

ORIGINAL ARTICLE

Antioxidant activity of aminodiarylamines in the thieno[3,2-*b*]pyridine series: radical scavenging activity, lipid peroxidation inhibition and redox profile

Ricardo C. Calhella^{1,2}, Daniela Peixoto², Miguel Vilas Boas¹, Maria-João R. P. Queiroz², and Isabel C. F. R. Ferreira¹

¹CIMO-ESA, Instituto Politécnico de Bragança, Campus de Sta Apolónia, Bragança, Portugal and ²Centro de Química, Universidade do Minho, Campus de Gualtar, Braga, Portugal.

Abstract

The antioxidant activity of the aminodi(hetero)arylamines, prepared by C–N coupling of the methyl 3-aminothieno[3,2-*b*]pyridine-2-carboxylate with bromonitrobenzenes and further reduction of the obtained nitro compounds, was evaluated by chemical, biochemical and electrochemical assays. The aminodi(hetero)arylamines with the amino group *ortho* to the NH and a methoxy group in *para*, was the most efficient in radical scavenging activity (RSA, 63 μ M) and reducing power (RP, 33 μ M), while the aminodiarylamines with the amino group in *para* to the NH, gave the best results in β -carotene-linoleate system (41 μ M) and inhibition of formation of thiobarbituric acid reactive substances in porcine brain cells homogenates (7 μ M), with EC₅₀ values even lower than those obtained for the standard trolox. This diarylamines also presented the lowest oxidation potential, lower than the one of trolox, and the highest antioxidant power in the electrochemical assays. The *para* substitution with an amino group enables higher antioxidant potential.

Keywords

Antioxidant activity, di(hetero)arylamines, electrochemical assays, thieno[3,2-*b*]pyridine

History

Received 10 December 2012
Revised 3 February 2013
Accepted 12 February 2013
Published online 11 March 2013

Introduction

The equilibrium between reactive oxygen species (ROS) production and antioxidant defences might be displaced either by the overproduction of ROS or by the loss of the cell antioxidant defences¹. This disequilibrium is known as oxidative stress, and the excess of ROS may oxidize and damage cellular lipids, proteins and DNA, leading to their modification and inhibiting their normal function². The discovery of the roles played by ROS on the cell has generated a great interest on compounds with antioxidant activity. There is no doubt about the beneficial effects of antioxidants on pathological conditions like ischemia-reperfusion, cancer and various types of inflammation, where ROS play a pivotal role in the disease onset³.

A few examples are the synthetic antioxidants edaravone⁴ and ebselen⁵ (Figure 1) used for improving neurologic recovery following acute cerebral infarctions. Edaravone was the first drug developed for treating acute cerebral infarctions that acts as scavenger of free radicals while ebselen is still on clinical trials for acute cerebral infarction as well as for the treatment of free radical cornea injury. Although antioxidants are used as therapeutic drugs and as ingredients in dietary supplements with the

purpose of maintaining health and preventing diseases, these claims are still subject to intense scientific debate.

Another application of antioxidants is as food additives such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), TBHQ (tertiary-butylhydroquinone), PG (propyl gallate), OG (octyl gallate) and EQ (ethoxyquin). Nevertheless, some of these compounds have raised concerns on possible health problems they may promote⁶.

For these reasons, the continuous discovery of new synthetic products with antioxidant activity is of primary importance as they may substitute with advantage the currently used compounds. Diarylamines have been described as good antioxidants, especially as radical scavengers, with the ability to act as chain-breaking compounds transferring one hydrogen from the N–H group to peroxy radicals (ROO), leading to the formation of an aminyl radical (-N \cdot)⁷. Radical scavenging activity (RSA)^{8,9}, redox properties^{10,11} and effects on mitochondrial bioenergetics¹² of these compounds has been demonstrated by our and other research groups.

In the present work, the antioxidant activity of aminodi(hetero)arylamines in the thieno[3,2-*b*]pyridine, previously synthesized by us¹³, was evaluated through their free radical scavenging effects, reducing power (RP), lipid peroxidation inhibition and electrochemical behavior. Electrochemical techniques, such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV), have been applied as alternative tools for the evaluation of antioxidant activity, expressed in terms of “antioxidant power”, and provide a deeper insight into the redox-processes of oxidative stressors and antioxidants^{11,14–16}.

