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Bis selenide alkene derivatives: A class of potential antioxidant and antinociceptive agents

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Abstract

Bis and tris–selenide alkene derivatives, a class of organoselenium compounds, were screened for antinociceptive and antioxidant activities. In vitro, bis–selenide alkene 1c (R=2,4,6-$Me_3C_6H_2$), 1d (R=4-ClC_6H_4) and 1e (R=4-$MeOC_6H_4$) protected against lipid peroxidation about 50%, whereas 1b (R=C_6H_5) and 1a (R=C_4H_9) protected only 23%. Compound 1d presented lesser IC_{50} against lipid peroxidation than other bis–selenide alkene compounds (1d>1e≥1c>1a=1b). The maximal inhibitory effect of tris–selenide alkenes on lipid peroxidation was in the following order 2c>2a=2b. Compound 1e increased the rate of GSH, but not DTT, oxidation. Tris–selenide alkene 2c (R=4-$MeOC_6H_4$) demonstrated the higher rate of thiol oxidation, while 2a (R=C_6H_5) did not change DTT oxidation but oxidized GSH. Conversely, compound 2b (R=4-ClC_6H_4) did not change the rate of GSH oxidation, but oxidized DDT. Bis–selenide alkene derivatives 1c, 1d and 1e were the most promising compounds tested in vitro. In vivo, compounds 1c and 1d (5–50 mg/kg, subcutaneously) produced significant inhibition of acetic acid- and capsaicin-induced pain. Compounds 1c and 1d increased the tail-flick response latency time. The antinociception effect of 1c and 1d was not abolished by naloxone (an antagonist of opioid receptor, 1 mg/kg, subcutaneously), suggesting that the antinociceptive effect is not influenced by the opioidergic mechanism.

Keywords: Organoselenium; Selenium; Antinociception; Antioxidant; Pharmacology

1. Introduction

Oxidative stress (OS) 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 (Arteel and Sies, 2001). Many diseases and degenerative processes can be associated with the overproduction of ROS, including inflammation, brain ischemia, mutagenesis, cancer, dementia and physiological aging (Ren et al., 2001).

Effective antioxidants able to counteract OS are therefore becoming increasingly important in disease prevention and therapy. Among them, compounds with glutathione peroxidase (GPx)-like activity are particularly interesting, since they catalytically remove oxidative stressors and can, therefore, be applied in small quantities (Collins et al., 2005).

Thus, to respond to ROS more effectively, compounds can be envisaged that combine a range of antioxidant activities in one chemically simple molecule. Organoselenium chemistry is a very broad and exciting field with many opportunities for research and development of applications. Organoselenium compounds have become attractive synthetic targets because of their chemio-, regio- and stereoselective reactions (Moro et al., 2005) and their useful biological activity (Nogueira et al., 2004). In fact, a variety of organoselenium compounds with potential antioxidant activity, including ebselen analogues, benzoselenazolinones, diaryl diselenides, selenamide and related derivatives have been reported (Sies, 1993; Yamagushi et al., 1998; Saito et al., 1998; Nogueira et al., 2004). Ebselen is a classical antioxidant and well known as the most important glutathione peroxidase mimetic agent (Muller et al., 1984; Daiber et al., 2000). Moreover, recent data from our laboratory have demonstrated that diphenyl diselenide, a simple

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organochalcogenide, is more active as a glutathione peroxidase mimic and less toxic to rodents than ebselen (Nogueira et al., 2003; Meotti et al., 2004). Furthermore, diorganoil chalcogenides have been described as good antioxidants in vitro and ex vivo (Rossato et al., 2002; Santos et al., 2004, 2005).

Therefore, a number of novel pharmaceutical agents which are selenium-based or which target specific aspects of selenium metabolism are under development (May, 1999; Nogueira et al., 2003; Meotti et al., 2004). Recently, our group reported that diphenyl diselenide produces dose-dependent antinociception in chemical and thermal models of nociception in mice (Nogueira et al., 2003).

Based on the organoselenium chemistry and pharmacological properties presented by synthetic organoselenium compounds, we appraised the antioxidant potential, thiol peroxidase and thiol oxidase activities of recently synthesized bis and tris–selenide alkene derivatives, in vitro. Considering the results obtained in vitro, a second objective of this study was to evaluate the antinociceptive activity induced by compounds 1c and 1d in chemical and thermal models of pain in mice. Moreover, we evaluated the possible involvement of the opioid system in the antinociception action of these compounds.

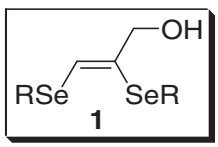
2. Materials and methods

2.1. Drugs

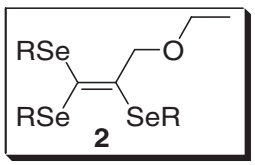
Bis 1a–e and tris–selenide alkene derivatives 2a–c (Scheme 1) were synthesized and characterized according to Moro et al. (2005). Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with their assigned structure. The chemical purity of bis and tris–selenide alkene derivatives (99.9%) was determined by GC/HPLC. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.2. Animals

Male adult Wistar rats (150–200 g) and adult Swiss mice (25–35 g) from our own breeding colony were used. The rats and mice were kept in separate animal rooms, on a 12 h light/dark cycle, at room temperature, with free access to food and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil.



