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Evaluation of in vitro antioxidant effect of new mono and diselenides

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ABSTRACT

This study was designed to examine the antioxidant activity in vitro of novel mono- and diselenide compounds. We compared whether the formation of p-methyl-selenol from compounds 1-phenyl-3-(p-tolylselanyl)propan-2-amine (C1) and 1,2-dip-tolyldiselenide (C4) and o-methoxy-selenol from compounds 1-(2-methoxyphenylselanyl)-3-phenylpropan-2-amine (C2) and 1,2-bis(2-methoxyphenyl) diselenide (C3) may be involved in their antioxidant effects. The compounds were tested against Fe(II) and sodium nitroprusside (SNP)-induced lipid peroxidation in rat brain and liver homogenates. Likewise, the antioxidant capacity of the compounds was assessed by their ability to decolorize the DPPH radical as well as the Fe(II) chelating assay through the reduction of molybdenum(VI) (Mo6+) to molybdenum(V) (Mo5+). This colorimetric assay was also used to quantify thiol peroxidase (GPx) and oxidase activity and thioredoxin reductase (TrxR) activity. The results showed that the novel selenide compounds inhibit the thiobarbituric acid reactive species (TBARS) induced by different pro-oxidants, but the monoselenides effects were significant only at concentrations higher than the concentrations of the diselenides. Similarly, the total antioxidant activity was higher in the diselenides. Moreover, GPx and TrxR activity was only observed for the diselenides, which indicates that these compounds are more stable selenol molecules than monoselenides

1. Introduction

Oxygen metabolism, which typically occurs in aerobic organisms, allows energy formation mediated by the mitochondrial electron transfer system (Puntel et al., 2013). However, oxygen metabolism also leads to the production of small quantities of reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) (Mugesh et al., 2001). Additionally, an aerobe is able to produce reactive nitrogen species (RNS), such as peroxynitrite ($ONOO^-$) and nitric oxide ($^{\cdot}NO$), which are also as strong biological oxidants (Nathan and Ding, 2010). Accordingly, the imbalance between ROS/RNS formation and the enzymatic/non-enzymatic antioxidant system is associated with many diseases, such as Alzheimer's, myocardial infarction, atherosclerosis, and Parkinson's, and in other pathological conditions, including senescence (Ji et al., 2003; Salmon et al., 2010; Schon and Przedborski, 2011).

Similarly, several research groups developed techniques to create potential antioxidant molecules using chalcogen elements in their structure, and thus, some of these new compounds were characterized as strong free radical scavengers. (Gutteridge and Halliwell, 1992). For example, the organoselenium compounds have shown mimetic glutathione peroxidase-like activity (GPx) and also act as substrates of thioredoxin reductase (TrxR). Therefore, these compounds might represent novel therapeutic targets for diseases caused by oxidative stress (Arteel and Sies, 2001).

The antioxidant effects of organoselenium compounds, such as ebselen and diphenyl diselenide (DPDS), have been shown to be due to their ability to generate a selenol/selenolate chemical form (Nogueira and Rocha, 2010). The selenolate group is a stronger nucleophile than its thiolate analog, which confers stronger reducing power to a given selenol group than the analog thiol group (Nogueira and Rocha, 2011).

However, although the selenol groups are less abundant than thiols and are found only in a small number of selenoproteins, they exhibit a stronger nucleophilicity than their sulfur analogs (Lu et al., 2009). In brief, the presence of selenium (Se) in selenocysteine reduces the enzymatic pK_a, compared to the sulfhydryl enzyme, and therefore leads to Se ionization, forming a selenol group (Gutteridge and Halliwell, 1992).

According to the proposed mechanism, the selenol complex (enzyme-SeH) could react with hydrogen peroxide or other hydroperoxides to produce selenic acid (enzyme-SeOH), which is capable

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when reacting with glutathione (GSH) to reclaim the selenol and form water (Nogueira and Rocha, 2010).

Previous studies reported that the DPDS antioxidant effect was better than that of ebselen, especially in the GPx-like action, and was mainly due to the formation of two selenol structures after interaction with reducing thiol groups (Nogueira et al., 2004). However, the instability of the selenol complex makes it difficult to detect any antioxidant effects during in vitro studies (Bhabak and Mugesh, 2010). Therefore, the emergence of classic, structural organoselenium compound analogs can promote the stability of the selenol (Balkrishna et al., 2011). Indeed, the structural inclusion of a basic amino acid nitrogen near the selenium can increase the antioxidant capacity to create a more stable selenol molecule (Hassan et al., 2012).