Address for correspondence: Isabel C. F. R. Ferreira, CIMO-ESA, Instituto Politécnico de Bragança, Campus de Sta Apolónia, Apartado 1172, 5301-855 Bragança, Portugal. Tel: +351273303219. Fax: +351273325405. E-mail: iferreira@ipb.pt

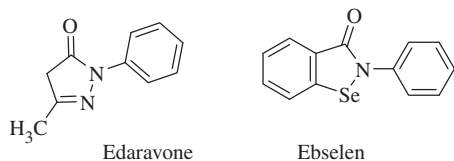
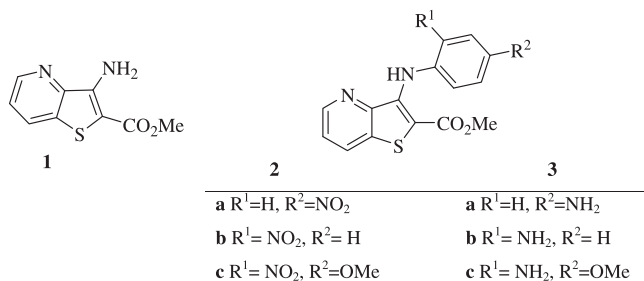


Figure 1. Structures of the synthetic antioxidants edaravone and ebselen.

Figure 2. Structures of the precursor methyl 3-aminothieno[3,2-*b*]pyridine-2-carboxylate **1**, of the nitrodi(hetero)arylamines **2** and of the corresponding aminodi(hetero)arylamines **3**.

Materials and methods

Aminodi(hetero)arylamines

The aminodiarylamines **3** were previously synthesized by Calhella et al.¹³ by a palladium-catalyzed C–N Buchwald-Hartwig coupling of the methyl 3-aminothieno[3,2-*b*]pyridine-2-carboxylate **1** with bromonitrobenzenes, and further reduction of the obtained nitrodi(hetero)arylamines **2** (Figure 2).

Standards and reagents

Methanol was analytical grade from Panreac (Lisbon, Portugal). Acetonitrile, analytical grade (Fisher Scientific, Brebieres, France), was double distilled over sodium perchlorate monohydrate, purity grade, purchased from Fluka (Sigma Chemical Co., St. Louis, MO). Tetrabutylammonium perchlorate (TBAP), electrochemical grade, was purchased from Fluka and kept at 30 °C before use. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA). Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, Greenville, SC).

Solutions of the compounds

Stock solutions of the tested aminodiarylamines were prepared in methanol and acetonitrile for antioxidant activity and electrochemical assays, respectively, and kept at –20 °C. Appropriate dilutions were prepared in the corresponding solvent prior to the assays.

Evaluation of antioxidant activity

DPPH radical-scavenging activity

This assay was performed in 96-well microplates using an ELX800 Microplate Reader (Bio-Tek Instruments, Bedfordshire, UK). Each one of the different concentrations of diarylamines solutions (30 μL) was mixed with methanolic solution containing DPPH radicals (6 × 10^{–5} mol/L; 270 μL). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 515 nm.

The RSA was calculated as a percentage of DPPH discoloration using the equation: % RSA = [(A_{DPPH} – A_C)/A_{DPPH}] × 100, where A_C is the absorbance of the solution when the compound has been added at a particular concentration, and A_{DPPH} is the absorbance of the DPPH solution^{8,9}. The concentration of diarylamine solution providing 50% of RSA (EC₅₀) was calculated from the graph of RSA percentage against compound concentration. Trolox was used as standard.

Reducing power

This assay was also performed using the Microplate Reader described above. Each one of the different concentrations of diarylamines solutions (0.5 mL) was mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). The mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured into the wells of a 48-well microplate, along with deionized water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm (8,9). The concentration of diarylamine solution providing 0.5 of absorbance (EC₅₀) was calculated from the graph of absorbance at 690 nm against compound concentration. Trolox was used as standard.

Inhibition of β-carotene bleaching

The antioxidant activity of the compounds was evaluated by the β-carotene linoleate model system (CLS). Two millilitres of a solution of β-Carotene in chloroform (0.2 mg/mL) were mixed with linoleic acid (40 mg) and Tween 80 emulsifier (400 mg). After chloroform evaporation (40 °C, under vacuum), distilled water (100 mL) was added and the mixture was vigorously shaking. Aliquots (4.8 mL) of this emulsion were transferred into test tubes containing each one of the concentrations of diarylamines solutions (0.2 mL). The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm (Analytikjena 200 spectrophotometer, Jena, Germany)^{8,9}. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2 h of assay/initial β-carotene content) × 100. The concentration of diarylamine solution providing 50% antioxidant activity (EC₅₀) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage (CLS) against extract concentration. Trolox was used as standard.

