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ORIGINAL ARTICLE

Antioxidant effect of functionalized alkyl-organotellurides: a study *in vitro*

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Abstract

We evaluated the *in vitro* antioxidant effect of alkyl-organotellurides **A–D** on lipid peroxidation and protein carbonylation in rat liver homogenates. The thiol oxidase and thiol peroxidase-like activities of compounds were investigated. δ-Aminolevulinic acid dehydratase (δ-ALA-D) activity was determined in rat liver homogenates. Compounds **A–D** protected against lipid peroxidation induced by Fe^{2+}/EDTA and sodium nitroprusside (SNP). According to the confidence limits of the IC_{50} values of compounds **A–D**, the IC_{50} values for organotellurides followed the order: **C** (0.30 μM) ≤ **B** (0.40 μM) < **D** (0.68 μM) < **A** (2.90 μM), for Fe^{2+}/EDTA, and **B** (0.21 μM) ≤ **C** (0.33 μM) ≤ **D** (0.43 μM) < **A** (1.21 μM) for SNP-induced lipid peroxidation. Compounds **A–D** reduced protein carbonyl content to control levels. The results demonstrated an inverse correlation between thiol oxidase and δ-ALA-D activities. This study supports an antioxidant effect of organotellurides **A–D** on rat liver.

Keywords: *Functionalized alkyl-organotellurides; antioxidant; tellurium; liver lipid peroxidation*

Introduction

Evidence has been provided that organochalcogenides, such as organoselenium and organotellurium compounds, are promising pharmacological agents[1–4]. Organotellurium compounds have been synthesized since 1840[5], and it is known that these compounds are much more effective bioactive agents than their corresponding selenium and sulfur analogs, with evident chemoprotective, antioxidative, and cytotoxic properties[3,6], making these compounds extremely attractive in medical therapies. However, little is known about the pharmacological and toxicological effects of these compounds[7,8]. Engman *et al.*[1,9] have investigated the possible anti-cancer properties of organotellurium compounds.

Several authors have reported that organotellurium compounds can exhibit antioxidant activity because of their glutathione peroxidase (GPx) mimetic properties[10] and peroxynitrite scavenger abilities[11]. Moreover, studies have shown an antioxidant activity *in vitro*[11–13] and low toxicity *ex vivo*[11,13,14] of organotellurium compounds.

Oxidative stress is characterized by a significantly increased concentration of intracellular oxidizing species, such as reactive oxygen species (ROS), and is often accompanied by the simultaneous loss of antioxidant defense capacity[15,16]. In pathologic conditions an overproduction of ROS can occur[17]. Besides that, ROS overproduction has been implicated in the installation and/or progression of a variety of human diseases[18,19] including diabetes[20], cancer[21], and various neurodegenerative diseases[22]. Thus, interest in the synthesis of antioxidant compounds that could potentially retard the development of diseases associated with oxidative stress has grown considerably in recent decades[23,24].

In this study, we evaluated the *in vitro* antioxidant effect of functionalized alkyl-organotellurides **A–D** against lipid peroxidation and protein carbonylation in rat liver homogenates. In addition, we examined the thiol peroxidase-like activity of functionalized alkyl-organotellurides **A–D** to determine the possible mechanism by which these compounds demonstrate antioxidant property. Furthermore, δ-aminolevulinic dehydratase (δ-ALA-D) and thiol oxidase activities were determined in rat liver homogenates.

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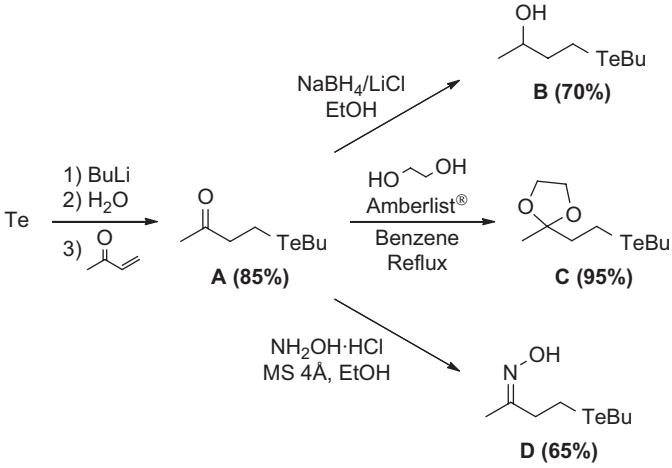
Materials and methods

Synthesis of alkyl-organotellurides

Telluro-ketone **A** was synthesized by hydrotelluration of methyl vinyl ketone in 86% isolated yield by reacting elemental tellurium with *n*-butyllithium, followed by the addition of deoxygenated water and the enone as reported previously[25,26]. Tellurides **B** and **C** were prepared in good yields according to our previous protocols[27–31] from telluride **A**. Finally, telluride **D** was prepared (65% isolated yield) from compound **A** by reacting with hydroxylamine hydrochloride[32]. Scheme 1 summarizes the preparation of the mentioned compounds.

