European Journal of Medicinal Chemistry 58 (2012) 44–49

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Microwave-assisted synthesis of hydroxyphenyl nitrones with protective action against oxidative stress

Cecilia Chavarría [a,b], Daniel I. Perez [c], Concepción Pérez [c], Jose A. Morales Garcia [d,e], Sandra Alonso-Gil [d,e], Ana Pérez-Castillo [d,e], Carmen Gil [c], José M. Souza [b,**], Williams Porcal [a,*]

[a] Grupo de Química Medicinal, Laboratorio de Química Orgánica, Facultad de Ciencias-Facultad de Química, Universidad de la República, Iguá 4225, 11400 Montevideo, Uruguay
[b] Departamento de Bioquímica and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Av. Gral. Flores 2125, 11800 Montevideo, Uruguay
[c] Instituto de Química Médica (IQM-CSIC), Juan de La Cierva 3, 28006 Madrid, Spain
[d] Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC), Arturo Duperier 4, 28029 Madrid, Spain
[e] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain

ARTICLE INFO

Article history:
Received 28 July 2012
Received in revised form
26 September 2012
Accepted 27 September 2012
Available online 5 October 2012

Keywords:
Hydroxyphenyl nitrones
Microwave-assisted synthesis
Alpha-synuclein
SH-SY5Y human neuroblastoma cells
Primary neural cell

ABSTRACT

Oxidative stress plays an important role in neuronal death in neurodegenerative disorders such as Parkinson's disease (PD). Hydroxyphenyl nitrones, derivatives of the nitrone spin trap alpha-phenyl-*N*-*tert*-butylnitrone (PBN), were synthesized and their antioxidant, anti-inflammatory and neuroprotective activity in neural cells evaluated. These hydroxyphenyl nitrones **5**–**7** were synthesized by reaction of the corresponding hydroxybenzaldehyde with *N*-*tert*-butyl hydroxylamine under microwave irradiation. They showed good peroxyl free radical scavenger capacities, analyzed by oxygen radical absorbance capacity (ORAC). Also inhibited peroxynitrite-mediated tyrosine nitration of alpha-synuclein *in vitro* and protected human neuroblastoma (SH-SY5Y) cells against SIN-1 and 6-OHDA toxicity when micromolar concentrations were used. Besides, the hydroxyphenyl nitrones evaluated showed anti-inflammatory activity modulating nitrite production in primary neural cell cultures of astrocytes and microglia treated with lipopolysaccharide (LPS), a potent inflammatory agent. These experimental data suggest a potential therapeutic use of these hydroxyphenyl nitrones against oxygen and nitrogen reactive species involved in neurodegenerative pathology.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder involving the progressive degeneration of dopamine neurons arising in the substantia nigra (SN). Although PD has been heavily researched in the last decades, the precise etiology of the disease is still unknown. However, research in recent years has provided substantial evidence supporting the hypothesis that oxidative stress plays a major role in disease pathogenesis [1–3]. The generation of reactive oxygen (ROS) and nitrogen species (RNS), and oxidative damage in the central nervous system (CNS) are major events occurring in many neurodegenerative disorders, besides Parkinson's disease (PD) including Alzheimer's disease, amyotrophic lateral sclerosis, as well as in the aging processes [4–6]. It is suggested that biological oxidation in the human body generates highly pathogenic ROS and RNS such as hydroxyl free radical (•OH), superoxide anion ($O_2^{\bullet -}$), nitric oxide (•NO), peroxynitrite ($ONOO^-$) and lipid peroxide free radicals (ROO•), causing cellular injury [7–9]. Nitric oxide also reacts with superoxide anion radical to produce significant amounts of peroxynitrite ($ONOO^-$), a potent oxidizing agent that can cause DNA fragmentation, protein oxidation and nitration and lipid peroxidation [10]. Although the body has developed several defense mechanisms to counteract oxidative stress, the brain appears to be more susceptible to this damage than any other organ. Studies in Parkinson proposed a possible role for ROS and/or RNS in selective loss of SN dopaminergic neurons in the disease [11,12]. Enhancing antioxidant and radical scavenger capabilities in the SN therefore constitutes a rational approach to prevent or slow ongoing damage of dopaminergic neurons. In the last decade, nitrone spin traps, α-phenyl-*N*-*tert*-butylnitrone (PBN, Fig. 1) and structurally related compounds, have shown their utility in the treatment of neurodegenerative diseases as well as in the prolongation of life span due to their neuroprotective effects [13,14]. Neuroprotective

* Corresponding author. Tel.: +598 2525 8618/216; fax: +598 2525 0749.
** Corresponding author. Tel.: +598 2924 9562; fax: +598 2924 9563.
E-mail addresses: jsouza@fmed.edu.uy (J.M. Souza), wporcal@fq.edu.uy (W. Porcal).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.09.044