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances

Porcine brains were dissected and homogenized with a polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000g for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the compound concentration (0.2 mL) in the presence of FeSO₄ (10 μM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37 °C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 mL), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000g for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)–TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] × 100, where A and B were the absorbance of the control and the diarylamine solution, respectively^{8,9}. The concentration of diarylamine solution providing 50% of lipid peroxidation

inhibition (EC_{50}) was calculated from the graph of antioxidant activity percentage against compound concentration. Trolox was used as standard.

Evaluation of oxidation potentials and antioxidant power

Instrumentation

CV and DPV measurements were performed on an Autolab PGSTAT 302 potentiostat/galvanostat using a closed standard three electrode cell. A platinum disk (BAS, $\varnothing = 0.071 \text{ cm}^2$) was used as the working electrode and a Pt foil as the counter electrode. All potentials are referred to an Ag/AgCl 3M KCl reference electrode (Metrohm, Herisau, Switzerland). Prior to use, the working electrode was polished in an aqueous suspension of $0.3 \mu\text{m}$ alumina (Beuhler, Lake Bluff, IL) on a Master-Tex (Beuhler) polishing pad, then rinsed with water. Subsequently, in a chemical treatment, the electrode was sonicated in 6M HCl for 1 min, and then in methanol. This cleaning procedure was applied always before any electrochemical measurements.

Procedure

The diarylamines and the standard trolox were studied in acetonitrile solutions containing 1 mM of the compound and 0.1 M TBAP as the supporting electrolyte. All the solutions were analyzed immediately after preparation and the electrochemical responses recorded after the Pt electrode immersion, to minimize adsorption of species onto the electrode surface prior to the run. The voltammetric profiles were then acquired in different intervals in the range between -0.5 and 1.2 V for compounds **3a–c** or -0.5 and 1.6 V for compounds **2a–c**, using scan rates from 0.01 to 1 V/s . The first and subsequent cycles were recorded in order to access any adsorption phenomena common in organic compounds¹¹. The antioxidant power was evaluated by DPV using the following operating conditions: 0.06 V pulse amplitude and 0.02 V/s as scan rate.

A calibration curve was prepared by plotting the concentration of trolox solutions between 0.05 and 2.00 mM against the current intensity of the respective DPV signals measured at peak maxima. The analytical signal of compounds (current density) was measured, converted to equivalents of trolox (mM), and expressed in terms of equivalents of trolox per mM of diarylamine. The sum of the values calculated at peak maxima for each electrochemical oxidation process was used to express the Total Electrochemical Antioxidant Power of the compound.

Results and discussion

Antioxidant activity

The evaluation of the antioxidant activity of the aminodi(hetero)arylamines **3a–c** was performed using different methods: scavenging effect on DPPH radicals, RP assay, inhibition of lipid peroxidation using the CLS and TBA reactive substances (TBARS) assay. The results are displayed in Figure 3. Furthermore, the antioxidant trolox was used as standard, and its results were compared with those of the compounds in terms of EC_{50} (Table 1).

The RSA values of methanolic solutions of the aminodi(hetero)arylamines **3a–c** were examined and compared (Figure 3A). Results are expressed as a percentage of the ratio of the decrease

Table 1. EC_{50} values^a (μM) obtained for the antioxidant activity of the diarylamines **3a–c** and the standard trolox (mean \pm SD; $n = 3$).

	3a	3b	3c	Trolox
RSA	131.51 ± 26.22	158.86 ± 9.33	62.62 ± 0.77	171.91 ± 6.84
RP	59.12 ± 0.39	295.15 ± 4.31	33.18 ± 2.85	118.35 ± 12.59
CLS	41.30 ± 3.44	196.15 ± 3.99	43.95 ± 2.78	10.52 ± 0.57
TBARS	7.27 ± 0.22	16.58 ± 0.40	11.97 ± 1.19	14.90 ± 7.60

^aResults are given in concentrations providing 50% of antioxidant activity in RSA, CLS and TBARS assays, and 0.5 of absorbance in RP assay.

