

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/266028934>

Synthesis and biological evaluation of new nitrogen-containing diselenides

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · SEPTEMBER 2014

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2014.09.022 · Source: PubMed

CITATIONS

8

READS

78

9 AUTHORS, INCLUDING:



[Emily Pansera Waczuk](#)

Universidade Federal de Santa Maria

18 PUBLICATIONS 75 CITATIONS

[SEE PROFILE](#)



[Joao Batista Teixeira da Rocha](#)

Universidade Federal de Santa Maria

575 PUBLICATIONS 13,026 CITATIONS

[SEE PROFILE](#)



[Antonio L Braga](#)

Federal University of Santa Catarina

275 PUBLICATIONS 5,267 CITATIONS

[SEE PROFILE](#)



Original article

Synthesis and biological evaluation of new nitrogen-containing diselenides



Vanessa Nascimento^a, Natasha L. Ferreira^a, Rômulo F.S. Canto^a, Karen L. Schott^b, Emily P. Waczuk^b, Luca Sancineto^c, Claudio Santi^c, João B.T. Rocha^b, Antonio L. Braga^{a,*}

^a Laboratório de Síntese de Substâncias de Selênio Bioativas, Centro de Ciências Físicas e Matemáticas, Departamento de Química, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, SC, Brazil

^b Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil

^c Dipartimento di Scienze Farmaceutiche, Università di Perugia, Via del Liceo 1, 06134 Perugia, Italy

ARTICLE INFO

Article history:

Received 16 March 2014

Received in revised form

6 September 2014

Accepted 6 September 2014

Available online 8 September 2014

Keywords:

Diselenides

Amphetamine

Glutathione peroxidase

Antioxidant

Selenium

Ebselen

ABSTRACT

The antioxidant properties of organoselenium compounds have been extensively investigated with the aim of developing new drugs, since oxidative stress is responsible for a variety of chronic human diseases. Herein, we reported the synthesis of new nitrogen-containing diselenides by a simple and efficient synthetic route. The products were obtained in good to excellent yields and their identification and characterization were achieved by NMR and HRMS techniques. The new derivatives may represent promising structures with different biological activities, which can act against oxidative stress through diverse mechanisms of action. The glutathione peroxidase-like assay (GPx-like activity) of the new synthesized compounds indicated that they reduced H₂O₂ to water at the expense of PhSH. The best results were obtained with diselenide **2b**, which was 9 times more active than the standard organoselenium drug ebselen and, in contrast, this compound was not reduced by hepatic TrxR. All of the new compounds inhibited Fe(II)-induced TBARS.

© 2014 Elsevier Masson SAS. All rights reserved.

1. Introduction

In the past few decades, interest in the chemistry of organoselenium compounds has significantly increased due to the discovery of a series of selenoproteins, which are involved in a number of physiological processes [1–4]. Selenoenzymes are an important class of mammalian antioxidant enzymes that protect biomembranes and other cellular components from oxidative stress [5–9]. This condition can be generated during oxygen metabolism where reactive oxygen species (ROS) are produced and these can

damage essential cell structures. A number of pathologies are linked to the overproduction of ROS, among them neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, as well as inflammatory processes and physiological signaling pathways [10–14]. Nevertheless, our organism has a refined defense mechanism against ROS and the enzyme glutathione peroxidase (GPx) is part of a complex detoxification system. The structural determination of GPx revealed the presence of selenocysteine (Sec) which, together with Trp153 (tryptophan) and Gln79 (glutamine), form the catalytic triad. The pivotal role of GPx relies on the reduction of endogenous peroxides using, as a cofactor, glutathione (GSH) and affording, as byproducts, water or alcohols depending on the nature of the initial substrate [15–19]. While the presence of Sec has been demonstrated to be fundamental for the enzyme being directly involved in the reduction process, tryptophan and glutamine allow Sec to remain in its selenol/active form.

Besides GPx, thioredoxin reductase (TrxR) is another selenoenzyme which plays a key role within the antioxidant machinery in the cell context. Mammalian TrxR maintains thioredoxin in the reduced form which, in turn, converts disulfide bridges into thiols, allowing target proteins to retain the native conformation.