4-(Butyltellanyl)butan-2-one (A)

In a two-necked 50 mL flask under a nitrogen atmosphere were added sequentially elemental tellurium (0.511 g, 4.0 mmol) and dry tetrahydrofuran (THF; 5 mL). To the resulting mixture under vigorous stirring, at room temperature, was added *n*-butyllithium (4.0 mmol, 1.6 mol L^{-1} solution in hexane, 1.0 eq.). To the resulting pale yellow clean solution was added deoxygenated water (0.18 mL, 10 mmol). Methyl vinyl ketone (0.280 g, 4.0 mmol) was added in a portion at room temperature with vigorous stirring. The resulting mixture was stirred for 1 h at room temperature then quenched with water and diluted with hexane/ethyl acetate (5 mL, 1:1). The organic and aqueous phases were separated and the organic phase was washed with NH_4Cl solution (3 × 15 mL) and brine (3 × 15 mL) and then dried with $MgSO_4$. The solution was filtered and the solvent was evaporated. The residue was purified by silica gel column chromatography, eluting first with hexane and then with hexane/ethyl acetate (10:1). Yield: 0.87 g (86%). ^{1}H NMR: (300 MHz, $CDCl_3$, ppm) δ 0.92 (t, *J* = 7.4 Hz, 3H), 1.38 (sext, *J* = 7.4 Hz, 2H), 1.73 (q, *J* = 7.4 Hz, 2H), 2.15 (s, 3H), 2,69 (m, 4H), 3.03 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR: (75 MHz, $CDCl_3$, ppm) δ 207.8, 46.4, 34.3, 29.8, 25.0, 13.3, 3.4, –7,0. ^{125}Te MNR: (157 MHz, $CDCl_3$, ppm, $(PhSe)_2$) δ 279.6. LRMS: *m/z* (70 eV, rel. int.): 258 (M^+, 7), 71 (40), 57 (24), 55 (26), 43 (100). IR: (ZnSe, cm^{-1}) 2955, 2925, 2865, 1715. Anal. Calc.: C, 37.56; H, 6.30. Found: C, 37.32; H, 6.02%.



Scheme 1. Chemical structures of functionalized alkyl-organotellurides **A**, **B**, **C**, and **D**.

4-(Butyltelluro)butan-2-ol (B)

To a 125 mL round-bottomed flask were added telluride **A** (1.28 g, 5 mmol) and ethanol (20 mL). To the resulting solution was added an ethanolic solution of $NaBH_4$ (0.21 g, 5.5 mmol in 6 mL ethanol). The mixture was stirred at room temperature and the progress of the reaction was monitored by thin layer chromatography (TLC). The reaction mixture was quenched with water (50 mL) and diluted with a mixture of hexane/ethyl acetate 1:1 (50 mL). The phases were separated and the aqueous phase was extracted with a mixture of hexane/ethyl acetate (1:1, 2 × 50 mL). The organic phases were combined, dried with $MgSO_4$, and filtered and the solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography eluting with a mixture of hexane/ethyl acetate (15:1). Yield: 0.89 g (70%). ^{1}H NMR: (200 MHz, $CDCl_3$, ppm) δ 0.85 (t, *J* = 7.2 Hz, 3H), 1.13 (d, *J* = 6.3 Hz, 3H), 1.31 (sext, *J* = 7.2 Hz, 2H), 1.65 (quint, *J* = 7.2 Hz, 2H), 1.76–1.85 (m, 2H), 2.53–2.69 (m, 4H), 3.75 (sext, *J* = 6 Hz, 1H). ^{13}C NMR: (50 MHz, $CDCl_3$, ppm) δ 69.1, 41.1, 34.2, 25.0, 23.2, 13.4, 2.7, –2.3. ^{125}Te NMR: (157 MHz, $CDCl_3$, ppm, $(PhTe)_2$) δ 251.43. LRMS: *m/z* (70 eV, rel. int.) 260 (M^{+2}, 13), 258 (M^+, 13), 256 (7), 255 (3), 254 (2), 215 (3), 186 (8), 72 (5), 57 (73), 55 (100), 45 (44).

2-(Butyltellanyl)ethyl-2-methyl-1,3-dioxolane (C)

To a 50 mL two-necked flask equipped with a Dean–Stark reflux apparatus were added telluride **A** (1.28 g, 5 mmol), benzene (10 mL), ethylene glycol (0.44 mL, 8 mmol) and Amberlist® (30 mg). The reaction mixture was refluxed under a nitrogen atmosphere for 5 h. The mixture was filtered and the residue was washed with ethyl acetate. The solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography eluting with a mixture of hexane/ethyl acetate (15:1). Yield: 1.41 g (96%). ^{1}H NMR: (300 MHz, $CDCl_3$, ppm) δ 0.92 (t, *J* = 7.3 Hz, 3H), 1.32 (s, 3H), 1.38 (sext, *J* = 7.3 Hz, 2 H), 1.72 (quint, *J* = 7.3 Hz, 2 H), 2.10–2.16 (m, 2 H), 2.60–2.66 (m, 4 H), 3.95 (m, 4 H). ^{13}C NMR: (75 MHz, $CDCl_3$, ppm) δ 110.2, 64.7, 42.1, 34.2, 25.0, 23.5, 13.3, 2.7, –5.6. ^{125}Te NMR: (157 MHz, $CDCl_3$, ppm, $(PhTe)_2$) δ 263.3. LRMS: *m/z* (70 eV, rel. int.) 302 (M^+, 3), 99 (4), 87 (100), 55 (15), 43 (58). IR: (film, cm^{-1}) 2979, 2958, 2927, 2874, 1457, 1378, 1246, 1212, 1040, 944. Anal. Calc.: C, 40.13; H, 6.68. Found: C, 40.35; H, 6.46%.