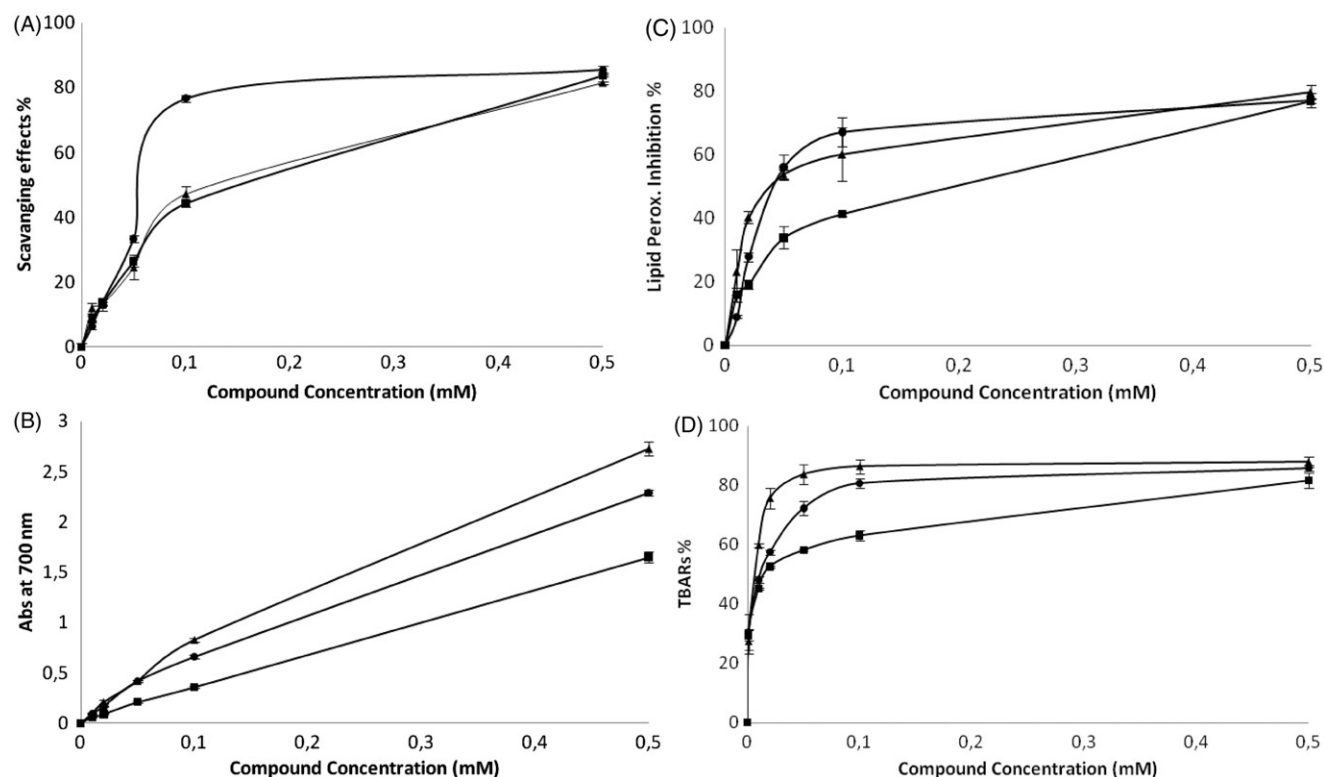


Figure 3. (A) Scavenging activity on DPPH radicals (RSA), (B) RP (Abs at 690 nm), (C) CLS and (D) TBARS assay using brain homogenized tissue for compounds **3a–c** (**3a** ▲; **3b** ■; **3c** ●). Results are the mean \pm SD of three independent experiments.

in the absorbance at 515 nm to the absorbance of DPPH solution in the absence of diarylamines at 515 nm. From the analysis of Figure 3(A), we can conclude that these diarylamines scavenging effects on DPPH radicals increase with the concentration and the results are very good for all the compounds specially for aminodi(hetero)arylamines **3c** (NH_2 in the *ortho* and OMe in the *para* position relative to the NH ; $85.62 \pm 0.23\%$ at 0.5 mM). The RSA slightly decreases for compounds **3b** (NH_2 in the *ortho* position; $83.37 \pm 0.22\%$ at 0.5 mM) and **3a** (NH_2 in the *para* position; $81.53 \pm 0.44\%$ at 0.5 mM). Nevertheless, at 0.1 mM the RSA of diarylamines **3b** and **3c** (<50%) is significantly lower than the RSA of diarylamine **3a** (~77%).

