3.86 (3H, s), 1.61 (9H, s); ^{13}C NMR (100

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MHz, $CDCl_3$) δ 157.2, 131.3, 128.8, 124.5, 120.9, 120.2, 109.7, 71.1, 55.7, 28.5. The spectral data was in agreement with the literature.[40]

***N-tert*-butyl-α-(3-methoxy)phenylnitrone (2).** The synthetic procedure was essentially the same as for compound **1**. m-anisaldehyde (1.39 g, 10.2 mmol) and 2-methyl-2-nitropropane (2.2 mL, 20.4 mmol) were used as starting materials. The reaction time was 24h. The crude mixture was purified by flash chromatography (EtOAc/cyclohexane, 4/6) followed by two successive crystallisations from EtOAc/*n*-hexane to afford 1.76 g of the title compound (8.5 mmol, yield 83%) as a white powder. R_f (EtOAc/cyclohexane, 4/6) = 0.28. ^{1}H NMR (400 MHz, $CDCl_3$,) δ 8.34 (1H, s), 7.54 (1H, s), 7.48 (1H, d, *J* = 7.6 Hz), 7.31 (1H, t, *J* = 8.0 Hz), 6.97 (1H, dd, *J* = 8.0 and 2.4 Hz), 3.86 (3H, s), 1.62 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.7, 132.4, 130.3, 129.3, 122.1, 117.6, 112.3, 71.1, 55.5, 28.5. The spectral data were in agreement with the literature.[41]

***N-tert*-butyl-α-(4-methoxy)phenylnitrone (3).** The synthetic procedure was essentially the same as for compound **1**. p-anisaldehyde (1.42 g, 10.4 mmol) and 2-methyl-2-nitropropane (2.3 mL, 20.8 mmol) were used as starting materials. The reaction time was 26h. The crude mixture was purified by flash chromatography (EtOAc/cyclohexane, 5/5) followed by two successive crystallisations from EtOAc/*n*-hexane to afford 1.70 g of the title compound (8.2 mmol, yield 79%) as a white powder. R_f (EtOAc/cyclohexane, 5/5) = 0.18. ^{1}H NMR (400 MHz, $CDCl_3$,) δ 8.27 (2H, d, *J* = 9.2 Hz), 7.45 (1H, s), 6.91 (2H, d, *J* = 8.0 Hz), 3.83 (3H, s), 1.59 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.9, 130.8, 129.5, 124.2, 113.8, 70.2, 55.4, 28.4. The spectral data were in agreement with the literature.[40]

***N-tert*-butyl-α-(2,6-dimethoxy)phenylnitrone (4).** The synthetic procedure was essentially the same as for compound **1**. 2,6-dimethoxybenzaldehyde (890 mg, 5.36 mmol) and 2-methyl-2-nitropropane (1.2 mL, 10.72 mmol) were used as starting materials. The reaction time was 21h. The crude mixture was purified by flash chromatography (EtOAc/MeOH, 98/2) followed by two successive crystallisations from EtOAc/*n*-hexane to afford 944 mg of the title compound (3.98 mmol, yield 74%) as a white powder. R_f (EtOAc/MeOH, 95/5) = 0.32. ^{1}H NMR (400 MHz, $CDCl_3$,) δ 7.54 (1H, s), 7.27 (1H, t, *J* = 8.4 Hz), 6.55 (2H, d, *J* = 8.4 Hz), 3.82 (6H, s), 1.61 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 130.7, 124.9, 109.1, 104.0, 70.1, 56.1, 28.5. HR-MS (ESI+, m/z) calcd for $C_{13}H_{20}NO_3$ [$(M + H)^+$] 238.1443, found 238.1449.

***N-tert*-butyl-α-(2,4,6-trimethoxy)phenylnitrone (5).** Under argon atmosphere and stirring, 2,4,6-trimethoxybenzaldehyde (1.01 g, 5.17 mmol, 1.0 equiv) and *N*-(*tert*-butyl)hydroxylamine acetate (770 mg, 5.17 mmol, 1.0 equiv) were dissolved in dry DCM (0.33 M). Pyrrolidine (0.5 mL, 6.20 mmol, 1.2 equiv) was added and the mixture was stirred at room temperature for 1h. The solvent was removed under vacuum and the crude mixture was purified by flash chromatography (EtOAc/MeOH, 95/5) followed by two successive crystallisations from EtOAc/*n*-hexane to afford 0.81 g of the title compound (3.03 mmol, yield 59%) as a white powder. R_f (EtOAc/MeOH, 95/5) = 0.26. ^{1}H NMR (400 MHz, $CDCl_3$,) δ 7.47 (1H, s), 6.12 (2H, s), 3.81 (3H, s), 3.80 (6H, s), 1.59 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 159.7, 124.7, 102.4, 91.0, 69.8, 56.0, 55.6, 28.6. The spectral data were in agreement with the literature.[42]