1a - R = C_4H_9
1b - R = C_6H_5
1c - R = 2,4,6-$Me_3C_6H_2$
1d - R = 4-ClC_6H_4
1e - R = 4-$MeOC_6H_4$



2a - R = C_6H_5
2b - R = 4-ClC_6H_4
2c - R = 4-$MeOC_6H_4$

Scheme 1.

2.3. General methods

On the day before behavior tests, mice were habituated for 20 min to the experimental cage (20 × 20 × 20 × 20 cm) to avoid any effect of novelty (Vendite et al., 1990).

2.4. In vitro

2.4.1. Lipid peroxidation

$FeCl_2$ and EDTA were used as classical inductors of lipid peroxidation. Animals were decapitated and whole liver tissues were rapidly homogenized in 50 mM Tris–HCl, pH 7.5 (1/10, w/v), and centrifuged at 4000 *g* at 4 °C for 10 min to yield a low-speed supernatant fraction (S_1). An aliquot of 200 μL of S_1 was added to the reaction mixture containing: 50 μM $FeCl_2$, 100 μM EDTA, bis (1 a–e) or tris–selenide alkene derivatives (2 a–c) at different concentrations (10, 100, 200, 300 and 400 μM). Afterward the mixture was pre-incubated for 1 h/37 °C. After pre-incubation, 500 μL TBA (0.8%), 200 μL SDS (8.1%) and 500 μL acetic acid were added to the reaction medium and the mixture was incubated for 2 h/95 °C. The formation of thiobarbituric acid-reactive species (TBARS) in the reaction was measured by the method of Ohkawa et al. (1979) using MDA as an external standard.

2.4.2. Thiol oxidase activity

Thiol oxidase activity was examined by the investigation of the pro-oxidant property of these compounds. The rate of thiol oxidation was determined in the presence of 50 mM Tris–HCl, pH 7.5, and bis (1 a–e) and tris–selenide alkene derivatives (2 a–c) at different concentrations (10–400 μM). Incubation at 37 °C was initiated by the addition one of the following thiol compounds: reduced glutathione, GSH, (1.0 mM) or dithiotreitol, DTT, (0.5 mM). 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 (1959).

2.4.3. Thiol peroxidase activity

The catalytic effect of bis (1 a–e) and tris–selenide alkene derivatives (2 a–c) on the reduction of H_2O_2 by reduced glutathione were assessed using the rate of GSH oxidation. Free-SH groups were determined according to Ellman (1959).

Bis and tris–selenide alkene derivatives were incubated in the medium containing GSH (1.0 mM) with and without H_2O_2 (0.3 mM). At 0, 30, 60 and 120 min, aliquots of the reaction mixture (200 μL) were checked for the amount of GSH.

2.5. In vivo

Bis selenide 1c and 1d demonstrated the best results in vitro, thus the antinociceptive experiments were performed only with these compounds.

2.5.1. Acetic acid-induced abdominal constriction

The abdominal constriction was induced according to procedures described previously (Nogueira et al., 2003) and resulted in the contraction of the abdominal muscle together with a stretching of the hind limbs in response to an intraperitoneal injection of acetic acid (1.6%) at the time of the test. Dose-response curves determined at the time of the peak effect were constructed in order to assess the antinociceptive activity of the pretreatment compounds 1c and 1d. Thus, the mice were pre-treated with compounds 1c and 1d (5–50 mg/kg) by subcutaneous route (s.c) 30 min before the irritant injection. Control animals received a similar volume of vehicle (1 mL/kg, canola oil).

In the experiment designed to evaluate the possible participation of the opioid system in the antinociceptive effect, animals received a single (s.c) injection of naloxone or saline 40 min before acetic acid administration. The compounds were administered 10 min after naloxone administration and the dose of compounds 1c and 1d used for this set of experiments was based on the mean of ID_{50} values.

After the challenge, mice were individually placed in separate boxes and the abdominal constrictions were counted cumulatively over a period of 20 min. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions, i.e. the difference between control animals (mice pre-treated with vehicle) and animals pre-treated with drugs.

2.5.2. Capsaicin-induced nociception

To evaluate the possible analgesic effect of compounds 1c and 1d on neurogenic pain, we investigated whether compounds 1c and 1d antagonized capsaicin-induced pain in the mouse paw. The procedure was similar to that described by Sakurada et al. (1993). Animals were observed individually for 5 min after capsaicin injection (1.6 μg/paw). The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. Animals were treated with vehicle (canola oil, s.c) or compounds 1c and 1d (5–50 mg/kg, s.c) 30 min before capsaicin injection.

2.5.3. Tail-immersion

The tail immersion test was carried out as described by Janssen et al. (1963). The lower 3.5 cm portion of the tail was marked and the animals were then injected (s.c) with compounds 1c and 1d (5–50 mg/kg) or vehicle (canola oil) 30 min before the test. The reaction was determined by immersing the lower 3.5 cm of the tail into a cup freshly filled with water from a large constant-temperature (55°) bath until the typical tail withdrawal response was observed. A 7-s cut-off was imposed to avoid tail damage by heat. Changes in tail-flick latency, Δt (s), was calculated for each animal according to the formula: Δt (s) = post-drug latency − pre-drug latency (Pinardi et al., 2003).