4-(Butyltellanyl)butan-2-one oxime (D)

To a 50 mL round-bottomed flask were added telluride **A** (1.28 g, 5 mmol), hydroxylamine hydrochloride (0.552 g, 7.5 mmol), molecular sieves 3Å (1.25 g), and ethanol (20 mL). The mixture was stirred at room temperature, and the progress of the reaction was monitored by TLC. The mixture was filtered and the residue washed with hexane/ethyl acetate 1:1 (20 mL). The organic phase was sequentially washed with $NaHCO_3$ (sat. sol., 2 × 20 mL), water (2 × 20 mL), and brine (20 mL). The organic phases were combined, dried with $MgSO_4$, and filtered and the solvents were removed under reduced pressure. The residue was purified by silica gel column chromatography, eluting with a mixture of hexane/

ethyl acetate (15:1). Yield: 0.88 g (65%). ^{1}H NMR: (300 MHz, $CDCl_3$, ppm) δ 0.91 (t, *J* = 7.2 Hz, 3H), 1.38 (sext, *J* = 7.2 Hz, 2H), 1.73 (quint, *J* = 7.2 Hz, 2H), 1.90 (s, 3H), 2.61–2.76 (m, 6H). ^{13}C NMR: (75 MHz, $CDCl_3$, ppm) δ 158.3, 79.4, 38.7, 34.1, 24.9, 13.4, 13.2, 2.8, –3.4. LRMS: *m*/*z* (70 eV, rel. int.) 271 (M^{+1}, 11), 256 (6), 216 (44), 199 (7), 185 (5), 171 (4), 157 (2), 145 (6), 130 (8), 86 (44), 70 (16), 57 (100).

Chemicals

The chemical purity of organotellurides (99.9%) was determined by gas chromatography/high performance liquid chromatography (GC/HPLC). δ-Aminolevulinic acid (δ-ALA), *p*-dimethylaminobenzaldehyde, and reduced glutathione (GSH) were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers. Alkyl-organotellurides were dissolved in dimethylsulfoxide (DMSO).

Animals

Male adult Wistar rats, weighing 200–300 g, from our own breeding colony were used. The animals were kept in a separate animal room, on a 12 h light/dark cycle, at a room temperature of 22 ± 2°C, with free access to food (Guabi, RS, Brazil) and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, Federal University of Santa Maria, Brazil.

Tissue preparation

Rats were euthanized and the liver tissue was rapidly dissected, placed on ice, and weighed. The liver tissue was immediately homogenized in cold 50 mM Tris-HCl, pH 7.4 (1/10, w/v). An aliquot of homogenate was used for the protein carbonyl assay. The homogenate was centrifuged at 2400 × *g* at 4°C for 10 min and a low-speed supernatant (S_1) was used for lipid peroxidation and δ-ALA-D assays.

Thiol peroxidase-like and thiol oxidase assays were performed in the absence of S_1.

Lipid peroxidation

This assay was carried out to determine the *in vitro* antioxidant effect of functionalized alkyl-organotellurides **A–D**. $FeCl_2$/ethylenediaminetetraacetic acid (EDTA) or sodium nitroprusside (SNP) was used as the classical inductor of lipid peroxidation. Thiobarbituric acid reactive species (TBARS) were used as a measure of lipid peroxidation and determined as described by Ohkawa *et al*.[33]. An aliquot of 200 µL of S_1 was added to the reaction mixture containing: 100 µM $FeCl_2$/50 µM EDTA or 50 µM SNP, 50 mM Tris-HCl, pH 7.4, and alkyl-organotellurides **A–D** at different concentrations (0.1–1 µM). The range of concentrations used for compound **C** was 0.001–1 µM. Compound **A** was used in a different range of concentrations in the lipid peroxidation assay induced with $FeCl_2$/EDTA (0.1–5 µM), and the range of concentrations for compound **B** was 0.01–1 µM for lipid peroxidation induced with SNP. Vitamin E and trolox (0.1–50 µM) were used as positive controls. Afterward the mixture was pre-incubated for 1 h at 37°C. The reaction product was determined using 500 µL thiobarbituric acid (0.8%), 200 µL 8.1% sodium dodecyl sulfate (SDS), and 500 µL acetic acid buffer (pH 3.4), and incubated at 95°C for 2 h. The absorbance of samples was determined at 532 nm. Results are reported as nmol MDA (malondialdehyde) per gram of tissue.

Protein carbonyl content

Oxidatively modified proteins present an enhancement of carbonyl content[34]. This assay was utilized to determine the *in vitro* antioxidant effect of functionalized alkyl-organotellurides **A–D** against protein carbonylation induced by SNP. Carbonyl content was assayed by a method based on the reaction of protein carbonyls with 2,4-dinitrophenylhydrazine (DNPH) forming dinitrophenylhydrazone, a yellow compound[35]. Briefly, homogenate was diluted 1:10 (v/v) and incubated for 2 h with alkyl-organotellurides **A–D** at different concentrations (0.001–1 µM) and SNP (50 µM). Vitamin E (0.5 µM–5 mM) and trolox (0.5–100 µM) were used as positive controls. Then, 1 mL aliquots were mixed with 200 µL of 10 mM DNPH or 200 µL 2M HCl. After incubation at room temperature for 1 h in a dark environment, 500 µL of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing 3% SDS), 1.5 mL of heptane (99.5%), and 1.5 mL of ethanol (99.8%) were added sequentially, and mixed with vortex agitation for 40 s and centrifuged for 15 min. Next, the protein isolated from the interface was washed twice with 1 mL of ethyl acetate/ethanol 1:1 (v/v) and suspended in 1 mL of denaturing buffer. Each DNPH sample was read at 370 nm in a spectrophotometer against the corresponding HCl sample (blank), and the total carbonylation was calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1} according to Levine *et al*.[35]. The results are expressed as nmol carbonyl per millgram of protein.

Thiol peroxidase-like activity

The thiol peroxidase-like activity was examined to determine the possible mechanism by which functionalized alkyl-organotellurides **A–D** demonstrate antioxidant property. Free –SH groups were determined according to Ellman[36]. The catalytic effect of alkyl-organotellurides **A–D** on the reduction of H_2O_2 by GSH was assessed using the rate of GSH oxidation. Alkyl-organotellurides **A–D** at different concentrations (0.1–100 µM) were incubated in the medium containing GSH (1.0 mM), with and without H_2O_2 (0.3 mM). At 120 min, aliquots of the reaction mixture (200 µL) were checked for the amount of GSH. The results are expressed as percentage of control.