Consequently, this study evaluates two different classes of organoselenium compounds, monoselenides (β-selenoamines) and diselenides (analogs of DPDS), using various antioxidant assays. The β-selenoamine chemical structure includes amino groups (C1 and C2) and the diselenides consist of methyl or methoxy group modifications (C3 and C4, respectively) (Fig. 1).

The aim of this study was to evaluate the antioxidant capacity using in vitro models of the compounds cited above and to associate the effects with the capacity of these molecules to form a more stable selenol once the theoretical compounds C1 and C4 generate p-methyl-selenol and compounds C2 and C3 form o-methoxy-selenol.

2. Materials and methods

2.1. Animals

Male, adult Wistar rats (200–250 g) from our own breeding colony were used. The animals were maintained on a 12-h light: 12-h dark cycle, at a room temperature of 22–24 °C and with free access to food and water. The animals were treated according to standard guidelines of the Committee on Care and Use of Experimental Animal Resources.

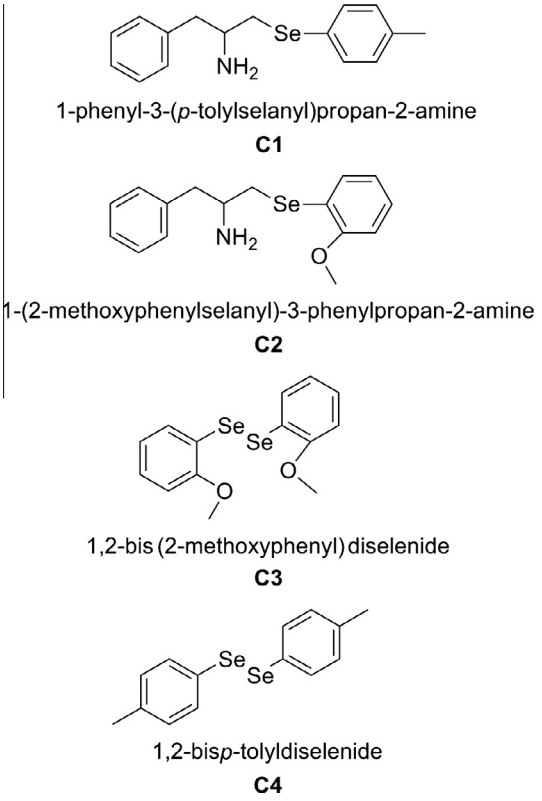

Fig. 1. Chemical structure and nomenclature of the β-selenoamines (C1 and C2) and analogs of diphenyl diselenide (C3 and C4).

2.2. Chemicals

Thiobarbituric acid (TBA), malonldialdehyde (MDA), diphenyl-2'picrylhydrazyl (DPPH), adenine dinucleotide phosphate (NADPH), benzenethiol, Tris–HCl, sodium dodecyl sulfate (SDS), ethylene diamine tetra acetic acid (EDTA) and dimethyl sulfoxide (DMSO) were obtained from Sigma (St. Louis, MO). Fe(II) sulfate, sodium nitroprusside (SNP), ascorbic acid, hydrogen peroxide, acetic acid, 5.5'-dithiobis(2-nitrobenzoate) (DTNB), NaCl, KCl, Na_2HPO_4, KH_2PO_4 and ethanol were obtained from Merck (Rio de Janeiro, RJ, Brazil).

2.3. Compounds

The mono- and diselenides were prepared following previously described methods (Salman et al., 2012), and the purity of the products was accessed by hydrogen and carbon nuclear magnetic resonance and gas chromatography. The compounds tested were 1-phenyl-3-(*p*-tolylselanyl)propan-2-amine (C1), 1-(2-methoxyphenylselanyl)-3-phenylpropan-2-amine (C2), 1,2-bis(2-methoxyphenyl)diselenide (C3), and 1,2-bis*p*-tolyldiselenide (C4). All the compounds are dissolved in DMSO.

2.4. Tissue preparation

Animals were sacrificed by decapitation. The brain and liver tissues were removed and immediately placed on ice. The tissues were homogenized in Tris–HCl 10 mM and centrifuged for 10 min at 2000 rpm. The supernatant fraction (S1) was collected immediately for the assays.

2.5. Sample preparation

Heparinized venous blood previously obtained from healthy volunteer donors from the Hospital of Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil. The study protocol was reviewed and approved by the appropriate institutional review board following the Guidelines of the Committee of UFSM (0089.0.243.000-07). The erythrocytes were separated by centrifugation (480*g* for 10 min at room temperature) and the plasma was aspirated. The cell pellet was washed three times with phosphate buffer-saline (6.1 mM and pH 7.4, containing 150 mM NaCl). The leukocytes were separate and utilized in the cell viability analysis.