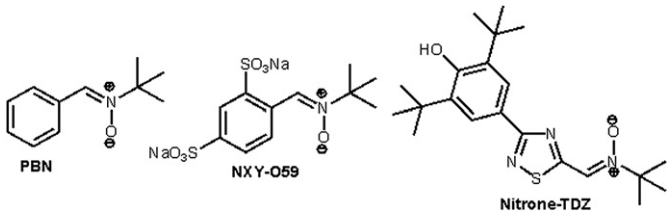
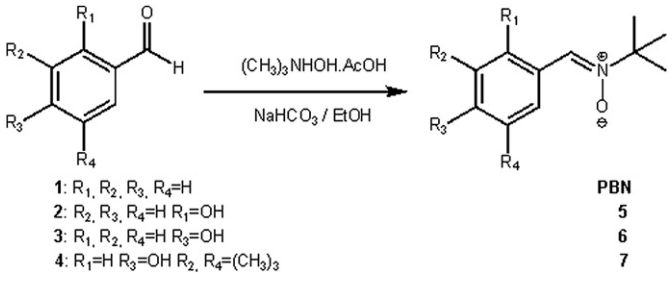


Fig. 1. Chemical structure of PBN, NXY-059 and Nitrone-TDZ.

mechanism of action postulated for PBN involved its ability to inhibit signal transduction processes such as suppression of proinflammatory cytokines, genes, and mediators associated with enhanced neuroinflammatory processes [15]. In an effort to optimize the biological profile of PBN, a wide structural diversity of PBN-like nitrones has been designed and synthesized [16]. The nitrone NXY-059 (Fig. 1) was evaluated in clinical trials phase III but unfortunately no positive effects was found in ischemic stroke patients [17]. Despite this clinical result, the hypothesis of using nitrones as neuroprotective agents remains viable. In particular, a phenolic nitrone-TDZ (Fig. 1) showed excellent free radical scavenger capacity and good neuroprotective effects without cellular toxicity [15]. Furthermore, recently experimental *in vitro* antioxidant properties, as well as preliminary *in vivo* pharmacological activities of nitrone derivatives of Trolox (α-tocoferol derivative) were reported, showing that nitrones bearing free phenol groups exerted the best antioxidant values [17,18]. Following our ongoing research on nitrones with neuroprotective properties, we proposed the synthesis of new molecules keeping the nitrone moiety, but combining it with antioxidant fragment able to stabilize the generated free radical [15,19]. Thus we described here an efficient and rapid synthetic route to phenolic PBN analogs based on microwave irradiation. The hydroxyphenyl nitrones developed were evaluated as antioxidant and neuroprotective agents on a human neuroblastoma (SH-SY5Y) cell model as well as anti-inflammatory agents in primary cultures of neural cells.

2. Chemistry

Although several methods have been reported to prepare nitrones [19,20], the selected hydroxyphenyl nitrones **5**–**7** (Scheme 1) and PBN were synthesized through the condensation between the corresponding phenolic aldehyde and *N*-*tert*-butyl hydroxylamine. Previous studies indicate that aromatic aldehydes with electron donors groups react slower than those substituted by electron withdrawing groups with *N*-*tert*-butyl hydroxylamine [20–22]. The main disadvantage of this synthesis is the use of 2 equiv of *N*-*tert*-Butyl hydroxylamine, an expensive reagent, and long reaction times to go to completion [21,23]. In this context, in recent years the microwave assisted organic synthesis has been



Scheme 1. Synthetic methodology of hydroxyphenyl nitrones **5-7** and PBN.

widely used especially applicable to small organic molecules synthesis [23,24]. Frequent issues such as time consumption and excess use of reagents found during traditional synthesis, can be solved with microwave heating application. Based on this, a comparison between yields obtained with conventional procedure and microwave heating were done (Table 1). In both cases the yields obtained for the desired nitrones were similar, but under microwave irradiation the reactions time were shorter. Aldehydes **3** and **4** (Scheme 1) were less reactive and the formation of the nitrones **6** and **7** required reflux in EtOH for several hours (Table 1, entries e and g). With the procedure here described, the reactions studied were very fast, with no need to use an excess of the expensive hydroxylamines and several hours refluxing to obtain good yields. A significant problem associated with many reactive nitrones is dimerization. No dimerization products were obtained under our reaction conditions. Therefore, the procedure is significantly more energy-efficient than conventional heating with enhanced reaction rates and higher purity of final products. Known nitrones **5**, **6** and PBN showed spectroscopic data in good agreement with those reported in the literature [20–22], while structures of new nitrone **7** were confirmed unequivocally using analytical and spectroscopic techniques. The proton and carbon assignment of the different nitrones was confirmed by ^{1}H NMR, COSY, and HSQC, HMBC spectra. The ^{1}H NMR analysis confirmed that only one stereoisomer was formed in all cases. The *Z*-configuration of nitrones synthesized was confirmed by NOE experiments.