Abbreviations: NMR, nuclear magnetic resonance; HMRS, high resolution mass spectrometry; GPx, glutathione peroxidase; TrxR, thioredoxin reductase; TBARS, thiobarbituric acid reactive substances; ROS, reactive oxygen species; Trp, tryptophan; Gln, glutamine; Sec, selenocysteine; GSH, glutathione; (PhSe)₂, diphenyl diselenide; SAR, structure activity relationship; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide; DCC, N,N'-dicyclohexylcarbodiimide; CDI, 1,1'-carbonyldiimidazole; m.p., melting point; T₅₀, time required, in min, to reduce the thiol concentration by 50%; UV, ultraviolet; LDH, lactate dehydrogenase; ALA-D, aminolevulinic acid dehydratase; S1, brain homogenates; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; MDA, malondialdehyde.

* Corresponding author.

E-mail addresses: albraga1@gmail.com, braga.antonio@ufsc.br (A.L. Braga).

TrxR is characterized by a broad substrate specificity, being able to reduce not only thioredoxin but also a number of substrates including organic and inorganic selenium compounds, increasing the pool of free selenols within the cell context and thus improving, through peroxides degradation, the antioxidant capacity of the cellular environment [20–22].

Several researchers have dedicated considerable effort to the design and synthesis of organoselenium compounds capable of reproducing the catalytic activity of GPx and, at the same time, act as a substrate for TrxR, thus operating as strong antioxidants, in an attempt to mimic the activity of endogenous antioxidant machinery and provide therapeutic options for oxidative stress-related diseases [23–30]. In this regard, the synthetic organoselenium derivative first reported and most extensively studied is ebselen [31–33]. This compound exhibits antioxidant and anti-inflammatory activities besides other therapeutic applications, for instance, anti-atherosclerotic and cytoprotective behavior [1,33]. Inspired by these studies, a number of new selenium-containing compounds with antioxidant activity have been successfully developed as potential therapeutic agents and also, from a purely synthetic point of view, as new green bio-mimetic catalysts [34–41]. The antioxidant properties of ebselen and other organoselenium compounds, such as diphenyl diselenide (PhSe_2), are mainly related to their glutathione peroxidase-like activities. Some studies have also shown that these compounds are substrates for the mammalian TrxR, which allows the formation of selenol intermediates, hence improving the degradation of peroxides [42,43].

However, the exact contribution to the antioxidant activity of each pathway is still unclear, but from a theoretical point of view the operation of the two routes can be considered advantageous in living cells, because it increases the range of reducing equivalent donors (i.e., thiols and NADPH) capable of synthesize selenol intermediates. Theoretically, the operation of the two pathways is expected to increase the steady state levels of selenol intermediate of given diselenide in the cellular environment. Therefore, these organoselenium compounds might represent novel therapeutic agents for the treatment of diseases caused by oxidative stress [44].

Among organoselenium derivatives, diselenides are particularly attractive given their relatively easy synthesis and stability; consequently, they have often been object of biological investigations [45]. Selenoamides [46,47], such as ebselen or selenides [48–50], have been widely explored in terms of antioxidant activity. Diselenides containing side moiety with heteroatoms in a suitable position to establish with the selenium atom a non-covalent interaction are a potentially fertile field of research in several aspects, including some biological issues such as antioxidant and anticancer activities and toxicity.

In this context, we recently reported some ephedrine-based seleno derivatives in an attempt to develop novel chiral catalysts while assessing their biological profile [51]. Among the derivatives, diselenide **1** appeared to be of interest since it showed a GPx-like activity better to that displayed by other derivatives and even superior than that of diphenyl diselenide, in the same assay. It has been found that selenium atom often interacts with a nearby heteroatom (N, O, S etc.) [52] and in the case of glutathione peroxidase mimics, the increased activity of diselenide derivatives is attributed to this beneficial non-covalent interactions, which occur during the course of the reaction.

It is known from the literature that diselenides are better antioxidants than ebselen and that diselenides with non-covalent interaction between selenium and a heteroatom present even better antioxidant activity [1]. This non-covalent interaction plays a major role in the stabilization of the selenium atom, mainly by preventing the over oxidation and increasing the electrophilicity of the selenium in the dimer [52]. In an attempt to reproduce this

pivotal interaction and gain some insight into the structure activity relationship (SAR) of this class of compounds, we designed a small set of phenylethylamine derivatives, selecting, as the joining moiety, the amidic group, mainly because of its presence in ebselen. In this way, our compound is designed to be a diselenide substituted with biological relevant amines in the form of amides that can interact with selenium and improve its antioxidant activity. Moreover, amide-diselenides obtained from simple amines [53] or amino acids [54–56] have been previously described as good GPx mimics, indicating that the amide group chelation with the diselenide function has a positive influence on the antioxidant activity (Fig. 1).