2.6. Purification of hepatic TrxR

The rat livers were homogenized in buffered saline (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4 – pH 7.3) and centrifuged at 13,000*g* for 30 min at 4 °C. The supernatant fraction was collected for TrxR isolation and dialyzed against buffered saline for 24 h to remove low molecular weight thiols. The dialysate was heated at 55 °C for 10 min, cooled, and centrifuged at 13,000*g* for 30 min (Wagner et al., 2010). The supernatant was used for the TrxR assay.

2.7. TBARS assay

The capacity to prevent end products of lipid peroxidation was determined in tissue samples as previously described (Ohkawa et al., 1979). Aliquots of brain and liver supernatants (100 μL of S1) were incubated for 60 min with freshly prepared Fe(II) (10 μM) or SNP (5 μM) in the absence or presence of different concentrations of the compounds C1–C4 (6.25, 12.5, 25, 50 μM) in a

medium containing Tris–HCl buffer 10 mM pH 7.4. The reaction was stopped by the addition of SDS (final concentration of 1.35%), and lipid peroxidation products were measured by the addition of acetic acid/HCl buffer, pH 3.4 and 0.6% TBA, pH 6.0. The color reaction was developed by incubating tubes in boiling water for 60 min. TBARS levels were measured at 532 nm.

2.8. DPPH· radical scavenging method

The radical scavenging activities of the compounds were determined as previously described (Brand-Williams et al., 1995). Each compound was tested at 6.25, 12.5, 25, 50, 100, 200, and 400 μM in 10% DMSO. Seven different concentrations of ascorbic acid (6.25; 12.5; 25; 50; 100; 200; 400 μM) were used as positive controls. DPPH· (diluted in ethanol) was added to final concentration of 0.3 mM and allowed to react at room temperature for 30 min in dark conditions. The absorbance was measured at 518 nm using Spectra Max Plate Reader® M2 (Molecular Devices), Sunnyvale, California, USA.

2.9. Total antioxidant capacity assay

The total antioxidant potential of the mono- and diselenides was evaluated by the phosphomolybdenum method as previously described (Prieto et al., 1999). A sample solution aliquot in ethanol (0.3 ml) was combined in a vial with reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate, 3 ml). The compounds were tested a concentration of 400 μM. The vials were capped and incubated in a water bath at 95 °C for 90 min. After cooling the mixture to room temperature, the absorbance was measured at 695 nm against a blank control.

2.10. Thiol-peroxidase-like activity assay

The GPx catalytic activity of mono- and diselenides was evaluated utilizing 10 mM benzenethiol (PhSH) as a substrate, as previously described (Iwaoka and Tomoda, 1994). The H_2O_2 reduction was monitored at 305 nm for 150 s. The compounds were tested at concentrations of 200 and 400 μM. DMSO was used as a negative control (vehicle).

2.11. Thiol-oxidase activity assay

Thiol oxidase activity of 200 and 400 μM concentrations of the compounds (C1–C4) was determined in a medium containing 10 mM Tris/HCl buffer (pH 7.4) and 1 mM glutathione or PhSH. An aliquot of 100 μL was removed at different time points (0, 30, 60 and 120 min) and added to a solution containing 0.5 mM DTNB and 10 mM Tris/HCl buffer (in the absence of thiol oxidation a maximum of 100 nmol of –SH/ml can be found). The absorbance of each sample was measured at 412 nm (Ellman, 1959).

2.12. NADPH oxidation by TrxR using the selenide compounds as substrates

The reduction of mono- and diselenides (15 μM) by rat hepatic TrxR was performed by a modification of the method previously described by Holmgren and Bjornstedt (1995). TrxR was mixed with a medium containing 10 mM Tris–HCl, 1 mM EDTA, pH 7.5, in the presence or absence of selenide compounds and then, the reaction was started by adding NADPH (final concentration 120 μM).

2.13. Fe(II)-chelating assay

The Fe(II)-chelating ability of compounds was determined using a modified method of Puntel et al. (2005). Freshly prepared 500 μmol/L Fe(II) (150 μL) was added to a reaction mixture containing 168 μL of 0.1 mol/L Tris–HCl (pH 7.4), 218 μL saline and the compounds (100 μM). The reaction mixture was incubated for 5 min prior to the addition of 13 μL of 0.25% 1,10-phenanthroline (w/v). The absorbance was then measured at 510 nm in a spectrophotometer.