3. Results and discussion

3.1. Antioxidant capacity of hydroxyphenyl nitrones

The antioxidant activity of the hydroxyphenyl nitrones **5**–**7** synthesized was determined *in vitro* by using the oxygen radical absorbance capacity (ORAC) assay. This assay measures peroxyl free radical scavenger compounds capability and uses fluorescein (FL) as the fluorescent probe [15]. Results of the experiment were expressed as Trolox equivalents (μmol of Trolox equivalents per μmol of tested compound) (Table 2). The peroxyl free radical scavenger properties of the hydroxyphenyl nitrones **5**–**7** were better than Trolox. The 4-hydroxyphenyl nitrone **6** showed the highest $ORAC_{FL}$ value (3.2 Trolox equiv), whereas the parent nitrone PBN showed the lowest $ORAC_{FL}$ value (0.014 Trolox equiv).

3.2. Hydroxyphenyl nitrones prevent protein nitration

Due to the ability of the hydroxyphenyl nitrones **5**–**7** to act as scavengers of free radicals, we next evaluated their ability to prevent protein nitration. The formation of 3-nitrotyrosine (3-NT) in proteins occurs as an *in vivo* posttranslational modification with

Table 1
Synthesis of nitrones **5**–**7** and PBN, conditions and yields.

Entry	Nitrone	Conditions		Yields (%)
		Temperature (°C)	Time (min)	
a	PBN	60	180	64
b		100[a]	10	72
c	**5**	78	180	78
d		100[a]	10	90
e	**6**	78	1800	42
f		i) 100[a]	10	35
		ii) 150[a]	30	
g	**7**	78	180	49
h		110–120[a]	20	47

[a] Heating under microwave irradiation.

Table 2
Antioxidant capacity and permeability assay.

Nitrone	ORAC (Trolox equiv)[a]	Permeability (P_e)[b]
PBN	0.014 ± 0.001	Nd
5	2.6 ± 0.2	37.5 ± 1.3 (CNS+)
6	3.2 ± 0.3	0.2 ± 0.1 (CNS−)
7	2.0 ± 0.1	10.2 ± 0.9 (CNS+)

nd: not determinate.
[a] Data are expressed as μmol of Trolox equiv/μmol of tested nitrones and are the mean ($n = 3$) ± SD.
[b] Prediction of the brain penetration using PAMPA-BBB assay (CNS+: compounds able to cross the BBB).

important pathophysiological consequences. Elevated levels of 3-NT have been detected in a myriad of pathological conditions including inflammatory, neurodegenerative, and cardiovascular disorders, among others [25,26]. Western blot analysis was employed to demonstrate the ability of these nitrones to prevent peroxynitrite-dependent tyrosine nitration of alpha-synuclein (αS). αS is a major fibrillar component of Lewy bodies and the aggregation of this protein represents a critical step in the pathogenesis of Parkinson's disease (PD) and other α-synucleinopathies. It has been demonstrated that exposure of human recombinant αS to nitrating agents induces formation of nitrated αS monomers and oligomers [26]. The nitrated oligomers are highly stabilized due to covalent cross-linking via the oxidation of tyrosine to form o,o-dityrosine [26,27]. An *in vitro* screening of hydroxyphenyl nitrones **5**–**7** was performed to evaluate them as potential inhibitors of the nitration and oxidation of αS (Fig. 2). The bands at about 15 kDa correspond to αS monomers whereas the 34 kDa bands indicate the presence of nitrated and aggregated forms of the protein. Derivatives of phenolic nitrones with the hydroxyl group in *para* position to the nitrone moiety (compounds **6**–**7**), show the best behavior preventing αS tyrosine nitration and also the formation of high molecular weight aggregates of αS. Using densitometry analysis in anti-nitrotyrosine western blots a dose–response curve of inhibition of nitration of αS by nitrone **6** was built. The treated αS with compound **6** markedly inhibited the nitration in a dose-dependent manner (see Supplementary data). However, these properties of nitrones as agents that prevent αS nitration could be extended to other proteins. Thus the activity of hydroxyphenyl nitrones **6**–**7** to prevent peroxynitrite-dependent tyrosine nitration was performed using western blot analysis. Small proteins, with tyrosine residues exposed to the solvent and accessible for modification by nitrating agents, like cytochrome c and RNAse A were used (Fig. 3). Results shown that, the hydroxyphenyl nitrones also prevent peroxynitrite-dependent protein tyrosine nitration of cytochrome c and RNAse A. Based on these results, we can extrapolate the antioxidants properties of the hydroxyphenyl nitrones **6**–**7** to other protein and not only to αS.