The RP of compounds **3a–c** was examined as a function of their concentration in methanolic solutions (Figure 3B). The RP of the compounds increases with an increase in concentration. A high value of absorbance at 690 nm is related to a high RP. For diarylamines **3a** and **3c** the absorbance values at 690 nm are the highest (2.73 ± 0.07 and 2.29 ± 0.03 at 0.5 mM, respectively) while for compound **3b** are the lowest (1.65 ± 0.05). Despite the highest absorbance observed for diarylamine **3a** at the highest concentration tested (Figure 3B), compound **3c** revealed the lowest EC_{50} value (Table 1).

The β -carotene undergoes a rapid decolorization in the absence of an antioxidant since the free linoleic acid radical attacks the β -carotene molecule, which loses the double bonds and, consequently loses its orange color. For compound **3a** (NH_2 in the *para* position; $79.83 \pm 2.19\%$ at 0.5 mM), the percentages of β -carotene bleaching inhibition are the highest while for compound **3b** are the lowest (Figure 3C).

The TBARS assay measures the MDA formed as the split product of an endoperoxide of unsaturated fatty acids resulting from an oxidation of a lipid substrate. The MDA reacts with TBA to form a pink pigment that is measured spectrophotometrically at 532 nm. The substrate is oxidized with the addition of an iron ion and the extent of the oxidation is determined by an addition of TBA and spectrophotometric measurement of the product.

Oxidation is inhibited by the addition of an antioxidant and therefore a reduction in the absorbance is observed. The results are typically quantified in terms of percentage of inhibition of the oxidation. The inhibition of lipid peroxidation by the three diarylamines using the TBARS assay was high (Figure 3D; >80%), but the highest value was obtained for compound **3a** ($87.89 \pm 1.83\%$ at 0.5 mM).

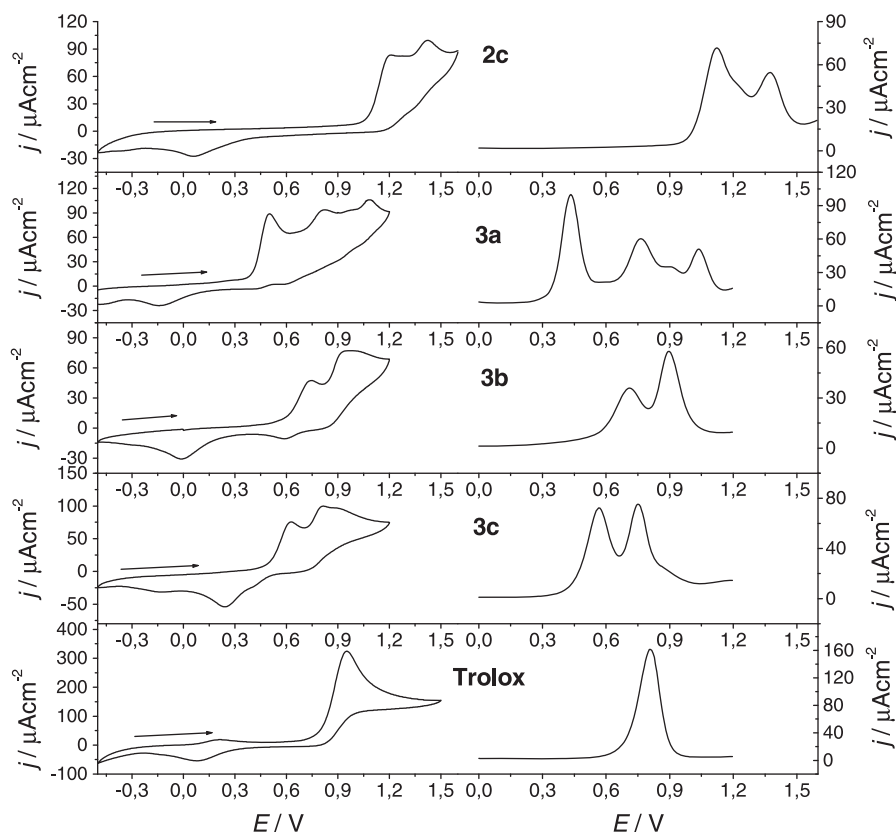
The DPPH scavenging activity and RP of the nitrodi(hetero)arylamines **2a–c** were also assessed, but these compounds did not show any activity. Probably, the presence of nitro groups (electro-withdrawing groups) in the phenyl ring stabilizes the N–H, avoiding the formation of the aminil radical (-N-) involved in antioxidant potential.