2.14. Cell viability analysis

The percentages of viable and nonviable leukocytes in samples incubated (90 min) with the compounds (100 μM) were determined by Trypan blue following the method of Mischell and Shiigi (1980). Cell viability was calculated as the number of living cells divided by the total number of cells multiplied by 100 (Mischell and Shiigi, 1980).

2.15. Protein quantification

The protein concentration was estimated by the Bradford method using bovine serum albumin as the standard (Bradford, 1976).

2.16. Statistical analysis

Individual dependent variable data were analyzed statistically by one-way (TBARS, DPPH levels, phosphomolybdenum, Fe^{2+}-chelating ability and cell viability) or two-way (thiol peroxidase, thiol oxidase and TrxR activity) analysis of variance (ANOVA), followed by Duncan's multiple range test when appropriate. Differences between groups were considered to be significant when $p < 0.05$. Data are expressed as means ± SEM and each experimental procedure was performed in at least 4 individual experiments with 3 replicates each. The compound concentration that causes 50% inhibition (IC_{50}) and the maximal inhibition of compounds (I_{max}) was determined by linear regression analysis from 4 individual experiments, using Graph Pad Prism software.

3. Results

3.1. Effect of compounds on lipid peroxidation induced by Fe(II) and SNP in rat brain

We induced lipid peroxidation in rat brain (Fig. 2) homogenates with Fe(II) (10 μM) and SNP (5 μM), and the antioxidant effect of selenium compounds on these homogenates was investigated. C1 had a protective effect against lipid peroxidation at the concentration range (25–50 μM), while the other compounds (C2, C3 and C4) demonstrated a significant effect from the lowest concentration tested (Fig. 2A). In SNP-induced rat brain homogenates, the monoselenides presented a significant antioxidant effect at the concentration range (12.5–50 μM) for C1 and (25–50 μM) for C2, while the diselenides showed a significant effect at 6.25 μM (Fig. 2B).

The IC_{50} values of the compounds followed the order C4 < C3 < C2 < C1 against Fe(II)-induced lipid peroxidation (Table 1). For SNP-induced lipid peroxidation, the IC_{50} values of the compounds followed the order C4 < C3 < C2 < C1 (Table 1).

The I_{max} values of the compounds against Fe(II)-induced lipid peroxidation was 87%, 92%, 93% and 96% respectively of C1 to C4 (Table 3). For SNP-induced lipid peroxidation, the I_{max} values of the compounds was 83%, 90%, 91% and 92% respectively of C1 to C4 (Table 3).

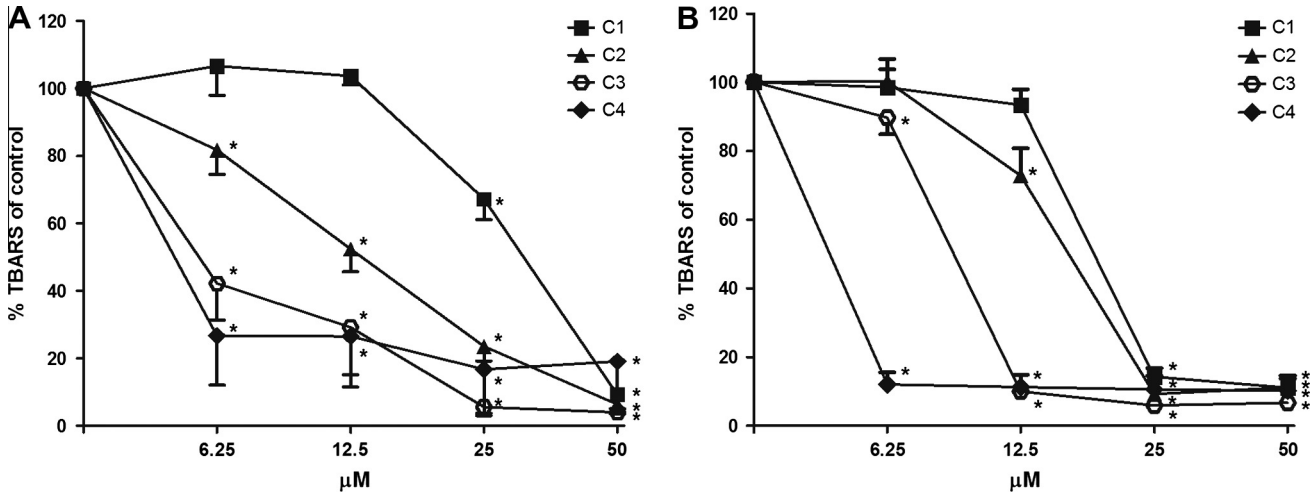

Fig. 2. Effect of the selenium compounds on TBARS production in the brain. The compounds were tested at final concentrations of 6.25, 12.5, 25 and 50 μM, and the results are expressed as a percentage (means ± SEM) relative to the Fe(II)-induced (A) or SNP-induced (B) control. Data is shown as means ± SEM of 4 individual experiments (3 replicates each). * Represents a significant difference when compared with the induced group by Duncan's multiple range test.