***N*-2-(diethoxyphosphoryl-propyl)-α-(4-methoxy)phenylnitrone (6).** Under argon atmosphere and stirring, *p*-anisaldehyde (565 mg, 4.15 mmol, 1.0 equiv) and diethyl [1-(hydroxyamino)-1-methylethyl]phosphonate[20] (876 mg, 4.15 mmol, 1.0 equiv) were dissolved in dry EtOH. The mixture was stirred at 60 °C for 22h. The solvent was removed under vacuum and the crude mixture was purified by flash chromatography (EtOAc/cyclohexane, 9/1) to afford 1.27 g of the title compound (3.86 mmol, yield 93%) as a colorless oil. R_f (EtOAc/cyclohexane, 9/1) = 0.22. ^{1}H NMR (400 MHz, $CDCl_3$,) δ 8.27 (2H, d, *J* = 8.8 Hz), 7.67 (1H, d, *J* = 2.8 Hz), 6.92 (2H, d, *J* = 9.2 Hz), 4.23-4.19 (4H, m), 3.84 (3H, s), 1.82 (6H, d, *J* = 14.8 Hz), 1.32 (6H, t, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.3, 133.1 (d, *J* = 4 Hz), 131.2, 123.9 (d, *J* = 2 Hz), 113.9, 72.3 (d, *J* = 153 Hz), 63.4 (d, *J* = 7 Hz), 55.5, 23.4, 16.6 (d, *J* = 6 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.38. HR-MS (ESI+, m/z) calcd for $C_{15}H_{25}NO_5P$ [$(M + H)^+$] 330.1470, found 330.1469.

***N*-2-(ethoxycarbonyl-propyl)-α-(4-methoxy)phenylnitrone (7).** Under argon atmosphere and stirring, ethyl 2-methyl-2-nitropropanoate[23] (2.00 g, 12.42 mmol, 1.0 equiv), p-anisaldehyde (1.8 mL, 14.90 mmol, 1.2 equiv) and NH_4Cl (930 mg, 17.39 mmol, 1.4 equiv) were dissolved in H_2O/MeOH (6/4, v/v). The mixture was cooled down to 0 °C, then zinc powder (1.61 g, 24.84 mmol, 2.0 equiv) was slowly added to keep the temperature below 15°C. The mixture was stirred at room temperature for 1h then heated at 60 °C in the dark for 18h. The reaction mixture was filtered off through a pad of Celite, and the solvent was removed under vacuum. The crude mixture was purified by flash chromatography (EtOAc/cyclohexane, 5/5) followed by two successive crystallisations from EtOAc/*n*-hexane to afford 1.49 g of the title compound (5.62 mmol, yield 45%) as a white powder. R_f (EtOAc/cyclohexane, 5/5) = 0.26. ^{1}H NMR (400 MHz, $CDCl_3$,) δ 8.27 (2H, d, *J* = 8.8 Hz), 7.40 (1H, s), δ 6.93 (2H, d, *J* = 8.8 Hz), 4.25 (2H, q, *J* = 7.2 Hz), 3.85 (3H, s), 1.81 (6H, s), 1.27 (3H, t, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 161.4, 131.2, 131.0, 123.5, 114.0, 76.5, 62.2, 55.5, 24.6, 14.1. HR-MS (ESI+, m/z) calcd for $C_{14}H_{20}NO_4$ [$(M + H)^+$] 266.1392, found 266.1398.

Determination of ClogP Values. The partition coefficient octanol/water (ClogP) was determined by using ALOGPS 2.1 software, which is available at www.vcclab.org/lab/alogps/.

Cyclic Voltammetric Measurements. The electrochemical experiments were carried out using a three-electrode cell under a dry argon atmosphere at room temperature. A silver wire electrode was used as the reference electrode and a platinum wire as the auxiliary electrode. The working electrode (glassy

carbon) was polished prior to each experiment using a 0.04 µm aqueous alumina slurry on a wetted polishing cloth.