δ-Aminolevulinic dehydratase activity

δ-ALA-D has been reported as being sensitive to pro-oxidants and heavy metals[37]. Since this enzyme is very sensitive to organochalcogen compounds, δ-ALA-D activity was used as a marker of toxicity. δ-ALA-D activity was assayed

by the method of Sassa[38] with some modifications. The principle of this method is based on enzyme incubation with an excess of δ-ALA. An aliquot of 200 µL of S_1 was pre-incubated for 10 min at 37°C in the presence or absence of functionalized alkyl-organotellurides **A–D** at different concentrations (0.1–100 µM). Enzymatic reaction was initiated by adding the substrate, δ-ALA, to a final concentration of 2.2 mM in a medium containing 45 mM potassium phosphate buffer, pH 6.8. The incubation was carried out for 1 h at 37°C and stopped by adding trichloroacetic acid solution (10% TCA) with 10 mM $HgCl_2$. Porphobilinogen (PBG), formed within a fixed time, was mixed with modified Ehrlich's reagent, and the color developed was measured photometrically (555 nm) against a blank. The results are expressed as nmol PBG per milligram of protein per hour.

Thiol oxidase activity

The thiol oxidase activity was examined to determine the pro-oxidant property of functionalized alkyl-organotellurides **A–D**. The rate of thiol oxidation was determined in the presence of 50 mM Tris-HCl, pH 7.5, and alkyl-organotellurides **A–D** at different concentrations (0.1–100 µM). Incubation at 37°C was initiated by the addition of 1.0 mM reduced glutathione (GSH). Aliquots of the reaction mixture (200 µL) were checked for the amount of –SH groups at 412 nm. The rate of thiol oxidation was evaluated by measuring the disappearance of –SH groups. Free –SH groups were determined according to Ellman[36]. Results are expressed as percentage of control.

Correlation between thiol oxidase and δ-ALA-D

Since the mechanism of δ-ALA-D inhibition may be linked to the oxidation of its sulfhydryl groups[39], the correlation between δ-ALA-D activity and thiol oxidase property of functionalized alkyl-organotellurides **A–D** was performed. Through this relationship, we wanted to verify whether the mechanism of δ-ALA-D inhibition by organotellurides was related to the oxidation of cysteinyl residues located at the active site of the enzyme.

Protein determination

Protein was measured by the Coomassie blue method according to Bradford[40] using bovine serum albumin as standard.

Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test when appropriate. Results were considered significantly different at values of $p < 0.05$. The IC_{50} values (inhibitory concentrations) were calculated by the probit method using computer software SAS/STAT, and are reported as geometric means accompanied by their 95% confidence limits. Maximal inhibition (I_{max}) values were calculated at the most effective dose used. Pearson's correlation coefficient was used for the estimation of correlation between parameters analyzed.

Results

Effect of functionalized alkyl-organotellurides A–D on lipid peroxidation induced by Fe^{2+}/EDTA or SNP

Compound **A** at the concentration of 1.00 µM was effective in protecting against lipid peroxidation induced by Fe^{2+}/EDTA in the liver (Figure 1a). Compounds **D** and **B** were effective at concentrations equal to or greater than 0.50 and 0.25 µM, respectively (Figure 1d and b). Compound **C** at the concentration of 0.01 µM reduced lipid peroxidation induced by Fe^{2+}/EDTA (Figure 1c). According to the confidence limits of the IC_{50} values of compounds **A–D**, the IC_{50} values for functionalized alkyl-organotellurides followed the order: **C** ≤ **B** < **D** < **A**. Compounds **A** (IC_{50} = 2.90 µM), **B** (IC_{50} = 0.40 µM), **C** (IC_{50} = 0.30 µM), and **D** (IC_{50} = 0.68 µM) were more effective in reducing lipid peroxidation induced by Fe^{2+}/EDTA in liver than the classical antioxidants, vitamin E (IC_{50} = 2.47 µM) and trolox (IC_{50} = 22.96 µM) (Table 1). The I_{max} values for compounds **A**, **B**, and **C** were around 70–80%, while for compound **D** the value was 50% (data not shown).

Compound **A** at the concentration equal to or greater than 0.50 µM reduced lipid peroxidation induced by SNP in the liver (Figure 2a). Compounds **D** and **B** were effective at concentrations of 0.25 and 0.10 µM, respectively (Figure 2d and b). Compound **C** at the concentration of 0.01 µM reduced lipid peroxidation induced by SNP (Figure 2c). According to the confidence limits of the IC_{50} values of compounds **A–D**, the IC_{50} values for functionalized alkyl-organotellurides followed the order: **B** ≤ **C** ≤ **D** < **A**. Compounds **A** (IC_{50} = 1.21 µM), **B** (IC_{50} = 0.21 µM), **C** (IC_{50} = 0.33 µM), and **D** (IC_{50} = 0.43 µM) were more effective in reducing lipid peroxidation induced by SNP in liver than vitamin E (IC_{50} = 2.97 µM) and trolox (IC_{50} = 27.20 µM) (Table 1). The I_{max} values for compounds **A–D** were around 70–80% (data not shown).