Table 1
Calculated IC_{50} values of compounds on lipid peroxidation in rat brain.

Compound	IC_{50} (μM) for Fe(II)	IC_{50} (μM) for SNP
C1	27.67 ± 1.28	20.12 ± 0.44
C2	13.78 ± 2.28	18.77 ± 0.14
C3	5.90 ± 1.32	9.01 ± 0.12
C4	4.73 ± 1.17	3.56 ± 0.15

Data are expressed as mean ± S.E.M and are calculated for fourth independent assays.

Table 2
Calculated IC_{50} values of compounds on lipid peroxidation in rat liver.

Compound	IC_{50} (μM) for Fe(II)	IC_{50} (μM) for SNP
C1	33.71 ± 1.72	38.70 ± 0.93
C2	18.73 ± 1.25	17.26 ± 0.41
C3	28.93 ± 2.05	14.18 ± 0.23
C4	18.23 ± 1.12	3.38 ± 0.21

Data are expressed as mean ± S.E.M and are calculated for fourth independent assays.

3.2. Effect of compounds on lipid peroxidation induced by Fe(II) and SNP in rat liver

Rat liver homogenates were induced with Fe(II) or SNP to cause lipid peroxidation, and the effect of selenium compounds on this lipid peroxidation was investigated (Fig. 3). Both the monoselenides and the diselenides decreased the lipid peroxidation induced by Fe(II) at the concentration range (25–50 μM) (Fig. 3A). However, during SNP-induced lipid peroxidation (Fig. 3B), the C1 compound presented a significant effect only at the highest concentration tested, while compounds C2 and C3 had significant effects at the concentration range (25–50 μM). The C4 compound was effective in reducing the lipid peroxidation at the lowest concentration tested.

The IC_{50} values of the compounds followed the order: C4 < C2 < C3 < C1 against Fe(II)-induced lipid peroxidation (Table 2). For SNP-induced lipid peroxidation, the IC_{50} values of the compounds followed the order: C4 < C3 < C2 < C1 (Table 2).

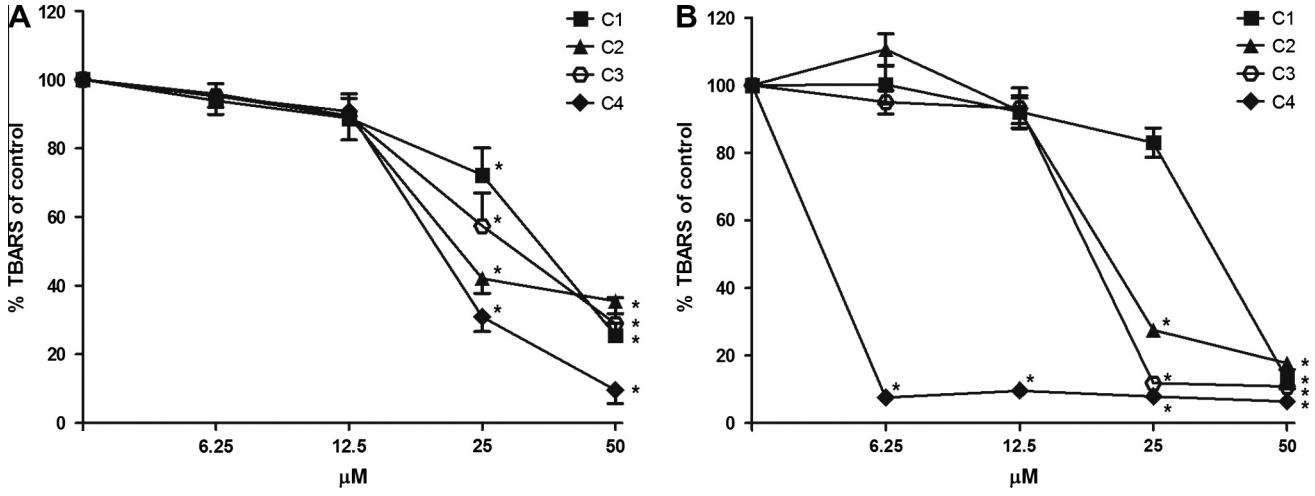

Fig. 3. Effect of the selenium compounds on TBARS production in the liver. The compounds were tested at final concentrations of 6.25, 12.5, 25 and 50 μM, and the results are expressed as a percentage (means ± SEM relative to the Fe(II)-induced (A) or SNP-induced (B) control. Data is shown as means ± SEM of 4 individual experiments (3 replicates each). * Represents a significant difference when compared with the induced group by Duncan's multiple range test.

