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1-(2-(2-(2-(1-Aminoethyl)phenyl)diselanyl)phenyl)ethanamine: An amino organoselenium compound with interesting antioxidant profile

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ABSTRACT

Free radical scavenging and antioxidant activities of 1-(2-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine (compound A) and diphenyl diselenide (PhSe)2 were examined and compared for inhibition of Fe(II) and sodium nitroprusside (SNP) stimulated lipid peroxidation in rat brain, interaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable free radical and their glutathione peroxidase (GPx) like antioxidant activities with H_2O_2 or tBuOOH as substrates and with PhSH as thiol co-substrates as well as their ability to oxidize mono- and di-thiols were also evaluated. This study revealed that an amino group in amino diselenide drastically enhances their catalytic activities in the aromatic thiol (PhSH) assay system. Compound A was ~2-fold more active than (PhSe)2 in both tBuOOH and H_2O_2 assay systems. In addition, the present results showed that (PhSe)2 exhibited an increased ability to oxidize di-thiols, compound A was not a good substrate for the oxidation of thiol used namely DTT, cystine and DMPS. The antioxidant potency against Fe(II) and SNP-induced brain TBARS were in this order [(compound A); IC_{50} 2 μM and 4 μM] > [(PhSe)2; IC_{50} 19 μM and 27.5 μM. Compound A showed DPPH radical-scavenging activity. This study provides in vitro evidence anti-oxidant action of the tested organoselenium compounds, that the nitrogen atom in the organochalcogens can have a profound effect on their antioxidant activity.

1. Introduction

Reactive species (RS) are commonly generated in normal cellular oxygen metabolism playing biological roles (Halliwell and Gutteridge, 2006). However, the imbalance between the antioxidant system and the over production of RS has been associated with a variety of human diseases such as atherosclerosis, cancer and neurodegenerative diseases (Gutteridge and Halliwell, 1994; Valko et al., 2006). Intracellular components could be damaged by an excess of reactive species generation, which could act as reducing (Halliwell and Gutteridge, 2006) or oxidizing (Augusto et al., 2002) agents of biomolecules. Thus, antioxidants are necessary for preventing the formation of free radicals and the deleterious actions of the RS (Perez-De La Cruz et al., 2006; Perez-Severiano et al., 2004).

Evidence has been provided in the last two decades indicating that organochalcogens are promising pharmacological agents and possess very interesting biological activities (Santamaría et al., 2003; Nogueira et al., 2004). Reports have shown that selenium-containing organic molecules are generally more potent antioxidants than "classical" antioxidants, and this fact serves as an impetus for an increased interest in the rational design of synthetic organoselenium compounds (Santamaría et al., 2003; Nogueira et al., 2004; Meotti et al., 2003; Braga et al., 2009).

The prototypes of this class of compounds are ebselen and (PhSe)2, antioxidant agents with thiol-peroxidase and thioredoxin reductase-like activity (Ibrahim et al., 2010, 2012a,b). Ebselen has been used with relative success in the treatment of acute human brain pathologies such as ischemia and stroke (Rossato et al., 2002; Saito et al., 1998). Amino groups that are capable of interacting with selenium, through Se–N non-bonded interactions

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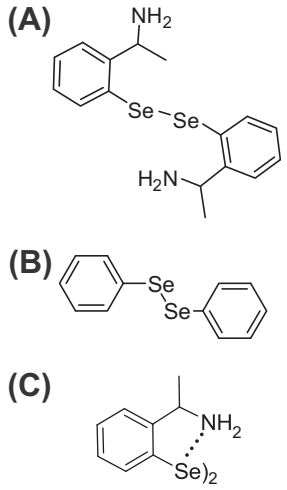

Fig. 1. (A) Chemical structure of compound A, i.e. 1-(2-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine, (B) diphenyl diselenide i.e. (PhSe)2 and (C) the proposed nonbonded interactions between the Sec-amino group and selenium.

(Fig. 1C), are known to play a significant role in modulating the redox proprieties of seleno based antioxidants (Meotti et al., 2003; Kondoh et al., 1999).