Effect of functionalized alkyl-organotellurides A–D on protein carbonyl content

Compounds **A** and **C** were effective in protecting against protein carbonylation induced by SNP in liver at the concentrations of 0.50 and 0.10 µM, respectively (Figure 3a and c). Compounds **B** and **D**, at the concentration of 0.25 µM, reduced protein carbonyl content (Figure 3b and d). According to the confidence limits of the IC_{50} values of compounds **A–D**, the IC_{50} values for functionalized alkyl-organotellurides followed the order: **A** = **D** ≥ **B** = **C**. Compounds **A** (IC_{50} = 0.81 µM), **B** (IC_{50} = 0.69 µM), **C** (IC_{50} = 0.71 µM), and **D** (IC_{50} = 0.83 µM) were more effective in reducing protein carbonyl formation in the liver than vitamin E (IC_{50} = 326.34 µM) and trolox (IC_{50} = 65.95 µM) (Table 1). The I_{max} values for compounds **A–D** were around 50% (data not shown).

Effect of functionalized alkyl-organotellurides A–D on thiol peroxidase-like activity

Functionalized alkyl-organotellurides **A–D** at different concentrations (0.1–100 μM) did not present thiol peroxidase-like activity (data not shown).

Effect of functionalized alkyl-organotellurides A–D on δ-ALA-D activity

Functionalized alkyl-organotellurides **A–D**, at the concentration of 100 μM, reduced significantly the δ-ALA-D activity in rat liver homogenates (data not shown).

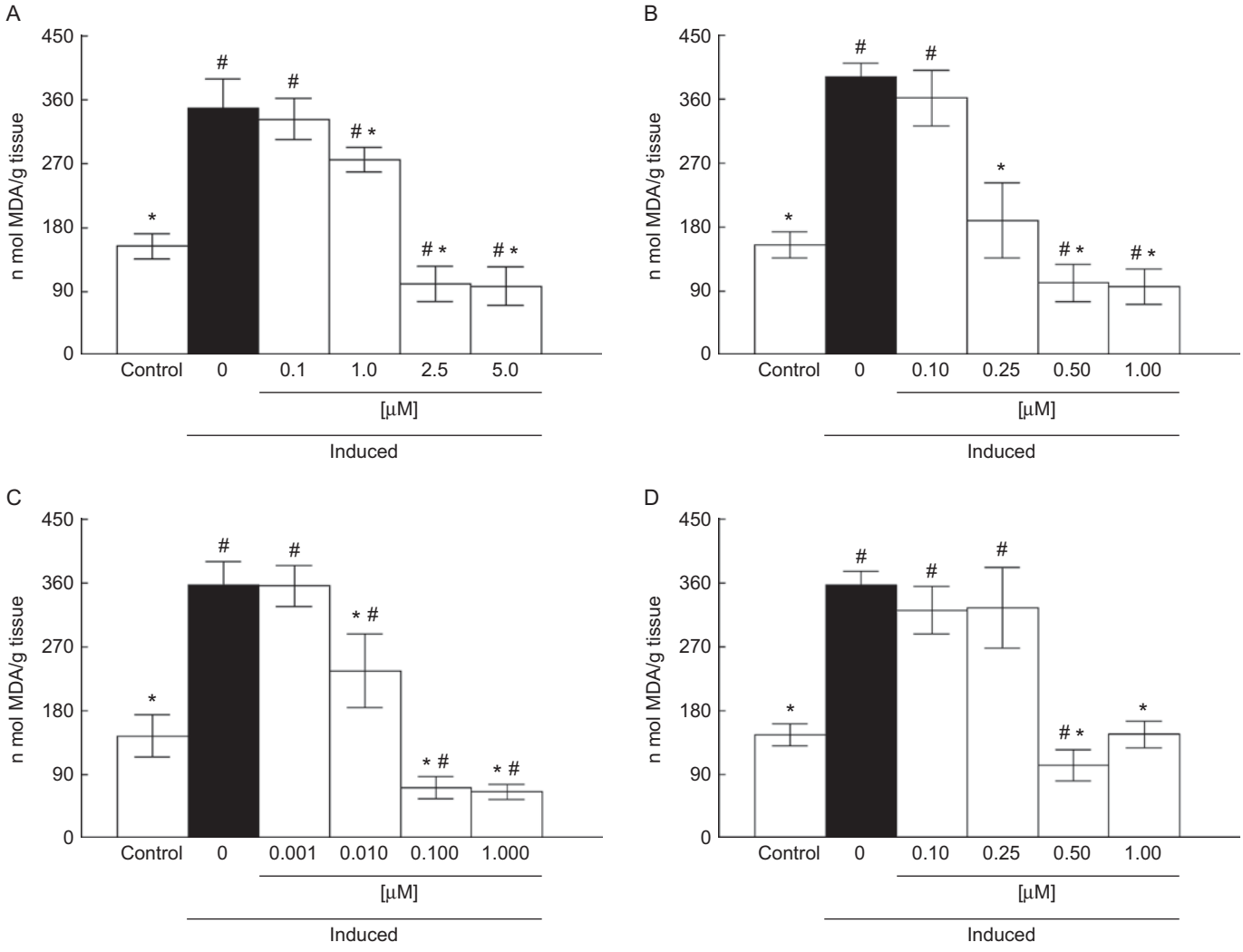


Figure 1. Effect of **A** (0.1–5 μM) (a), **B** (0.1–1 μM) (b), **C** (0.001– 1μM) (c), and **D** (0.1–1 μM) (d) on lipid peroxidation induced with Fe^{2+}/EDTA. Data are reported as mean ± SD for four independent experiments, each one in duplicate. *, Denotes $p < 0.05$ as compared to the sample with Fe^{2+}/EDTA (one-way ANOVA/Duncan). #, Denotes $p < 0.05$ as compared to control values (without Fe^{2+}/EDTA and compounds) (one-way ANOVA/Duncan).

Table 1. Calculated IC_{50} values (μM) for lipid peroxidation and protein carbonyl content of functionalized alkyl-organotellurides in rat liver.