Nitration of tyrosine primarily occurs via attack by the $^{\bullet}NO_2$ radical that originates from the decomposition of peroxynitrite [9]. Hydroxyphenyl nitrone **6** can prevent peroxynitrite-dependent protein nitration through different mechanisms. It could react and form a spin adduct with the radical derived from the decomposition of $ONOO^-$, react directly with $ONOO^-$ or reduce the tyrosyl radical in proteins. We assumed that the first hypothesis is the most plausible because the inhibition of protein tyrosine nitration by the nitrone it is similar in different proteins and phenolic groups did not react with $ONOO^-$ directly, they react with the radicals generated by $ONOO^-$ homolysis [28]. Through western blot analysis we demonstrate that nitrone **6** reacts with $^{\bullet}OH$, $CO_3^{\bullet-}$ and $^{\bullet}NO_2$ radicals (not shown). To investigate the possible mechanism by which hydroxyphenyl nitrones prevent peroxynitrite-dependent protein nitration compound **6** was treated with peroxynitrite ($ONOO^-$) and the product analyzed by HPLC. Peroxynitrite homolysis (in the absence of bicarbonate) results in equals amounts of $^{\bullet}OH$ and $^{\bullet}NO_2$. To especifically evaluated the reaction of nitrone **6** and $^{\bullet}NO_2$ radical the reaction mixture was preincubated with an excess of sodium nitrite (4 mM), which was rapidly oxidized by $^{\bullet}OH$ to $^{\bullet}NO_2$ [29]. An additional peak when nitrone **6** reacts with $ONOO^-$ as an indicator of a modified structure was observed (Fig. 4). This was studied by mass spectrometry analysis, nitrone **6**, presents a unique peak with a retention time of 18.7 min (m/z 194.4 Da, $M^{+\bullet} + H$) (Fig. 4A). Whereas a new peak appeared after the reaction between nitrone **6** and $^{\bullet}NO_2$ with a retention time of 26.9 min (m/z 239.4 Da, $M^{+\bullet} + H$) (which presents absorbance at 300 and 360 nm, not shown) associated with a mono nitrated form of nitrone **6** (Fig. 4B). In the absence of $NaNO_2$ there are several peaks including the peak with a retention time of 26.9 min. The other peaks could correspond to hydroxylated and nitrated forms of nitrone **6**. These peaks appear in small amount and are difficult to characterize.

3.3. Neuroprotective studies in vitro

The hydroxyphenyl nitrones were then evaluated for their activity *in vitro* as protectants against induced damage in human neuroblastoma (SH-SY5Y) cells by 6-hydroxydopamine (6-OHDA) and the peroxynitrite donor 3-morpholinosydnonimie (SIN-1) [30,31]. 6-OHDA is known to destroy dopaminergic neurons through free radical-mediated mechanisms and it is used as neurotoxin producing experimental model of Parkinson's disease (PD). SIN-1 generates superoxide anion ($O_2^{\bullet-}$) and nitric oxide ($^{\bullet}NO$) resulting in constant production of $ONOO^-$, and therefore serves as a model for physiological $ONOO^-$ production [32]. The rate of $ONOO^-$ formation by SIN-1 (0.5 mM) was measured following the DHR method obtaining $ONOO^-$ at a rate of 9 μM per minute [33]. To explore the effects of hydroxyphenyl nitrones **5**–**7** on 6-OHDA-injured SH-SY5Y cells, cell viability and cytotoxicity

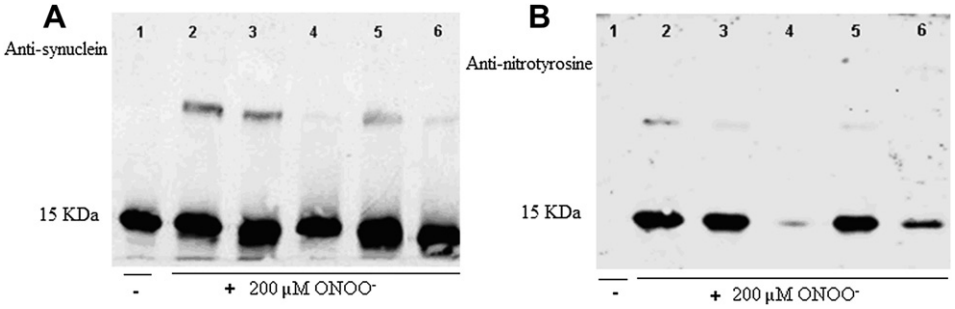


Fig. 2. Immunochemical analysis of peroxynitrite-treated αS and the hydroxyphenyl nitrones effects. 12% SDS-PAGE and western blot using antibodies anti-αS (A) and anti-3-nitrotyrosine (B). Lines 2–6: 5 μM αS treated with authentic 200 μM $ONOO^-$. 1: αS control (not treated), 3: +DMSO 1%, 4: +50 μM Nitrone **7**, 5: +50 μM Nitrone **5**, 6: +50 μM Nitrone **6**. Reverse addition of $ONOO^-$ is similar to lane 1 (not shown).