From a hypothetical point of view, the formation of stables selenolate (Se-1) ions can increase the reducing properties of these moieties on the organochalcogenides and hypothetically can increase their anti-oxidant properties. However, very few reports are available in the literature concerning the synthesis and GPx like evaluations of amine based diselenide. Thus, to get a deeper insight into the potential use of two organoselenium compounds 1-(2-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine (compound A) and (PhSe)2, (Fig. 1A and B) as a possible pharmacological agents we have studied *in vitro* their antioxidant activities for inhibition of Fe(II) and SNP stimulated lipid peroxidation in rat brain, interaction with 1,1-diphenyl-2-picrylhydrazyl stable free radical (DPPH) and thiol peroxidase-like activities by using H_2O_2 as substrate and PhSH as co-substrate and as well as their ability to oxidize mono- and di-thiols to better understand their pharmacological properties.

2. Materials and methods

2.1. Chemicals

Compound A (Fig. 1A) was synthesized according to literature methods (Braga et al., 2005) with little modifications and (PhSe)2, (Fig. 1B) was synthesized by previously described methods (Paulmier, 1986) and were dissolved in DMSO. Analysis of the ^1H NMR and ^{13}C NMR spectra showed that all the chalcogens presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of chalcogens (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 (200–250 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 and with free access to food and water (Guabi, RS, Brazil). 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.

2.3. Preparation of tissue homogenate for (TBARS) assay

Production of TBARS was determined using a modified method of Ohkawa et al., 1979. Rats were euthanized and the cerebral (whole brain) tissue was rapidly dissected, placed on ice and weighed. One gram quantities of tissues were homogenized in cold 100 mM Tris-buffer pH 7.4 (1:10 w/v) with ten up and down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenates were centrifuged for 10 min at 1400*g* to yield a pellet that was discarded and a low-speed supernatant (S1) used for the assay. The homogenates (100 μl) were incubated with or without 50 μl of the both freshly prepared oxidants iron(II) (final concentration (10 μM) and sodium nitroprusside (final concentration 5 μM), and different concentrations of both organocalcogen (1–45 μM) previously dissolved in DMSO). This was then used for lipid peroxidation determination, together with an appropriate volume of deionized water, to give a total volume of 300 μl at 37 °C for 1 h. The color reaction was carried out by adding 200, 500 and 500 μl each of the 8.1% sodium dodecyl sulfate (SDS), acetic acid (pH 3.4) and 0.6% TBA, respectively. The reaction mixtures, including those of serial dilutions of 0.03 mM standard MDA, were incubated at 97 °C for 1 h. The absorbance was read after cooling the tubes at a wavelength of 532 nm in a spectrophotometer.

2.4. DPPH· radical scavenging activity assay

In order to determine if both compound A and (PhSe)2 present antioxidant activity by the mechanism that involves the scavenging activity of DPPH radical, against the radical DPPH· was performed (Choi et al., 2002). Briefly, 85 μM DPPH· was added to a medium containing different concentrations of organoselenium compounds (1–100 μM). The medium was incubated for 30 min at room temperature. The decrease in absorbance measured at 518 nm depicted the scavenging activity of the organoselenium compounds against DPPH·. Ascorbic acid was used as positive control to determine the maximal decrease in DPPH· absorbance (Choi et al., 2002). The values are expressed in percentage of inhibition of DPPH· absorbance in relation to the control values without the compound A (ascorbic acid maximal inhibition was considered 100% of inhibition).

2.5. Oxidation of mono and di-thiols

The rate of thiol oxidation was determined in the presence of 50 mM Tris–Cl, pH 7.4, and 20 μM of organodiselenides. The rate of thiol oxidation was evaluated by measuring the disappearance of –SH groups. Free –SH groups were determined according to Ellman, 1959. Incubation at 37 °C was initiated by the addition of the thiol compounds. Aliquots of the reaction mixture (100 μL) were checked for the amount of –SH groups at 412 nm after 0–48 h interval with addition of color reagent 5′5′-dithio-bis(2-nitrobenzoic) acid (DTNB).

2.6. Thiol peroxidase activity

The catalytic activity of 1-(2-(2-(2-(1-aminoethyl)phenyl)diselanyl)phenyl)ethanamine, and (PhSe)2 at 1 μM as a GPx model enzyme, was evaluated according to the method of Iwaoka and Tomoda (1994) using PhSH (5 mM) as a glutathione alternative. The reduction of H_2O_2 (10.4 mM) was monitored through the UV absorption increase at 305 nm (3 min), at least more than three times under the same conditions.