2.5.4. Rotorod

The rotorod test was carried out to determine if the antinociception effect of compounds 1c and 1d, in chemical and thermal models of nociception, could be related to non-specific disturbances in the locomotor activity of the animal. Briefly, the rotorod apparatus consists of a rod 30 cm long and 3 cm in diameter that is subdivided into three compartments by discs 24 m in diameter. The rod rotates at a constant speed of 10 rpm. The animals were selected 24 h previously by eliminating those mice that did not remain on the bar for two consecutive periods of 60 s. Animals were treated with compounds (50 mg/kg, s.c) or with vehicle (oil canol, s.c) and were retested 30 min after. Time they remained on the rotating bar (maximum 60 s) was recorded (Santos et al., 1999).

2.6. Statistical analysis

The results in vitro are presented as means ± SD. Results of antinociception are expressed as mean ± S.E.M. of antinociception, except the ID_{50} and IC_{50} values which are reported as geometric means accompanied by their 95% confidence limits. The ID_{50} and IC_{50} values were determined by linear regression from individual experiments, using "GraphPad software" (GraphPad software, San Diego, CA). Maximal inhibition values were calculated at the most effective dose used. The statistically significant difference between groups was detected by ANOVA, followed Duncan's test when appropriate. Probability values less than 0.05 ($p < 0.05$) were considered as statistically significant.

3. Results

3.1. Effect of bis and tris–selenide alkene derivatives on lipid peroxidation

Bis–selenide alkene derivatives 1a–e reduced lipid peroxidation at concentrations higher than 200 μM (Table 1). The maximal inhibitory effect was in the following order 1c = 1d = 1e > 1a > 1b. In fact, at 400 μM, bis–selenide alkene 1c, 1d and 1e protected against lipid peroxidation about 50%, whereas 1b protected only 23% (Table 2).

Compound 1d presented lesser IC_{50} against lipid peroxidation than other bis–selenide alkene compounds (1d > 1e ≥ 1c > 1a = 1b) (Table 2).

Compounds 1a and 1b presented poor antioxidant profiles ($IC_{50} > 400$ μM) when compared to 1d, 1c, 1e (Table 2).

The maximal inhibitory effect of tris–selenide alkenes on lipid peroxidation was in the following order 2c ≫ 2a = 2b. In fact, tris–selenide alkene 2c protected (61%) against lipid peroxidation at 400 μM (Table 2) ($p < 0.05$ by Duncan's multiple range test).

Analogous compounds 2a and 2b inhibited lipid peroxidation in a similar manner ($IC_{50} > 400$ μM) (Table 2) reducing only 20% of lipid peroxidation induced by Fe–EDTA in the rat liver (Table 1) ($p < 0.05$ by Duncan's multiple range test).

Compound 2c demonstrated the best antioxidant potential ($IC_{50} = 319$ μM and maximal inhibitory effect was 61%) when compared to the other bis and tris–selenide alkene derivatives tested (Table 2).

Table 1
Effect of bis and tris–selenide alkene derivatives on lipid peroxidation in rat liver

	Basal	Fe^{2+}/EDTA	Concentrations (μM)				
			10	100	200	300	400
1a	41.5±8.8	100.5±0.4*	94.1±13*	87.6±7.2*	70.7±15.1*#	63.1±11.0*#	58.7±11.8#
1b	36.1±3.6	100.7±0.9*	100.2±4*	100.6±4.6*	89.6±5.0*#	84.7±2.2*#	77.2±6.2*#
1c	32.1±6.2	100.1±0.5*	106.1±4*	89.7±1.9*	65.9±13.5*#	56.7±7.4*#	47.4±4.7*#
1d	39.5±4.5	100.7±0.9*	104.2±9*	90.7±12.8*	73.7±16.5*#	55.5±9.1*#	47.1±7.2#
1e	43.1±8.1	100.4±0.4*	85.8±20*	68.1±24.2*	61.2±20.2*#	54.5±13.2*#	49.5±9.2#
2a	40.3±4.3	100.7±0.9*	106.2±9*	93.3±8.2*	84.7±7.3*#	80.1±8.2*#	72.9±10.2*#
2b	34.2±3.4	101.1±1.3*	99.5±4.7*	91.9±6.9*	78.4±13.9*#	68.2±13.2*#	74.2±6.5*#
2c	32.6 ±2.0	100.5±0.5*	99.2±3.2*	80.7±3.4*#	70.7±3.2*#	48.8±10.5*#	39.5±2.6#

Data are reported as mean±SD of five experiments. TBARS (thiobarbituric acid reactive species) are expressed as percentage of Fe–EDTA group (1200±200 nmol MDA/g tissue). (*) denotes $p<0.05$ as compared to basal (without Fe–EDTA or drug) and (#) to Fe–EDTA group (ANOVA/Duncan).

3.2. Effect of bis and tris–selenide alkene derivatives on thiol–peroxidase activity

Bis and tris–selenide alkene derivatives did not present thiol peroxidase activity (data not shown).

3.3. Effect of bis and tris–selenide alkene derivatives on thiol oxidase

Compound 1e increased the rate of GSH oxidation by only 20% at 120 min ($p<0.05$ by Duncan's multiple range test). Compounds 1a, 1b, 1c and 1d did not change the rate of GSH oxidation. Similarly, all bis–selenide alkene derivatives tested did not alter DTT oxidation (data not shown).