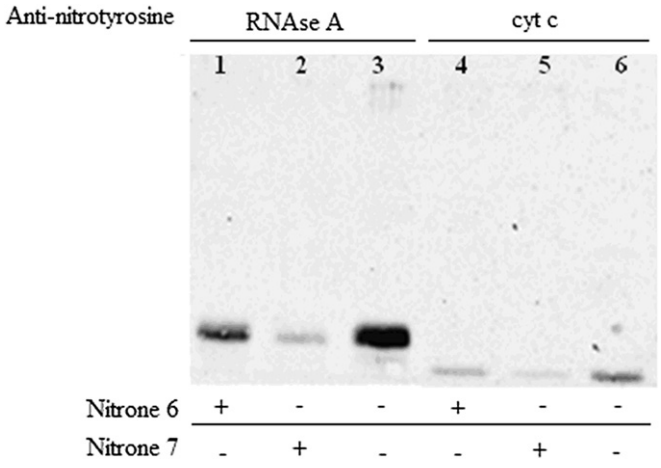


Fig. 3. Protein tyrosine nitration assessed in different proteins exposed to peroxynitrite. 12% SDS-PAGE and western blot using anti-3-nitrotyrosine antibody. Lines 1–6: different proteins (5 μM) treated with authentic $ONOO^-$ (200 μM). 1: RNase A + Nitrone **6**, 2: RNase A + Nitrone **7**, 3: RNase A control, 4: Cyt c + Nitrone **6**, 5: Cyt c + Nitrone **7**, 6: Cyt c control.

was determined using the MTT assay. First, SH-SY5Y cytotoxicity of the hydroxyphenyl nitrones **5**–**7** was evaluated. Different nitrones doses (10–100 μM) do not alter SH-SY5Y cell viability at 24 h (data not shown). The basal percentage of viable neurons was 75% at 24 h after 25 μM 6-OHDA treatment (Fig. 5A). Nitrones **5**–**7** showed significative protective effect at 10 μM as the viability of cells treated with these was approximately 100% (Fig. 5A). Cytoprotective properties of the phenolic nitrones against SIN-1 damage were evaluated using the MTT assay. The effects of the hydroxyphenyl nitrones **5**–**7** were evaluated on oxidative injury in SH-SY5Y cells using SIN-1 to generate $ONOO^-$ *in vitro*. Nitrones **5** and **7** did not show any significant protective effect (data not shown). In these conditions, only compound **6** showed protective effects against human neuroblastoma cell injury by SIN-1. Although the oxidative stress induced by 0.5 mM SIN-1 caused cell death of about 21%, treating cells with nitrone **6** markedly reverted cell death in a dose-dependent manner. On the other hand, at concentrations of 100 and 500 μM, the viability of cells treated with nitrone **6** was 91% and 100%, respectively (Fig. 5B).

3.4. Anti-inflammatory studies in vitro

Our next step was to explore whether the hydroxyphenyl nitrones present neuroprotective effects in different cell based assays. In this context, activation of astrocytes and microglia are involved on the neuronal death [34,35]. Thus, we used primary cultures of astrocytes and microglia treated with lipopolysaccharide (LPS), a potent inflammatory agent. The anti-inflammatory activity of the hydroxyphenyl nitrones was tested by evaluating the production of nitrites from primary cultured glial cells

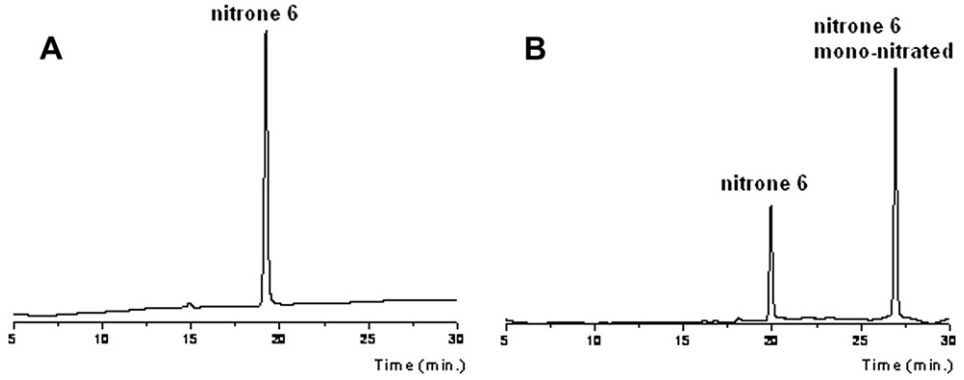


Fig. 4. Chromatograms of Nitrone **6** treated with peroxynitrite. (A) 50 μM Nitrone **6** (untreated) as a control. (B) 50 μM of Nitrone **6** was preincubated with an excess of sodium nitrite (4 mM $NaNO_2$) and then treated with 500 μM $ONOO^-$ in potassium phosphate buffer (50 mM KPO_4) at pH 7.4.