EPR Measurements and Spin-Trapping Kinetics. EPR measurements were carried out on a Bruker EMX spectrometer operating at X-band with 100 kHz modulation frequency. The general instrument settings used for spectral acquisition were as follows: microwave power, 20 mW; modulation amplitude, 1 G; received gains, 9×10^1 to 9×10^2; scan time, 5 s; sweep width, 100 G. Spectra were recorded at room temperature, and measurements were performed using a capillary tube. The spectrum simulation was carried out using the WINSIM program,[43] available as free software from Public Electron Paramagnetic Resonance Software Tools (http://www.niehs.nih.gov/research/resources/software/tox-pharm/tools/).

The solvents were of the highest purity grade and used without further purification. The trinitrone TN was synthesized and purified as previously described.[16] To generate the hydroxymethyl ($^{\bullet}CH_2OH$) radical, nitrone (100 mM) was dissolved in a Fenton system containing hydrogen peroxide (6%) and iron (II) sulfate (6 mM) in methanol. The method of kinetic competition permitted to evaluate the ratio of the second-order rate constants for the trapping of $^{\bullet}CH_2OH$ by one of the nitrone N of interest (k_N, corresponding to the compounds **1**–**7**) and TN (k_{TN}) or PBN (k_{PBN}) used as competitive inhibitors. Then, the commercially available PBN was tested versus TN to determine the ratio of the rate constants for the trapping of $^{\bullet}CH_2OH$ by PBN and by TN: *i.e.*, k_{PBN}/k_{TN} for nitrones **1**-**5** and **7**. The concentrations of the various nitrones were varied to keep the [N]/[TN] ratio between 2 and 5. For each nitrone, at least five experiments were repeated twice. In each case, a series of 10 EPR spectra was then recorded (scan time for a single spectrum; 20 s). The signal-to-noise ratio was improved using a SVD procedure, as described elsewhere.[44] The signal recorded exactly 2 min after the beginning of the reaction was then simulated using the WinSim software to determine the relative areas of the **N**-CH_2OH and **TN**-CH_2OH adducts. In this approach, the R/r ratio was evaluated as follows:

$$\frac{R}{r} = \frac{area\ of\ N{-}R\ signal + area\ of\ TN{-}R\ signal}{area\ of\ TN{-}R\ signal} \qquad (2)$$

General Computational Methods. Geometries and energies were optimized using DFT formalism with the hybrid meta-GGA (Generalized Gradient Approximation) M06-2X functional and the 6-31+G(d,p) basis set. Three other hybrid functionals were tested on PBN, taken as a reference compound, namely B3LYP, B3P86 and PBE1PBE (data not shown). No differences were observed in terms of atomic charges, one of the major parameters under scrutiny in this work, hence the M06-2X functional was chosen for all compounds, as a good standard.[45] The use of larger basis set (namely 6-311+g(2d,3pd)) did not exhibit significant differences in terms of atomic charges with respect to 6-31+G(d,p), suggesting the absence of basis set effect owing to the relatively small molecular size of the series. Therefore, the 6-31+G(d,p) basis set was used for all compounds. A frequency analysis was systematically performed at 298 K and 1 atm, at the M06-2X/6-31+G(d,p) level of theory confirming that optimized geometries at in a potential energy surface minimum owing to the absence of any imaginary frequency. Solvent (water) effects were considered by using the C-PCM (conductor-like polarizable continuum model) solvation model.[46,47] In continuum models, the solute is embedded into a shape-adapted cavity surrounded by a dielectric continuum characterized by its dielectric constant. Calculations were performed in water (ε = 78.35). The atomic charge analysis was performed using the natural population analysis (NPA) within the NBO (Natural Bond Orbital) framework. The IP was calculated as follows:

IP = E($ArNO^{\bullet+}$) – E(ArNO), where $ArNO^{\bullet+}$ is the radical cation obtained after electron withdraw from the neutral form of the PBN derivatives, named here ArNO. It is worth mentioning that IP corresponds to the adiabatic energy of electron withdraw energy, *i.e.*, considering the radical cation energy using the neutral form geometry. All calculations were performed with Gaussian 09 Rev. A2.[48]

Conflicts of interest

There are no conflicts to declare.

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MeO-derivatives of phenyl nitrones were synthesized and their electrochemical and spin-trapping properties were studied.