Tris–selenide alkene derivatives 2a (Fig. 1B) and 2c (Fig. 2B), at concentrations higher than 100 μM, significantly increased the rate of GSH oxidation. The maximal rate of GSH oxidation was attained at 400 μM for 2a (40%) and 2c (70%) ($p<0.05$ by Duncan's multiple range test).

Compound 2c demonstrated the highest rate of DTT oxidation (about 50%) (Fig. 2A), while 2a did not change DTT oxidation (Fig. 1A).

Compound 2b did not change the rate of GSH oxidation, but oxidized DDT (Fig. 3A and B) ($p<0.05$ by Duncan's multiple range test).

3.4. Acetic acid-induced abdominal constriction in mice

A time-course analysis of the antinociception effect of compounds 1c and 1d was accomplished. The antinociceptive effect of compounds 1c and 1d reached their peak 30 min after s. c administration and remained significant up to 45 min after the administration (data not shown). Thus, the time point (30 min) of the maximum effect of compounds 1c and 1d was chosen for all further studies. Administration of 1c and 1d in mice, at all tested doses, produced significant inhibition in acetic acid-induced abdominal constriction in comparison to the control group ($p<0.05$, Duncan's test) (Fig. 4A and B).

Table 2
Calculated IC_{50} for lipid peroxidation and maximal lipid peroxidation inhibition for bis or tris–selenide alkene derivatives

Compounds	Maximal inhibition[a] (%)	IC_{50} (μM)
1a	44	>400
1b	23	>400
1c	52	395
1d	51	360
1e	48	389
2a	28	>400
2b	23	>400
2c	61	319

[a] Lipid peroxidation maximal inhibition was calculated using 400 μM compounds.

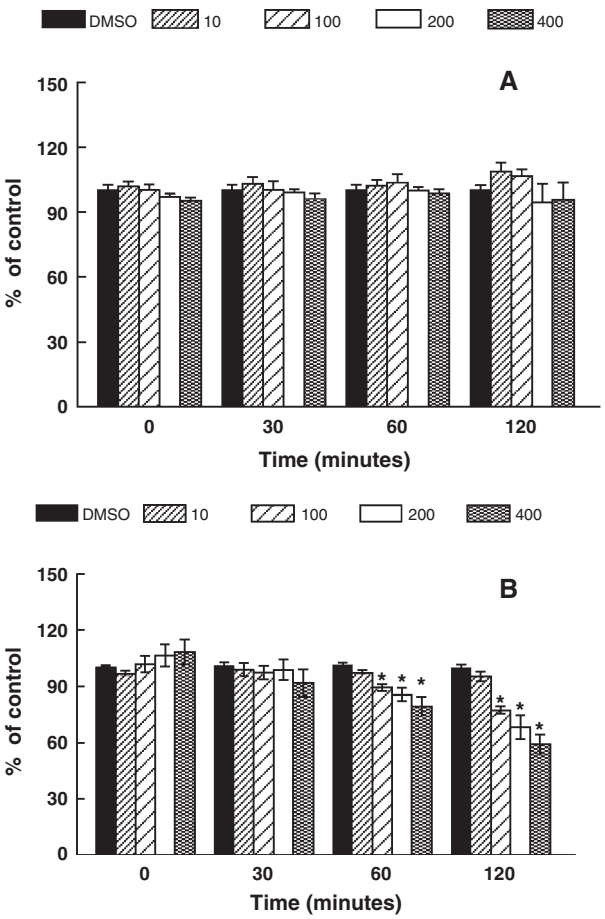


Fig. 1. Effect of compound 2a on the rate of DTT (A) and GSH (B) oxidation. The rate of thiol oxidation was determined in the presence of 50 mM Tris–HCl, pH 7.5, and tris–selenide alkene at different concentrations (10–400 μM). Data are mean±SD of five independent experiments. (*) denotes $p<0.05$ as compared to the control tube (one-way ANOVA/Duncan).

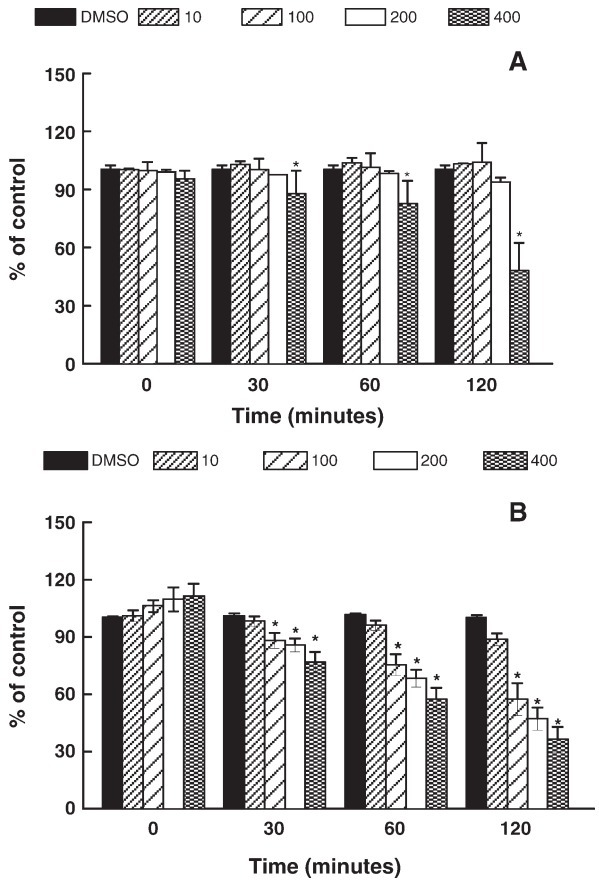


Fig. 2. Effect of compound 2c on the rate of DTT (A) and GSH (B) oxidation. The rate of thiol oxidation was determined in the presence of 50 mM Tris–HCl, pH 7.5, and tris–selenide alkene derivatives at different concentrations (10–400 μM). Data are mean±SD of five independent experiments. (*) denotes $p<0.05$ as compared to the control tube (one-way ANOVA/Duncan).