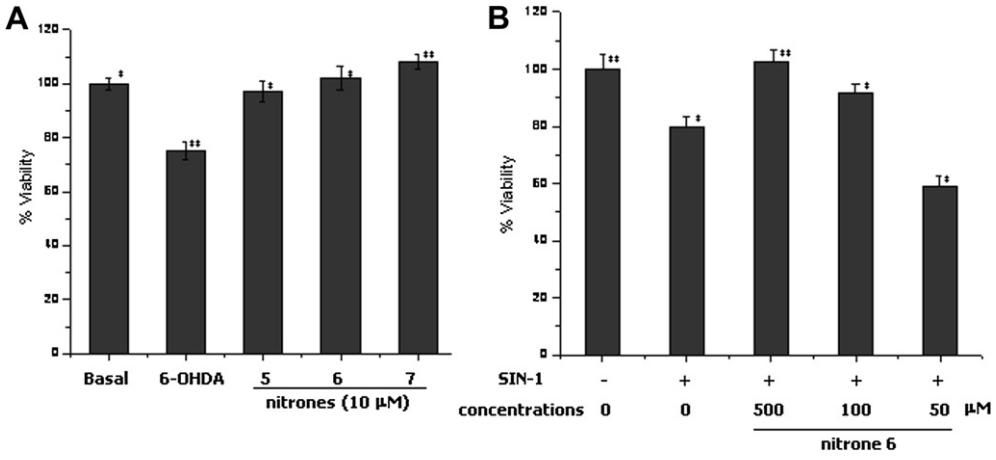


Fig. 5. (A) Neuroprotective effect of nitrones **5**–**7** on 6-OHDA-induced neurotoxicity in human neuroblastoma SH-SY5Y cells. SH-SY5Y cells were pre-incubated for 1 h with the indicated concentration of the test compounds and then stimulated with 6-OHDA (25 μM) or vehicle (mean ± SD values from three independent experiments). *, $p \leq 0.01$, **, $p \leq 0.001$. (B) Neuroprotective effect of nitrone **6** on SIN-1 induced neurotoxicity in human neuroblastoma SH-SY5Y cells. SH-SY5Y cells were pre-incubated for 1 h with the indicated concentrations of test compound and then stimulated with SIN-1 (0.5 mM) or vehicle (mean ± SD values from three independent experiments). *, $p \leq 0.01$, **, $p \leq 0.001$.

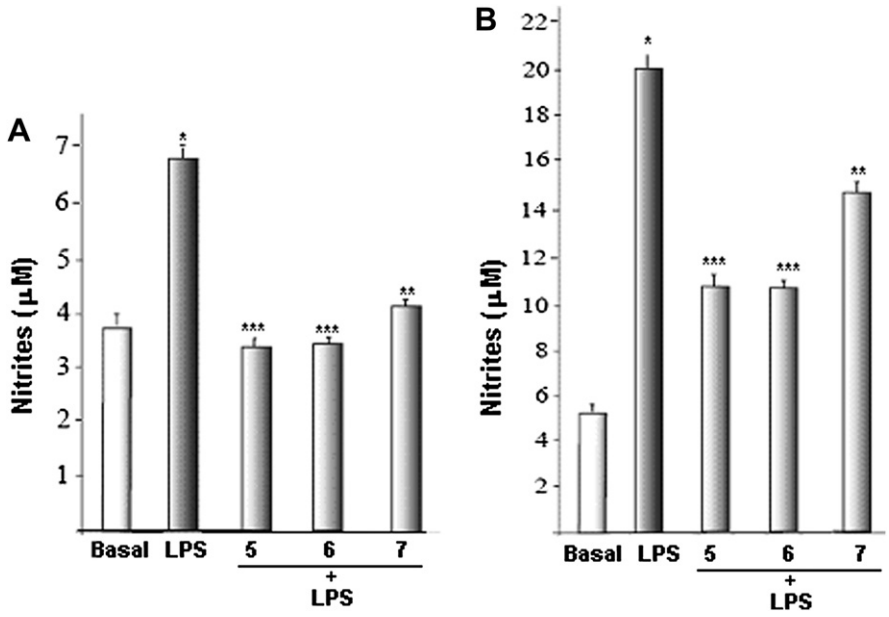


Fig. 6. (A) Effect of hydroxyphenyl nitrones on the inflammatory response of microglial cultures. (B) Effect of hydroxyphenyl nitrones on the inflammatory response of astrocyte cultures. Rat primary microglial and astrocyte cultures were treated for 24 h with LPS (10 μg/mL) in the absence or presence of nitrones **5**–**7** and the production of nitrite was evaluated by the Griess reaction. Values represent the means ± SD from three different experiments. ***, $p \leq 0.001$, **, $p \leq 0.01$, *, $p \leq 0.05$.