	Lipid peroxidation		Protein carbonyl
Compound	Fe^{2+}/EDTA	SNP	SNP
Vitamin E	2.47 (0.54–4.17)	2.97 (0.49–4.97)	326.34 (288.48–369.53)
Trolox	22.96 (19.92–26.45)	27.20 (18.76–39.55)	65.95 (60.67–71.70)
A	2.90 (2.60–3.20)	1.21 (1.05–1.40)	0.81 (0.72–0.92)
B	0.40 (0.37–0.44)	0.21 (0.19–0.22)	0.69 (0.47–1.02)
C	0.30 (0.24–0.54)	0.33 (0.12–0.89)	0.71 (0.58–0.89)
D	0.68 (0.65–0.71)	0.43 (0.36–0.51)	0.83 (0.70–0.98)

Note. 95% Confidence limit (μM) presented in parentheses.

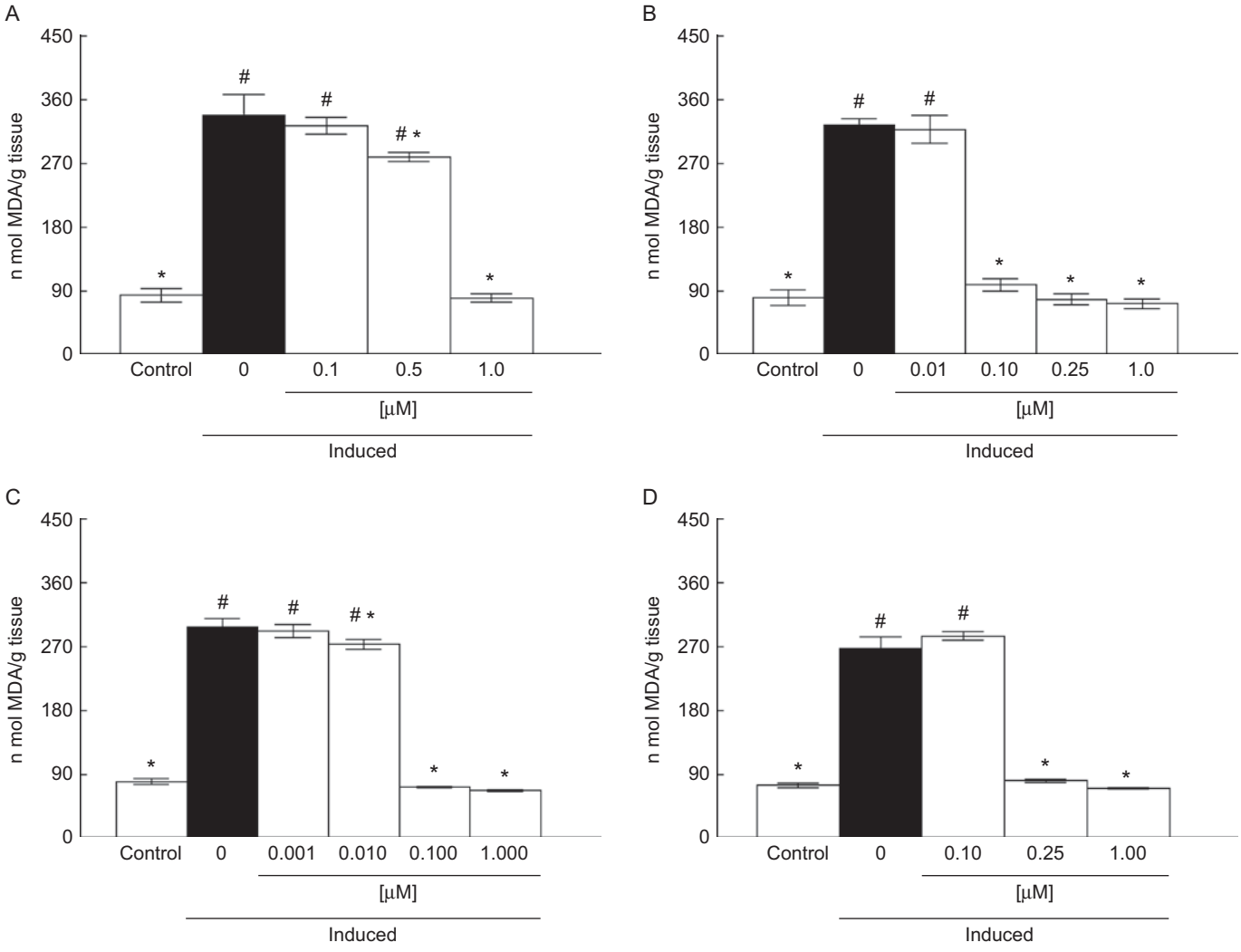


Figure 2. Effect of **A** (0.1–1 μM) (a), **B** (0.01–1 μM) (b), **C** (0.001–1 μM) (c), and **D** (0.1–1 μM) (d) on lipid peroxidation induced with SNP. Data are reported as mean ± SD for four independent experiments, each one in duplicate. *, Denotes $p < 0.05$ as compared to sample with SNP (one-way ANOVA/Duncan). #, Denotes $p < 0.05$ as compared to control values (without SNP and compounds) (one-way ANOVA/Duncan).

Effect of functionalized alkyl-organotellurides A–D on thiol oxidase activity

Functionalized alkyl-organotellurides **A–D**, at the concentration of 100 μM, increased the thiol oxidase activity (data not shown).

Correlation of thiol oxidase and δ-ALA-D activities

The results demonstrated a significant inverse correlation (r = 0.987, $p < 0.005$) between thiol oxidase and δ-ALA-D activity for compound **A** (Figure 4). The r values for alkyl-organotellurides **B**, **C**, and **D**, which also presented significant inverse correlation between these parameters, were r = 0.916, r = 0.970, and r = 0.972, respectively (data not shown).