The mean ID_{50} values from these data were >50 and 14.61 (7.33–29.13) mg/kg, and the maximal inhibition (Imax) attained were 42%±5% and 65%±6% for compounds 1c and 1d, respectively.

3.5. Capsaicin-induced nociception

Compound 1c caused an inhibition of the capsaicin-induced licking/biting response at the doses of 25 and 50 mg/kg ($p<0.05$, Duncan's test), with a mean ID_{50} value of 33.24 mg/kg (26.99–40.95) and an Imax value of 62%±6%. Compound 1d produced an inhibition of capsaicin-induced neurogenic pain at all tested doses ($p<0.05$, Duncan's test), as well (Fig. 5). The mean ID_{50} and Imax values from these results were 5.92 mg/kg (1.11–31.68) and 79%±3%, respectively.

3.6. Tail immersion

As demonstrated in Fig. 6, compounds 1c and 1d administered at doses of 10–50 mg/kg caused a significant increase in tail-flick response latency time as compared to control animals ($p<0.05$, Duncan's test).

3.7. Rotorod test

There was no significant difference between mice treated with the higher dose (50 mg/kg) of compounds (1c and 1d) and the control group in the rotorod test (Table 3).

3.8. Effect of the opioid receptor antagonist on antinociception induced by compounds 1c and 1d

Pre-treatment with naloxone (1 mg/kg, s.c), an antagonist of the opioid receptor, completely abolished the antinociceptive effect of morphine (positive control) at the dose of 2.5 mg/kg, s.c. The antinociceptive effect of compounds 1c (26.91 mg/kg, s.c) and 1d (14.61 mg/kg, s.c) was not prevented by pre-treatment with naloxone (Fig. 7A and B).

4. Discussion

All mammalian life contains reactive oxygen species as the metabolic by-product of O_2 that supports cellular respiration. In certain diseases, the production of reactive oxygen species is enhanced, resulting in reactive oxygen species-mediated cell injury (Ren et al., 2001). Therefore, the overproduction of

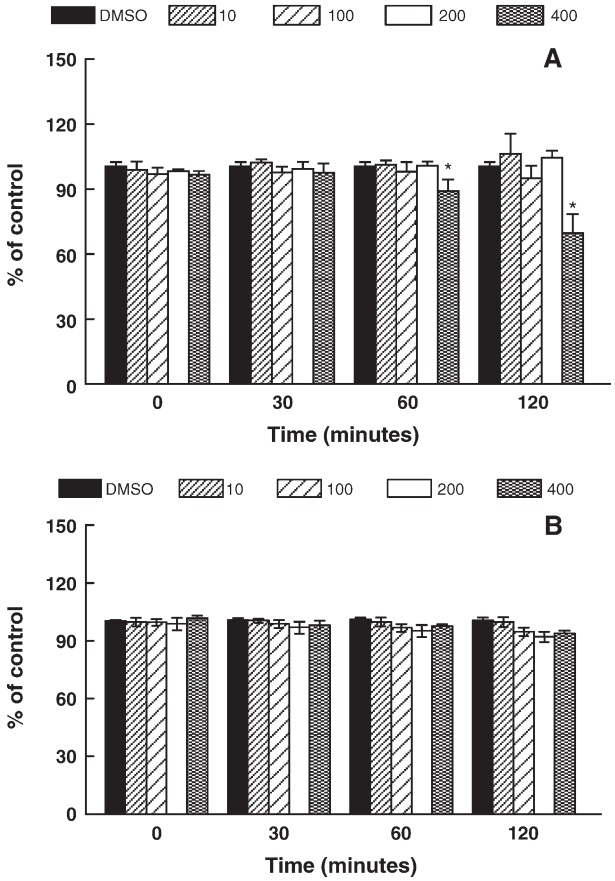


Fig. 3. Effect of compound 2b on the rate of DTT (A) and GSH (B) oxidation. The rate of thiol oxidation was determined in the presence of 50 mM Tris–HCl, pH 7.5, and tris–selenide alkene at different concentrations (10–400 μM). Data are mean±SD of five independent experiments. (*) denotes $p<0.05$ as compared to the control tube (one-way ANOVA/Duncan).