(astrocytes and microglia) [36]. Cultures were incubated with the indicated concentration of the nitrones **5**, **6** and **7** for 1 h, and then cells were cultured for another 24 h with LPS. When primary microglial and astrocytes cells were stimulated with LPS (Fig. 6) we observed a significant induction of nitrite production in the culture medium (1.7- and 4.0-fold, respectively), which was significantly diminished by hydroxyphenyl nitrones treatment. Nitrite production is mainly due to induction of inducible nitric oxide synthase (iNOS) enzyme which produces nitric oxide in LPS-treated cells. The treatment of primary cultured glial cells with phenolic nitrones **5**, **6** and **7**, resulted in a significant reduction in nitrite production. Additionally hydroxyphenyl nitrones cytotoxicity in astrocytes and microglia cultures was evaluated *in vitro* for 24 h. All the nitrones did not displayed cytotoxicity in primary glial cells, at assayed neuroprotective doses of 10 μM (data not shown).

3.5. Blood brain barrier permeation prediction

Early assessment of compound availability in the central nervous system (CNS) is essential for CNS drugs and useful for optimizing the toxicity profile of non CNS drugs. Drug's penetration through the blood brain barrier (BBB) is one of the major obstacles for the treatment of diseases in the CNS. For the hydroxyphenyl nitrones **5**–**7** the capacity to cross the blood–brain barrier (BBB) was evaluated employing a parallel artificial membrane permeation assay for BBB (PAMPA-BBB) using a lipid extract of porcine brain [37]. The assay validation was made by comparing experimental permeabilities of 10 commercial drugs to reported values [30,36]. A good correlation between experimental-described values was obtained P_e (exp) = 0.9479 (bibl) + 0.7528 (R^2 = 0.9723) (see Supplementary data). From this equation and following the pattern established in the literature for BBB permeation prediction we could classify compounds as CNS + when they present a permeability 4.54×10^{-6} cm s^{-1}. Based on these results we can consider that nitrones **5** and **7** are able to cross the BBB by passive permeation (Table 2).

4. Conclusions

We have reported a series of hydroxyphenyl nitrones, designed as multifunctional agents, since they combine hydroxyphenyl antioxidant with a neuroprotective and spin-trapping profile of nitrone moiety. Nitrones **5**–**7** were prepared in short time by condensation between the corresponding phenolic aldehyde and *N*-*tert*-butyl hydroxylamine under microwave irradiation. The biological evaluation showed that the hydroxyphenyl nitrones were more potent antioxidant than the reference compound PBN, probably due to the combination of aromatic hydroxy function and nitrone moiety. Hydroxyphenyl nitrones **6** and **7** avoid protein nitration and also the presence of high molecular aggregates forms of alpha-synuclein (αS). Nitrones **5**–**7** were also able to improve cell viability of SH-SY5Y cells damaged with 6-OHDA, whereas compound **6** was able to exhibited antioxidant effects against $ONOO^-$-induced oxidative stress. Moreover, these nitrones also showed significant anti-inflammatory effect, modulating nitrite production in neural cells. These results indicate that the hydroxyphenyl nitrones evaluated are promising anti-inflammatory and neuroprotective agents, with a potential use as therapeutic agents in neurodegenerative diseases.

Acknowledgments

This work was supported by Agencia Nacional de Investigación e Innovación (ANII, Fondo Clemente Estable 491), Comisión Sectorial de Investigación Científica (CSIC)-UdelaR (CSIC-N° 417), PEDECIBA-Química and project agreement UdelaR (Uruguay)-CSIC (Spain) (2009UY0006) and Ministerio de Ciencia y Tecnología (Spain) (SAF2010-16365, SAF2009-13015-C02-01). The authors acknowledge Mr. Horacio Pezzaroglo for recording the NMR spectra, Drs. Madia Trujillo and Gonzalo Peluffo for assistance with some experiments. We thank ANII for scholarship to CC. DIP acknowledges postdoctoral fellowship from the CSIC (JAE program).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.09.044.