Table 3
Calculated I_{max} values of compounds on lipid peroxidation in rat brain.

Compound	I_{max} (%) for Fe(II)	I_{max} (%) for SNP
C1	87.11 ± 5.1	83.03 ± 2.1
C2	92.66 ± 2.9	90.99 ± 1.1
C3	93.03 ± 3.2	91.01 ± 2.3
C4	96.37 ± 2.3	92.32 ± 2.6

Data are expressed as mean ± S.E.M and are calculated for fourth independent assays.

Table 4
Calculated Imax values of compounds on lipid peroxidation in rat liver.

Compound	I_{max} (%) for Fe(II)	I_{max} (%) for SNP
C1	67.18 ± 1.9	69.94 ± 3.4
C2	81.97 ± 1.7	79.63 ± 3.1
C3	72.29 ± 2.2	89.33 ± 2.8
C4	90.39 ± 2.1	93.21 ± 1.6

Data are expressed as mean ± S.E.M and are calculated for fourth independent assays.

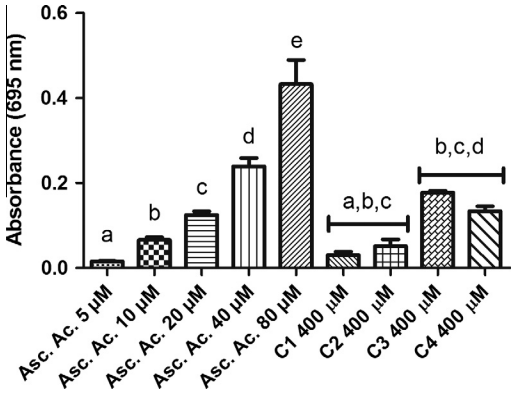

Fig. 4. Total antioxidant activity of the monoselenides and diselenides were measured by the phosphomolybdenum assay. The compounds (400 μM) were incubated with 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate for 90 min: (a–e) represent the effect of ascorbic acid. Values are expressed as absorbance means ± SEM of 4 individual experiments (3 replicates each).

The I_{max} values of the compounds against Fe(II)-induced lipid peroxidation was 67%, 81%, 72% and 90% respectively of C1 to C4 (Table 4). For SNP-induced lipid peroxidation, the I_{max} values of the compounds was 69%, 79%, 89% and 93% respectively of C1 to C4 (Table 4).

3.3. Fe(II)-chelating property, free radical scavenging (DPPH•), thiol-oxidase activity and cell viability

The organoselenium compounds did not show any significant effects in tests involving Fe(II)-chelating properties, free radical scavenging, thiol-oxidase activities and cellular viability (data not shown).

3.4. Total antioxidant activity

The curve of ascorbic acid was determine utilizing the concentration 5, 10, 20, 40 and 80 μM represented at Fig. 4 as the letters a–e. The diselenides at 400 μM showed total antioxidant activity similar to ascorbic acid at 10, 20 and 40 μM. Similarly, the monoselenides at 400 μM demonstrated an antioxidant effect equivalent to that of ascorbic acid at 5, 10 and 20 μM.

3.5. Determination of thiol-peroxidase-like activity

Fig. 5 demonstrates the GPx activity of the organoselenium compounds. The compounds C1 (Fig. 5A) and C2 (Fig. 5B) did not present any significant GPx activity when compared with the control group. DMSO alone had no significant effect on the GPx activity. However, our data reveals that DPDS, C3 (Fig. 5C) and C4 analogs (Fig. 5D) at both concentrations tested demonstrated GPx-like activity.

3.6. NADPH oxidation by TrxR using C1–C4 as substrates

The monoselenides did not show TrxR activity, while the diselenides demonstrated a significant difference compared to the control group. As shown in Fig. 6, C3 and C4 demonstrated 13 and 7 times higher TrxR activity, respectively, than the control.