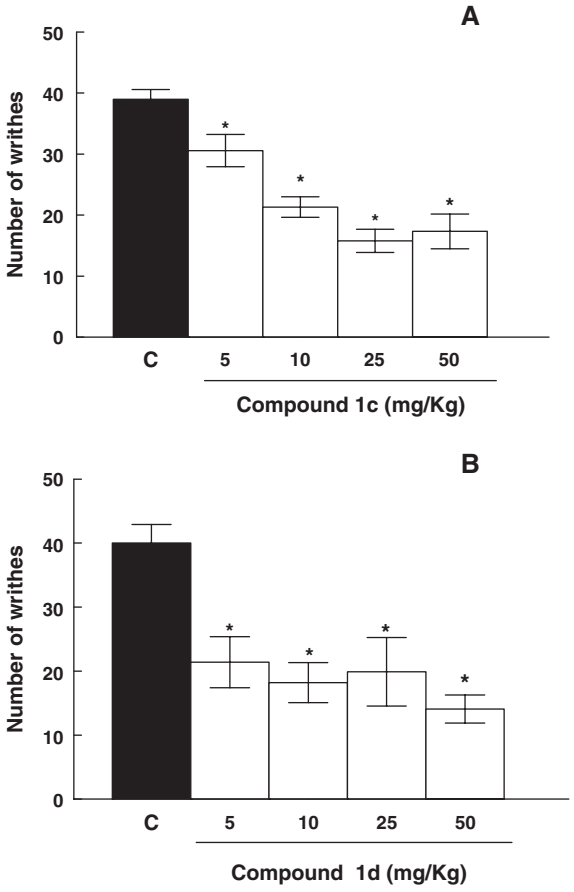


Fig. 4. Effect of compounds 1c (A) and 1d (B) on acetic acid-induced abdominal writhing in mice. Animals were pretreated subcutaneously with compounds at various doses (from 5 to 50 mg/kg) for 30 min prior to the acetic acid (1.6%, i.p). The number of writhing was counted for 20 min following acetic acid injection. Each column represents the mean with S.E.M. for 6–10 mice in each group. (*) denotes $p<0.05$ as compared to respective vehicle (one-way ANOVA/Duncan).

reactive oxygen species has been implicated in a variety of degenerative processes and diseases. Consequently, the interest in natural and synthetic antioxidant compounds that could potentially retard the development of these diseases has grown considerably in the scientific community in the last decades. Accordingly, in the present study we reported the antioxidant activity of bis and tris–selenide alkene derivatives.

A closer inspection of the results revealed that the antioxidant activity is sensitive to substituents on the aromatic ring. Therefore, the compounds containing an aromatic ring without substituents, 1a and 1b, gave the worst antioxidant activity (IC_{50} values are similar 1a = 1b).

The results also demonstrated that the antioxidant activity was significantly dependent on the electronic effects of the substituents on the aromatic ring. Accordingly, Galet and collaborators (1994) reported that electronic or steric parameters on the aromatic group seem to be important to the good antioxidant activity of benzoselenazolinones. Furthermore, literature data indicate that the chemical structure of compounds has an important role in establishing its applications as a biological antioxidant (Tiano et al., 2000). Thus, a compound having an electron-donating substituent bonded to the aromatic ring, such as 1e, gave lower antioxidant activity than that having an electron-withdrawing substituent, such as 1d. In fact, the lower IC_{50} values were found for bis–selenide alkenes with a Cl substituent on the aromatic ring and followed the order of $1d > 1e \geq 1c$.

Different from the bis, tris–selenide alkenes did not follow the rule described above. The best antioxidant profile was found for 2c, a compound with an electron-donating substituent on the aromatic ring. Conversely, compound 2a without a substituent and 2b with Cl substituted at the *para* position on the aromatic ring were less efficient in reducing lipid peroxidation than 2c. In this series, no clear structure-activity relationship emerged from the antioxidant studies. Thus, we can infer that the antioxidant activity of bis, but not of tris–selenide alkenes, is related to the presence of substituents on the aromatic ring.

The thiol–peroxidase like activity can explain, at least in part, the in vitro antioxidant properties of several compounds (Nogueira et al., 2004). Since the bis and tris–selenide alkene derivatives tested did not present thiol peroxidase activity, the antioxidant activity of these compounds could not be related to this activity.