For an overview of the results, Table 1 presents the EC_{50} values (mM) for the different antioxidant activity assays performed for compounds **3a–c** and the standard trolox. Diarylamine **3c** (NH_2 in the *ortho* and OMe in the *para* position relative to the NH) was the most efficient in RSA ($62.62 \pm 0.77 \mu\text{M}$) and RP ($33.18 \pm 2.85 \mu\text{M}$), while compound **3a** (NH_2 in the *para* position) gave the best results in CLS ($41.30 \pm 3.44 \mu\text{M}$) and TBARS ($7.27 \pm 0.22 \mu\text{M}$). Unless for CLS assay, the mentioned EC_{50} values were lower than those obtained for the standard trolox.

Oxidation potentials and antioxidant power

The cyclic voltammograms (CV) observed for the diarylamines and for the standard trolox are shown in Figure 4. Compounds **3b** and **3c** revealed an electrochemical behavior with two major quasi-reversible oxidation processes, while diarylamine **3a** shows three major irreversible oxidation processes. The nitrodi(hetero)arylamines **2** exhibit a similar behavior in comparison to the structural equivalent aminodi(hetero)arylamines **3**, however at a significantly higher potential (Figure 4). This can be justified due to the electro-withdrawing effect of the nitro group. Those electrochemical profiles are different from the one obtained for

Figure 4. Electrochemical responses for 1 mM nitrodi(hetero)arylamines **2**, the corresponding aminodi(hetero)arylamines **3** and trolox in 0.1 M TBAP/ CH_3CN solutions, with Pt electrodes: (left) cyclic voltammogram at 0.1 V s^{-1} between -0.5 and $1.2/1.5 \text{ V}$; (right) differential pulse voltammogram obtained with 0.06 V pulse amplitude at 0.02 V s^{-1} .



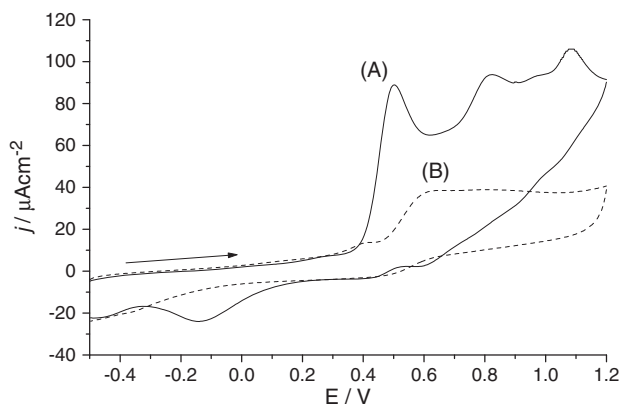


Figure 5. Cyclic voltammograms of 1 mM aminodi(hetero)arylamine **3a** in 0.1 M TBAP/CH₃CN solution, with Pt electrodes, between -0.5 and 1.2 V at 0.1 Vs⁻¹: (A) first scan; (B) after several scans.

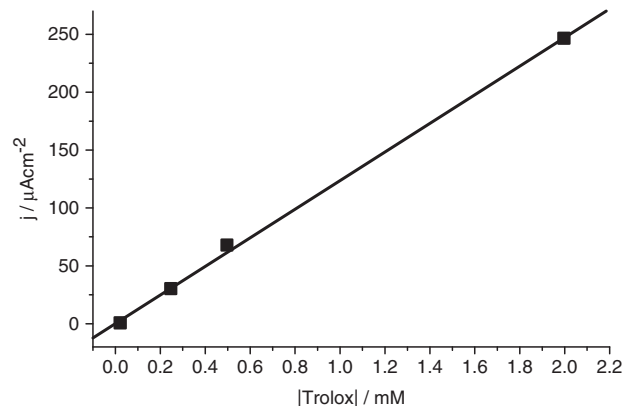


Figure 6. Variation of the oxidation peak height for different trolox concentration (0.05–2 mM), obtained by DPV of trolox/TBAP/CH₃CN solutions, with Pt electrodes between -0.5 and 1.2 V, with 0.06 V pulse amplitude at 0.02 Vs⁻¹.