4. Discussion

The present study aimed to investigate and clarify the antioxidant properties of novel mono- and diselenides compounds. Oxidative stress is involved in various metabolic disorders and in the normal process of aging (Giles et al., 2012; Mugesh et al., 2001). Additionally, antioxidant therapy has been used in an attempt to repair these harmful effects (Nogueira and Rocha, 2011; Zadra et al., 2012). In this context, lipid peroxidation products MDA and 4-hydroxynonenal have been shown to play significant roles in brain and liver toxicities and can serve as markers of oxidative damage (Chen et al., 2005). Prestes reported that monoselenides, which possess an amino group near the selenium, exhibited decreased MDA formation compared to that found for DPDS (Prestes et al., 2012). The novel mono- and diselenides compounds examined in our study demonstrated antioxidant activity against Fe (II)- and SNP-induced lipid peroxidation in rat brain and liver homogenates. We also showed that the β-selenoamines had a similar antioxidant effect as the diselenides in rat liver homogenates following Fe(II)-induced oxidation. The antioxidant effect on lipid peroxidation demonstrated by the diselenide compounds was more pronounced than that of the monoselenide compounds. These results support the assumption that the presence of the amino group decreases selenol formation.

Additionally, using a total antioxidant activity assay, we demonstrated that the diselenides presented a greater antioxidant activity than the monoselenides when compared with equivalents of ascorbic acid. The presence of an amino group in the structure of organoselenium compounds was shown to reduce their antioxidant activity (Sabir et al., 2012). Conversely, the inclusion of a methyl and a methoxy group in the diselenides C3 and C4 does not interfere in the antioxidant activity and most likely maintains the formation of the two selenol structures.

Similarly, the effect of antioxidant compounds on DPPH radical scavenging is involved with their capacity to donate a hydrogen atom. Ogunmoyole et al. reported that DPDS had no significant effect on ability to decolorize the DPPH•, and Prestes et al. reported that β-selenoamines had negligible antioxidant properties in the DPPH assay (Ogunmoyole et al., 2009; Prestes et al., 2012). Thus, in the present study, we also demonstrated that the novel mono- and diselenides did not present any scavenger effects on DPPH radicals, suggesting that the antioxidant mechanism of action of mono- and diselenides may not be related to their ability to donate an electron or hydrogen radical.

Similarly, reducing power is related to the mechanism by which antioxidant agents transfer an electron or hydrogen atom to oxidants or free radicals (Ogunmoyole et al., 2009). Thus, it is possible to assert that the compounds tested in the Fe(II)-chelating assay

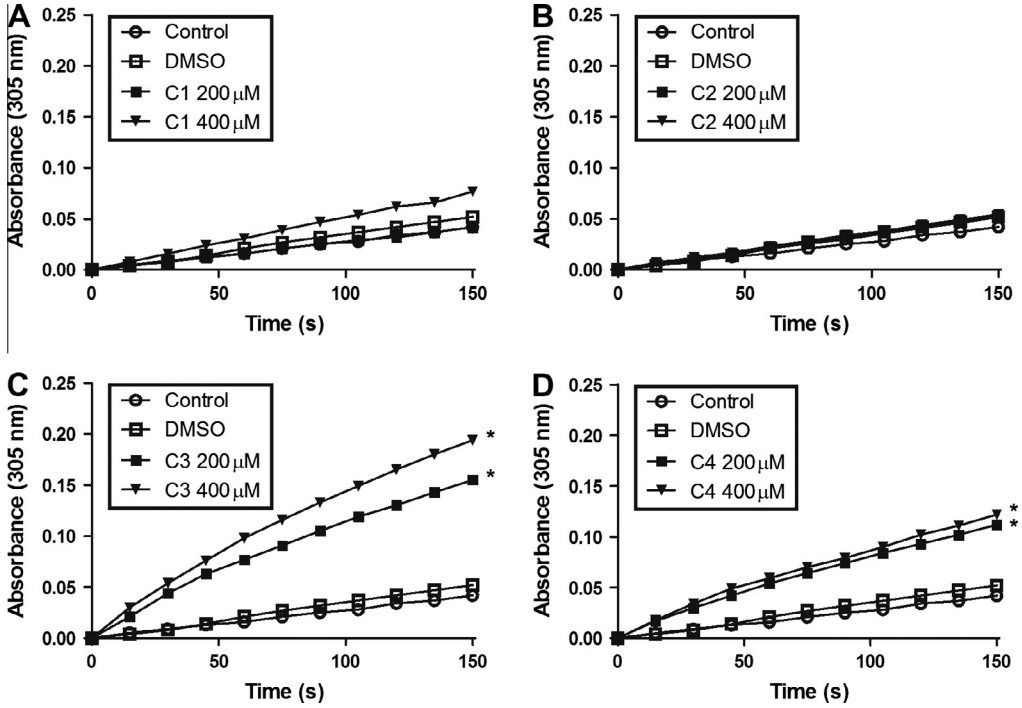

Fig. 5. Determination of thiol-peroxidase-like activity of the organoselenium compounds C1 (A), C2 (B), C3 (C) and C4 (D) at 200 and 400 μM. * Significant difference when compared with the control group by Duncan's multiple range test. Standard error values were omitted for the sake of clarity, and they were <5% of the respective means for 5 individual experiments (3 replicates each).