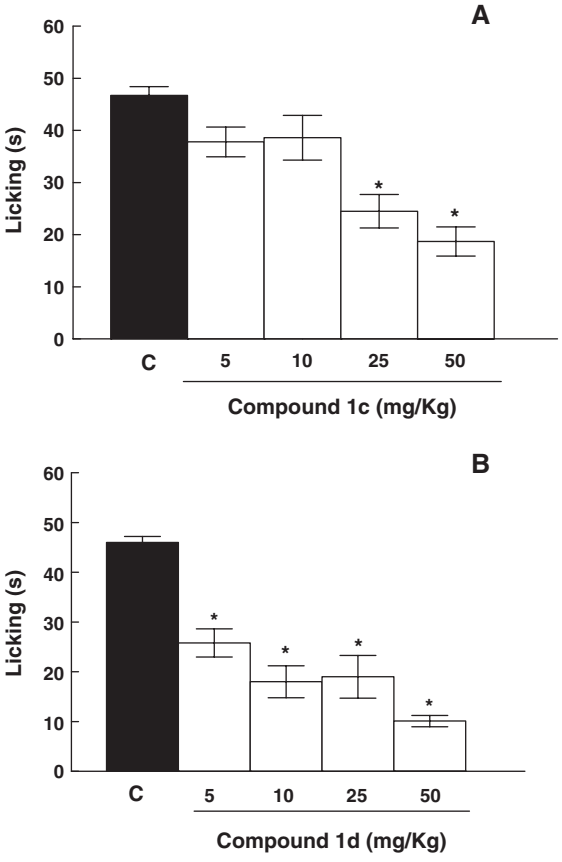


Fig. 5. Effect of compounds 1c (A) and 1d (B) on capsaicin-induced nociception in mice. Animals were pretreated subcutaneously with compounds at various doses (from 5 to 50 mg/kg) for 30 min prior to capsaicin (1.6 μg/paw). The total time (mean ± S.E.M.) spent licking the hind paw was measured during 5 min after s.c. injection of capsaicin in the hind paw. Each column represents the mean with S.E.M. of 8–12 animals. (*) denotes $p<0.05$ as compared to respective vehicle (one-way ANOVA/Duncan).

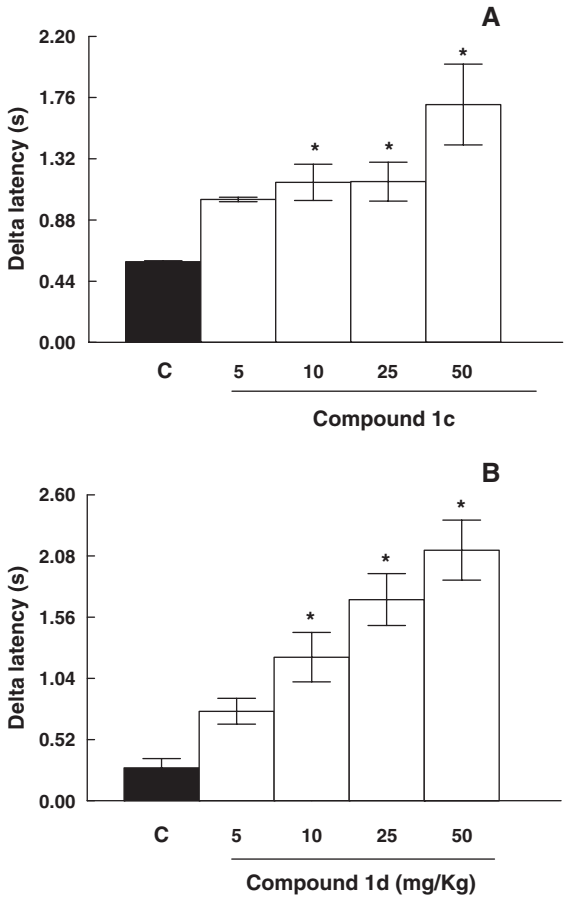


Fig. 6. Effect of compounds 1c (A) and 1d (B) on the tail-imersion test in mice. Animals were pretreated subcutaneously with compounds at various doses (from 5 to 50 mg/kg) for 30 min prior to tail-immersion at 55 °C. A cut-of time of 7 s was imposed on this measured. Δt (s) = post-drug latency − pre-drug latency. Each column represents the mean with S.E.M. for 6–10 mice in each group. (*) denotes $p < 0.05$ as compared to respective vehicle (one-way ANOVA/Duncan).

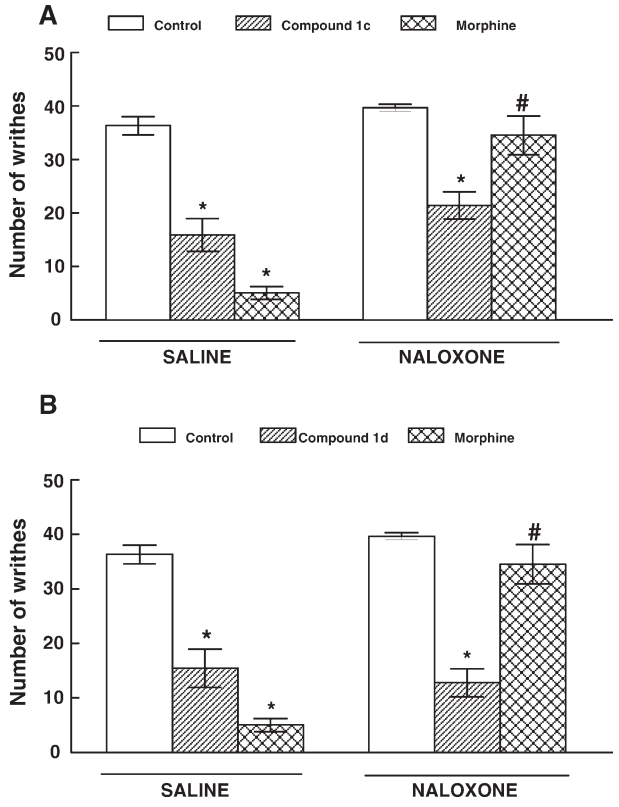


Fig. 7. Effect of naloxone (1 mg/kg, s.c) or 0.9% saline to mice treated with morphine (2.5 mg/kg, s.c), compounds 1c (26.91 mg/kg) (A) and 1d (14.91 mg/kg) (B) on acetic acid-induced abdominal writhing. Each column represents the mean with S.E.M. for 6–10 mice in each group. (*) denotes $p < 0.05$ as compared to respective vehicle (control group), (#) $p < 0.05$ as compared to saline pre-treatment.