Table 2. Peak potentials obtained for the electrochemical oxidation processes (CV and DPV) of the nitrodi(hetero)arylamines **2**, the corresponding aminodi(hetero)arylamines **3**, and the standard trolox.

	CV (V)			DPV (V)			EAP			
	Ep1	Ep2	Ep3	Ep1	Ep2	Ep3	p1	p2	p3	Total EAP
2a	–	1.32 ± 0.01	>1.60	–	1.24 ± 0.07	1.39 ± 0.07	–	0.30 ± 0.00	0.01 ± 0.0	0.31 ± 0.00
2b	–	1.35 ± 0.00	>1.60	–	1.27 ± 0.07	>1.60	–	0.33 ± 0.02	>1.60	0.33 ± 0.02
2c	–	1.19 ± 0.02	1.42 ± 0.01	–	1.12 ± 0.02	1.38 ± 0.00	–	0.40 ± 0.00	0.19 ± 0.02	0.59 ± 0.02
3a	0.49 ± 0.00	0.79 ± 0.00	1.10 ± 0.02	0.43 ± 0.00	0.77 ± 0.01	1.05 ± 0.01	0.71 ± 0.01	0.29 ± 0.01	0.23 ± 0.01	1.23 ± 0.01
3b	–	0.76 ± 0.01	0.94 ± 0.01	–	0.71 ± 0.00	0.90 ± 0.00	–	0.14 ± 0.01	0.31 ± 0.00	0.45 ± 0.01
3c	–	0.63 ± 0.02	0.81 ± 0.01	–	0.55 ± 0.02	0.74 ± 0.01	–	0.38 ± 0.02	0.46 ± 0.01	0.84 ± 0.02
Trolox	0.94 ± 0.01	–	–	0.81 ± 0.00	–	–	–	–	–	–

Electrochemical Antioxidant Power (EAP) expressed in mM of trolox equivalents per mM of diarylamine (mean ± SD; *n* = 3).

the amino precursor **1** (data not shown), leading us to associate the observed oxidation processes of the di(hetero)arylamines with the different substitutions in the phenyl ring. Furthermore, the presence of an amino group in the *para* position promotes significant changes in the electrochemical profile, with the appearance of a new oxidation process at lower potential, 0.5 V, and the inactivation of the electrode surface. This change did not occur if the *para* position is occupied by the nitro group, **2a**. In fact, with the continuous cycling, the electrochemical activity for the compound **3a** almost vanished, Figure 5, as observed in our previous work⁵ attributed to the adsorption of electroinactive species onto the electrode surface, requiring the physical polishing of the platinum electrode. For the other compounds, this behavior is not observed and after the second cycle the voltammogram reaches a repeatable electroactive profile. It seems that the presence of the amino/nitro group in the *ortho* position prevents somehow the low oxidative process/adsorption on the electrode surface, possible due to some steric hindrance caused by the substituent.

Peak potentials for the different compounds are presented in Table 2. The aminodi(hetero)arylamines **3a** presented the lowest oxidation potentials, even lower than the one of trolox. Comparing compounds **3b** and **3c**, or **2b** and **2c**, the presence of a methoxy group (**2c/3c**), decreases the oxidation potentials probably due to a resonance stabilization of the oxidized species through the electro-donating effect. This effect was also observed by us in a previous study with diarylamines in the 2,3-dimethylbenzo[*b*]thiophene series¹¹. The dynamic behavior of the oxidation processes, scan rate dependency, revealed a semi-diffusion controlled process for the second peak of compound **3a** and first peak of compounds **2a–c**, **3b** and **3c** (considered in

Table 2 as Ep2), with a linear response of the anodic current density versus $\nu^{1/2}$, as predicted by the Randles–Sevcik equation¹⁷. All the other anodic processes show a decline in the current densities for high scan rates, typical of a slow electron transfer mechanism. The presence of the amino group in the *para* position seems to be important for the antioxidant properties since it introduces an oxidation process at lower potentials.

The DPV, Figure 4, follow a pattern similar to that of CV, but enable an easier evaluation of the antioxidant properties of the compounds. Based on the linear relationship of current density with the concentration of the electrochemical standard trolox, Figure 6, diarylamine **3a** gave the lowest antioxidant potentials and the highest “electrochemical antioxidant power” (EAP), Table 2, measure as trolox equivalents.