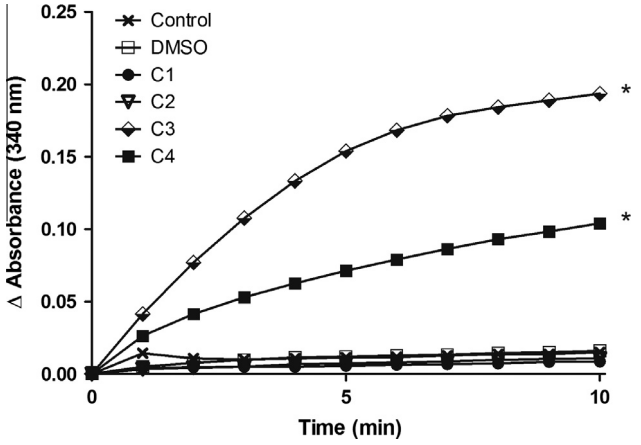

Fig. 6. TrxR activity of the compounds. * Significant difference when compared with the control group by Duncan's multiple range test. Standard error values were omitted for the sake of clarity, and they were <5% of the respective means for 5 individual experiments (3 replicates each).

did not generate significant results due to their inability to donate electron or hydrogen atoms.

Studies in the literature report that organoselenium compounds can cause several toxic effects. These effects are associated with the catalytic oxidation of thiol groups from GSH or from different proteins or enzymes (Meotti et al., 2003; Nogueira et al., 2003a,b). Thus, thiol group oxidation might cause enzyme activity inhibition and can contribute to cellular toxicity (Nogueira and Rocha, 2010). Santos suggested that organochalcogens exhibit hemolytic and genotoxic actions in blood cells, which are most likely linked to their thiol oxidase activity and preferential interaction with sulfhydryl groups critical to enzyme function (Santos et al., 2009). However, when we tested the novel mono- and diselenides, we did not observe any toxic effects in the cellular viability of human leukocytes. Similarly, the compounds examined in this study showed no significant difference in the thiol oxidase activity when compared with the basal group. In agreement with these observations, we propose that the novel mono- and diselenides compounds have a low toxic potential because they did not demonstrate any genotoxic effects and did not interact with sulfhydryl groups in the same way as the classic organochalcogens.

Furthermore, we showed that the novel diselenides demonstrated mimetic GPx-like activity as well as increased TrxR activity when analyzed in vitro. The GPx enzyme neutralizes the toxic or signaling effects of hydrogen and lipid peroxides (Arthur, 2000), which is consistent with the fact that the novel diselenides, by having GPx-like activity, also had a significant inhibitory effect on lipid peroxidation in brain and liver homogenates.

Similarly, TrxR exhibits a broad substrate specificity and can therefore reduce many low molecular weight compounds, including hydrogen peroxide and lipid hydroperoxides (Li et al., 2008). Thus, according to the results obtained for the diselenides, it is possible that increased TrxR activity can be associated with a lipid peroxidation inhibitory effect.

Therefore, we hypothesize that the effects presented in this study for the C3 and C4 compounds, the GPx mimetic effect, and the increased TrxR activity should most likely be attributed to the formation of selenol groups, such as p-methyl-selenol and o-methoxy-selenol.

However, the presence of the basic amino acid inclusion in the monoselenides did not allow the formation of selenol groups, which explains the lack of GPx and TrxR activity. Therefore, the monoselenide effects obtained in the TBARS assay as well as the total antioxidant capacity may simply be due to the nucleophilicity of the amino group near the selenium (Hassan et al., 2012).

In conclusion, structural additions made in classical organoselenium compounds allow the elucidation of antioxidant mechanisms involved in these compounds, enabling the discovery of new drugs. We observed that the inclusion of the amino group in the monosel-

enides resulted in an antioxidant effect, but this effect was not as significant as that observed for the diselenides, which most likely have a higher antioxidant effect due to the formation of selenol groups, as well as their mimetic GPx activity and their elevated TrxR activity.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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