Although the peroxidase-like activity of selenides may account for their antioxidant properties, the thiol–selenide exchange catalyzed by chalcogenides may contribute to their toxicological properties by oxidizing relevant thiol-containing metabolites and proteins without consuming toxic substances, such as peroxides (Wilson et al., 1989). In the presence of thiol, oxygen is reduced by selenides in a one-electron transfer process, leading to the production of reactive oxygen species (Chaudiere et al., 1992). The thiol oxidase activity is, therefore, an estimation of the cytotoxicity of molecules (Nogueira et al., 2004).

Upon the inspection of our data, we assume that there is no difference between bis–selenide alkene derivatives tested in thiol oxidase activity, demonstrating a poor potential toxicity of these compounds. Regarding tris–selenide, neither electron-withdrawing nor electron-donating substituents bonded on the aromatic ring caused a difference in the capacity of oxidizing thiol groups, suggesting that there is no relationship between thiol oxidase activity and the chemical structure of selenide alkene derivatives. Comparing the measured values for thiol oxidase activity, compounds 2a and 2b probably are more cytotoxic than the other alkene selenides tested. It is noteworthy that these compounds presented less antioxidant activity than the other selenide alkene derivatives.

Since 1c and 1d demonstrated the best antioxidant activity and negligible thiol oxidase activity, these compounds were chosen for screening the antinociceptive potential of selenide alkenes. The present results provide convincing evidence that compounds 1c and 1d exert some antinociception effect against acetic acid, capsaicin and tail-flick models of pain in mice at a dose that does not interfere with motor performance.

The acetic acid-induced writhing reaction in mice, described as a model for visceral pain, has long been used as a screening tool for the assessment of analgesic or anti-inflammatory properties of new agents (Vinegar et al., 1979; Tjϕlsen and Hole, 1997). Accordingly, compounds 1c and 1d presented an antinociceptive effect in this model of pain, however, 1d was a significantly more potent inhibitor of pain than 1c (compare ID_{50} 14.61 to 26.91 mg/kg for 1d and 1c, respectively). When

Table 3
Effect of compounds 1c and 1d (50 mg/kg) in rotorod test in mice

Treatments	Time of latency (s)
Control	50.7±2.86
1c	52.3±4.10
1d	49.0±7.5

Data are reported as mean±S.E.M. of 5 to 9 animals per group.

compared with some standard analgesic drugs, compounds 1c and 1d, even though administered by a different route of administration, were found to be about 2 to 4-fold more potent than aspirin and acetaminophen administered i.p (Vaz et al., 1996).

The role of opioid receptors in the modulation of nociceptive processing has been demonstrated (Sakurada et al., 2005). Contrarily, pre-administration of naloxone was unable to antagonize analgesia induced by compounds 1c and 1d in acetic acid-induced writhing, suggesting that nociception induced by these compounds is not mediated by opioid receptors. However, the possible interaction of compounds 1c and 1d with the opioid system should be further investigated in behavioral models which do not overlap with the acute-pain model.

It has been reported that capsaicin, the pungent algesic substance obtained from red hot chili peppers, is a valuable pharmacological tool for studying a subset of mammalian primary sensory C-fibers and Aδ afferent neurons, including polymodal nociceptors and warm thermoceptors (Jancsó, 1992). Another interesting finding of the current study is the demonstration that compounds 1c and 1d produced an antinociceptive effect on the capsaicin-induced neurogenic paw licking response. In this chemical model of pain, compound 1d was also more potent (ID_{50} = 5.92 mg/kg) than 1c (ID_{50} = 33.24 mg/kg) in inhibiting the neurogenic pain caused by capsaicin. However, these compounds were less potent than morphine (s.c), but they were more potent than dipyrone (i.p) in preventing the neurogenic antinociception when mediated by the capsaicin test in mice (Vaz et al., 1996).

Based on the results obtained with acetic acid- and capsaicin-induced pain, we can infer that the antinociceptive activity may significantly depend on the electronic effects of the substituents on the aromatic ring of the organoselenium group. These results are in accordance with previous data obtained from our research group (Nogueira et al., 2003). In line with this, the highest antinociceptive potency was attained by compound 1d, which has Cl as its substituent on the aromatic ring. Since the C–Se bond is more easily cleaved with an electron withdrawing group directly bonded to the aromatic ring, this could explain the superior effect of 1d (which has a withdrawing group) when compared to 1c (which has a donating group).

Compounds 1c and 1d, with a similar potency, prolonged the tail-flick latency, indicating the increase of the nociceptive threshold. The tail-flick response is believed to be a spinally mediated reflex (Chapman et al., 1985). Moreover, Grumbach (1966) has shown that the effectiveness of analgesic agents in the tail-flick pain model is highly correlated with relief of human pain.

In summary, the most relevant additional findings of the present study are that (i) bis–selenide alkene derivatives 1c, 1d and 1e are the most promising antioxidant compounds tested; (ii) compounds 1c and 1d caused significant reduction of the acetic acid-induced abdominal writhes, a visceral somatic model (tonic pain); (iii) compounds 1c and 1d inhibited the neurogenic pain caused by the activation of the vanilloid receptor agonist, capsaicin; (iv) compounds 1c and 1d produced an increase in tail-flick response latency time, a thermal test involving thermal stimuli (phasic pain); (v) compounds 1c and 1d did not cause gross motor locomotion.

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