Conclusions

The aminodi(hetero)arylamines **3c** with the amino group *ortho* to the NH and a methoxy group in *para*, was the most efficient in RSA (63 μM) and RP (33 μM), while compound **3a** with the amino group in *para* to the NH, gave the best results in CLS (41 μM) and TBARS (7 μM), with EC₅₀ values even lower than those obtained for the standard trolox. The di(hetero)arylamines **3a** also presented the lowest oxidation potential, lower than the one of trolox, and the highest antioxidant power in the electrochemical assays. The amino group in the *para* position relative to the NH, enables a higher antioxidant potential. Furthermore, it should be mentioned that the absence of antioxidant activity observed in the nitrodi(hetero)arylamines **2** is in agreement with the high oxidation potential induced by the nitro group and verified in electrochemical analyses.

Declaration of interest

The authors report no declarations of interest.

The authors are grateful to FCT and FEDER (European Fund for Regional Development)-COMPETE/QREN/EU for financial support through the research unities PEst-C/QUI/UI686/2011 and PEst-OE/AGR/UI0690/2011, the research project PTDC/QUI-QUI/111060/2009 and the post-Doctoral grant attributed to R.C.C. (SFRH/BPD/68344/2010).

References

- Halliwell B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiol* 2006;141:312–22.
- Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidant in normal physiological function and human disease. *Int J Biochem Cell Biol* 2007;39:44–84.
- Halliwell B. The wandering of a free radical. *Free Rad Biol Med* 2009;46:531–42.
- Toyoda K, Fujii K, Kamouchi M, et al. Free radical scavenger, edaravone, in stroke with internal carotid artery occlusion. *J Neurol Sci* 2004;221:11–17.
- Green AR, Ashwood T, Odegren T, Jackson DM. Nitrones as neuroprotective agents in cerebral ischemia, with particular reference to NXY-059. *Pharmacol Therapeut* 2003;100:195–214.
- Carocho M, Ferreira ICFR. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem Toxicol* 2013;51:15–25.
- Thomas JR. The identification of radical products from the oxidation of diphenylamine. *J Amer Chem Soc* 1960;82:5955–6.
- Ferreira ICFR, Queiroz M-JRP, Vilas-Boas M, et al. Evaluation of the antioxidant properties of diarylamines in the benzo[*b*]thiophene series by free radical scavenging activity and reducing power. *Bioorg Med Chem Lett* 2006;16:1384–7.
- Queiroz M-JRP, Ferreira ICFR, Calhella RC, Estevinho LM. Synthesis and antioxidant activity evaluation of new 7-aryl or 7-heteroarylamino-2,3-dimethylbenzo[*b*]thiophenes obtained by Buchwald–Hartwig C–N cross-coupling. *Bioorg Med Chem* 2007;15:1788–94.
- Esteves MA, Narender N, Marcelo-Curto MJ, Gigante B. Synthetic derivatives of abietic acid with radical scavenging activity. *J Nat Prod* 2001;64:761–6.
- Abreu RMV, Falcão S, Calhella RC, et al. Insights in the antioxidant activity of diarylamines from the 2,3-dimethylbenzo[*b*]thiophene through the redox profile. *J Electroanal Chem* 2009;628:43–7.
- Pinto-Basto D, Silva JP, Queiroz M-JRP, et al. Di(hetero)arylamines in the benzo[*b*]thiophene series as novel potent antioxidants. *Mitochondrion* 2009;9:17–26.
- Calhella RC, Ferreira ICFR, Peixoto D, et al. Aminodi(hetero)arylamines in the thieno[3,2-*b*]pyridine series: synthesis, effects in human tumor cells growth, cell cycle analysis, apoptosis and evaluation of toxicity using non-tumor cells. *Molecules* 2012;17:3834–43.
- Korotkova EI, Karbainov YA, Shevchuk A. Study of antioxidant properties by voltammetry. *J Electroanal Chem* 2002;518:56–60.
- Blasco AJ, Rogerio MC, González MC, Escarpa, A. Electrochemical Index'' as a screening method to determine ''total polyphenolics'' in foods: a proposal. *Analyt Chim Acta* 2005;539:237–44.
- Chevion S, Roberts MA, Chevion M. The use of cyclic voltammetry for the evaluation of antioxidant capacity. *Free Rad Biol Med* 2000;28:860–70.
- Bard AJ, Faulkner LR. *Electrochemical methods, fundamentals applications*. NY: Wiley; 2001.