Discussion

The results of the present study showed that functionalized alkyl-organotellurides **A–D**, at low μM range, displayed an *in vitro* antioxidant activity in rat liver homogenates. Alkyl-organotellurides were able to prevent lipid peroxidation induced by Fe^{2+}/EDTA and SNP in rat liver homogenates. It is known that iron complexes, such as Fe^{2+}/EDTA, stimulate lipid peroxidation in cells[41]. SNP has been suggested to cause cytotoxicity through the release of cyanide and/or nitric oxide[42]. In the present study, we demonstrated that low concentrations of functionalized alkyl-organotellurides were required to reduce lipid peroxidation induced by Fe^{2+}/EDTA or SNP in rat liver homogenates. The IC_{50} values for functionalized alkyl-organotellurides **A–D** of lipid peroxidation induced by Fe^{2+}/EDTA or SNP were close. In this way, we can infer that the antioxidant property of the studied alkyl-organotellurides did not depend on the inductor used. Moreover, compounds **B**, **C**, and **D** demonstrated higher efficiency in reducing lipid peroxidation induced by both inductors than compound **A**, as can be seen by the IC_{50} values. The IC_{50} values for functionalized alkyl-organotellurides followed the order **C** ≤ **B** < **D** < **A** for lipid peroxidation induced by Fe^{2+}/EDTA or SNP. However, it is difficult to clearly explain why compound **A** displayed the lowest efficiency against lipid peroxidation. Based on chemical structures of alkyl-organotellurides **A–D**

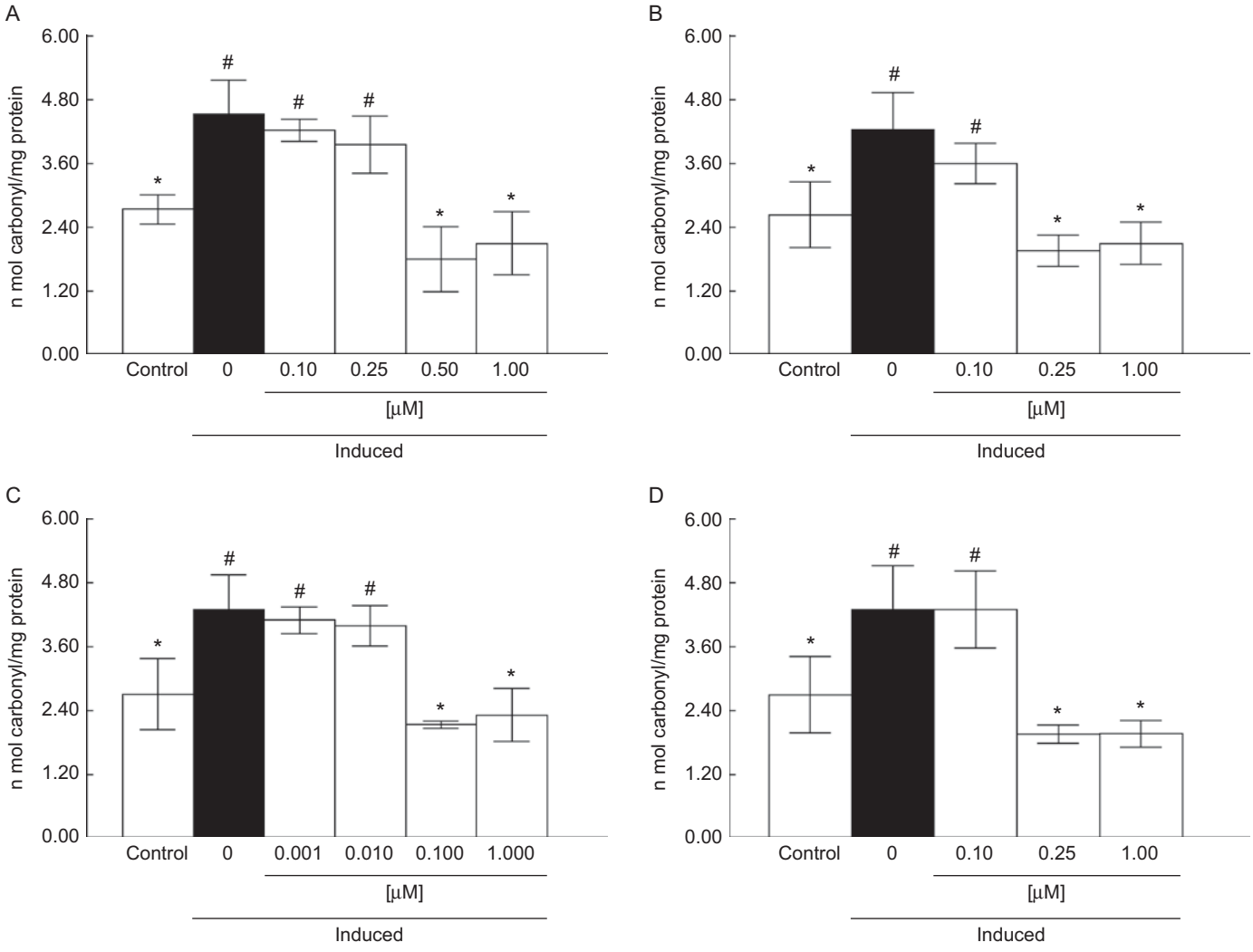


Figure 3. Effect of **A** (0.1–1 μM) (a), **B** (0.1–1 μM) (b), **C** (0.001–1 μM) (c), and **D** (0.1–1 μM) (d) on protein carbonyl content. Data are reported as mean ± SD for four independent experiments, each one in duplicate. *, Denotes $p < 0.05$ as compared to the sample with SNP (one-way ANOVA/Duncan). #, Denotes $p < 0.05$ as compared to control values (without SNP and compounds) (one-way ANOVA/Duncan).