References

[1] S.J. Chinta, J.K. Andersen, Biochim. Biophys. Acta 1780 (11) (2008) 1362–1367.
[2] B.I. Giasson, H. Ischiropoulos, V.M. Lee, J.Q. Trojanowski, Free Radic. Biol. Med. 32 (12) (2002) 1264–1275.
[3] V.M. Lee, J.Q. Trojanowski, Neuron 52 (1) (2006) 33–38.
[4] M. Aslan, T. Ozben, Curr. Alzheimer Res. 1 (2) (2004) 111–119.
[5] R.A. Floyd, K. Hensley, Oxidative stress in brain aging, Neurobiol. Aging 23 (5) (2002) 795–807.
[6] B.J. Tabner, S. Turnbull, O.M. El-Agnaf, D. Allsop, Free Radic. Biol. Med. 32 (11) (2002) 1076–1083.
[7] D.J. Betteridge, Metabolism 49 (2 Suppl. 1) (2000) 3–8.
[8] K. Hensley, K.A. Robinson, S.P. Gabbita, S. Salsman, R.A. Floyd, Free Radic. Biol. Med. 28 (10) (2000) 1456–1462.
[9] R. Radi, Proc. Natl. Acad. Sci. U. S. A. 101 (12) (2004) 4003–4008.
[10] P. Pacher, J.S. Beckman, L. Liaudet, Physiol. Rev. 87 (1) (2007) 315–424.
[11] E. Grunblatt, S. Mandel, M.B. Youdim, Ann. NY Acad. Sci. 899 (2000) 262–273.
[12] X.R. Shi, Z.Y. Hong, H.R. Liu, Y.C. Zhang, Y.Z. Zhu, Neurochem. Int. 58 (8) 851–860.
[13] R.A. Floyd, K. Hensley, M.J. Forster, J.A. Kelleher-Andersson, P.L. Wood, Mech. Ageing Dev. 123 (8) (2002) 1021–1031.
[14] R.A. Floyd, K. Hensley, F. Jaffery, L. Maidt, K. Robinson, Q. Pye, C. Stewart, Life Sci. 65 (18–19) (1999) 1893–1899.
[15] W. Porcal, P. Hernandez, M. Gonzalez, A. Ferreira, C. Olea-Azar, H. Cerecetto, A. Castro, J. Med. Chem. 51 (19) (2008) 6150–6159.
[16] S. Goldstein, P. Lestage, Curr. Med. Chem. 7 (12) (2000) 1255–1267.
[17] Y. Sun, J. Jiang, Z. Zhang, P. Yu, L. Wang, C. Xu, W. Liu, Y. Wang, Bioorg. Med. Chem. 16 (19) (2008) 8868–8874.
[18] G.T. Balogh, K. Vukics, A. Konczol, A. Kis-Varga, A. Gere, J. Fischer, Bioorg. Med. Chem. Lett. 15 (12) (2005) 3012–3015.
[19] Y. Sun, G. Zhang, Z. Zhang, P. Yu, H. Zhong, J. Du, Y. Wang, Bioorg. Med. Chem. 20 (12) (2012) 3939–3945.
[20] Z. Patai AR, Nitrones, Nitronates and Nitroxides, New York, 1989.
[21] A.G. Dias, C.E. Santos, F.Z. Cyrino, E. Bouskela, P.R. Costa, Bioorg. Med. Chem. 17 (11) (2009) 3995–3998.
[22] S. Kim, A.V.G.V. de, J. Bouajila, A.G. Dias, F.Z. Cyrino, E. Bouskela, P.R. Costa, F. Nepveu, Bioorg. Med. Chem. 15 (10) (2007) 3572–3578.
[23] M.M.B. Andrade, M.T. Pinto, R. C. Tetrahedron 64 (2008) 10521–10530.
[24] C.O. Kappe, Angew. Chem. Int. Ed. Engl. 43 (46) (2004) 6250–6284.
[25] B.I. Giasson, J.E. Duda, I.V. Murray, Q. Chen, J.M. Souza, H.I. Hurtig, H. Ischiropoulos, J.Q. Trojanowski, V.M. Lee, Science 290 (5493) (2000) 985–989.
[26] J.M. Souza, G. Peluffo, R. Radi, Free Radic. Biol. Med. 45 (4) (2008) 357–366.
[27] J.M. Souza, B.I. Giasson, Q. Chen, V.M. Lee, H. Ischiropoulos, J. Biol. Chem. 275 (24) (2000) 18344–18349.
[28] L.J. Ignarro, Nitric Oxide. Biology and Pathobiology, ELSEVIER, 2009.
[29] S. Bartesaghi, M. Trujillo, A. Denicola, L. Folkes, P. Wardman, R. Radi, Free Radic. Biol. Med. 36 (4) (2004) 471–483.
[30] X.L. Piao, M.H. Jang, J. Cui, X. Piao, Bioorg. Med. Chem. Lett. 18 (6) (2008) 1980–1984.
[31] Z.H. Wen, C.H. Chao, M.H. Wu, J.H. Sheu, Eur J Med. Chem. 45 (12) 5998–6004.
[32] M. Feelisch, J. Ostrowski, E. Noack, J. Cardiovasc. Pharmacol. 14 (Suppl. 11) (1989) S13–S22.
[33] H. Ischiropoulos, A. Gow, S.R. Thom, N.W. Kooy, J.A. Royall, J.P. Crow, Methods Enzymol. 301 (1999) 367–373.
[34] M. Pehar, M.R. Vargas, P. Cassina, A.G. Barbeito, J.S. Beckman, L. Barbeito, Neurodegener. Dis. 2 (3–4) (2005) 139–146.
[35] S. Singh, S. Swarnkar, P. Goswami, C. Nath, Int. J. Neurosci. 121 (11) (2011) 589–597.
[36] M. Redondo, J.G. Zarruk, P. Ceballos, D.I. Perez, C. Perez, A. Perez-Castillo, M.A. Moro, J. Brea, C. Val, M.I. Cadavid, M.I. Loza, N.E. Campillo, A. Martinez, C. Gil, Eur. J. Med. Chem. 47 (1) (2011) 175–185.
[37] L. Di, E.H. Kerns, K. Fan, O.J. McConnell, G.T. Carter, Eur. J. Med. Chem. 38 (2003) 223–232.