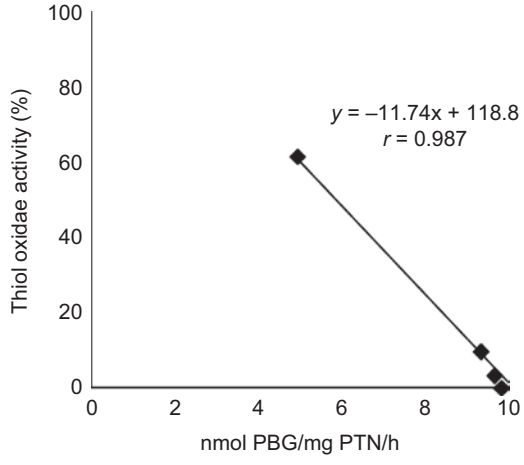


Figure 4. Correlation between thiol oxidase property and δ-ALA-D activity of alkyl-organotelluride **A**.

there is not an obvious explanation for the lowest antioxidant activity of compound **A**. Conversely, the literature data indicate that the chemical structure of organochalcogens has an important role in establishing their application as biological antioxidants[43,44].

Different from the results for lipid peroxidation, alkyl-organotellurides **A**, **B**, **C**, and **D** demonstrated similar effects on protein carbonyl content, suggesting that structural differences did not affect the antioxidant effect on proteins. Furthermore, carbonyl protein is the most used biomarker for protein oxidative damage, and it reflects the cellular damage induced by multiple forms of ROS[45]. Taken together, the results of the current study demonstrated that functionalized alkyl-organotellurides, at low concentrations, displayed an antioxidant effect on lipid and protein oxidation.

Some authors have demonstrated that tellurium compounds are effective antioxidant agents, even more active than their selenium and sulfur analogs[1,46]. The antioxidant activity of diorganoyl tellurides and diselenides[8] has been

attributed, at least in part, to the glutathione peroxidase-like activity of these compounds[10,43]. In contrast, functionalized alkyl-organotellurides **A–D** did not display glutathione peroxidase-like activity, discarding that the antioxidant activity of these compounds could be related to this mechanism. However, other mechanisms could be involved in the antioxidant property of the studied functionalized alkyl-organotellurides[11]. In fact, more studies are necessary to elucidate the exact mechanism involved in the antioxidant effect caused by alkyl-organotellurides **A–D**.

Although functionalized alkyl-organotellurides presented an antioxidant effect, the literature data report that they induce toxicity[7]. In this way, previous works have indicated that alkyl-organotellurides inhibit cysteinyl-containing enzymes, such as Na^+/K^+ ATPase[47], δ-ALA-D[37,39], and squaleno monooxygenase[48]. Thus, the toxicity of organotellurium compounds can be related to interaction with the thiol groups of important biomolecules[8], the replacement of selenium in selenoproteins, such as thioredoxin[1], and the capacity of tellurium compounds to induce the formation of ROS[49].

Surprisingly, the present study demonstrated that functionalized alkyl-organotellurides a–d were good antioxidants, and affected δ-ALA-D activity exclusively at high concentrations. In fact, δ-ALA-D is a group of sulfhydryl-containing enzymes[50], being extremely sensitive to the presence of pro-oxidant elements, and numerous compounds that oxidize sulfhydryl groups, such as selenium and tellurium compounds[39,51,52], modify its activity. Particularly, organotellurium compounds may interact with thiol groups of this enzyme, oxidizing sulfhydryl groups of the active site, changing the configuration of the enzyme and impairing its activity. A previous study from our research group reported that organochalcogen compounds, at low concentrations, reduced δ-ALA-D activity[37,39,51]. Conversely, we demonstrated here that functionalized alkyl-organotellurides a–d did not inhibit δ-ALA-D activity at concentrations for which they displayed antioxidant activity. In fact, these compounds inhibited enzyme activity at the concentration of 100 μM, a concentration 100 times higher than those at which alkyl-organotellurides presented the antioxidant effect. This result highlights this class of compounds as good antioxidant agents with low potential toxicity, demonstrated in rat liver homogenates *in vitro*. The thiol oxidase activity is an estimation of the cytotoxicity of molecules[8]. Organotellurides **A–D** showed thiol oxidase activity only at the concentration of 100 μM, indicating an advantage regarding the possible toxicological properties of these compounds. Recently, our research group reported that the mechanism involved in δ-ALA-D inhibition caused by telluroacetylenes *in vitro* is related to their ability to oxidize sulfhydryl groups from the enzyme structure[52]. In conformity, our results demonstrated an inverse correlation between thiol oxidase and δ-ALA-D activities, meaning that δ-ALA-D activity decreases as the compound's ability to oxidize sulfhydryl groups increases. Therefore, at the concentration for which compounds have the thiol oxidase property, they also inhibit δ-ALA-D activity, suggesting that oxidation of enzyme sulfhydryl groups could be involved in the inhibition caused by functionalized alkyl-organotellurides. Thus, similar to other organochalcogens[7,39,51–53], functionalized alkyl-organotellurides oxidize thiol groups to disulfides[8], clearly demonstrated by the thiol oxidase activity and the results obtained with δ-ALA-D activity in rat liver homogenates *in vitro*.

In conclusion, the results of the present investigation support the *in vitro* antioxidant effect of functionalized alkyl-organotellurides **A–D** in rat liver homogenates. Of particular importance, functionalized alkyl-organotellurides **A–D** inhibited δ-ALA-D activity at a concentration 100 times higher than those for which they displayed the antioxidant effect. Additional studies are necessary to investigate the possible antioxidant effect of these compounds in an *in vivo* model of oxidative damage.

Declaration of interest

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