In particular, the aromatic derivative **2a** and its methyl analog **2b** were designed in order to constrain the amidic nitrogen and keep it close to the diselenide group, thus increasing the probability of the occurrence of the non-covalent interaction and allowing its effect on the antioxidant activity to be verified. Aliphatic derivatives **3a–b** were designed with the interatomic distance of only a methylene linker. **3c–d** were conceived, keeping the distance between the selenium and nitrogen constant but introducing a certain degree of flexibility. The aim of this approach was to provide some insight into the interaction vs. activity relationship of the diselenides with amide bonds.

Modulation of multiple targets along the same biological pathway could potentially lead to disease modification rather than just control of symptoms and the development of new drugs is being focused on multi-potent molecules acting in a complementary manner [57]. The whole set of compounds was investigated in depth in terms of the antioxidant capacity, through different *in vitro* tests, and the inherent SAR is also discussed. In particular, the glutathione peroxidase-like activity was initially assayed and the inhibition of lipid peroxidation in brain homogenates was then evaluated. Furthermore, all of the compounds were investigated for their capacity to act as substrates for the enzyme thioredoxin reductase (TrxR). The antioxidant action of the new nitrogen-containing diselenides (**2a–b** and **3a–d**) was compared with that

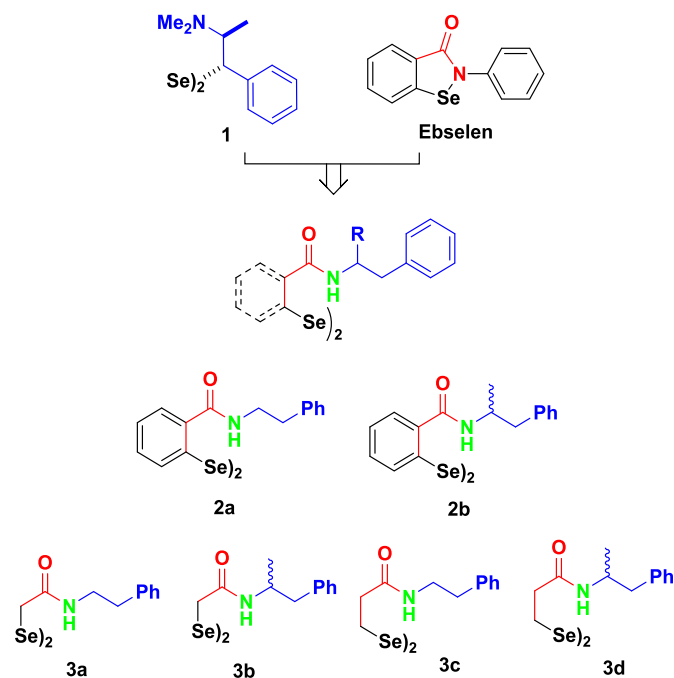


Fig. 1. Structures of nitrogen-containing diselenides reported herein.

of the standard organoselenium drug ebselen, used as the positive control.

2. Results and discussion

2.1. Chemistry

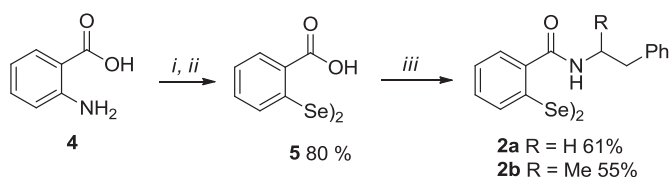
Initially, we synthesized the carboxylic acid diselenide derivatives that were used as starting materials to prepare the target compounds. The aromatic acid diselenide **5** was synthesized in good yield through diazotization of anthranilic acid and substitution with a freshly prepared solution of Na_2Se_2 , as shown in Scheme 1 [58]. The aliphatic acid diselenides **7a–b** were synthesized by reacting the appropriate bromo-carboxylic acids with Na_2Se_2 as outlined in Scheme 2 [59]. The 1-phenylpropan-2-amine used in this study was prepared as described by Swern et al. [60] and Collins et al. [61].

After preparing the starting materials we promoted amide bond formation between the acid diselenides and amines, through (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) (EDC)-mediated coupling reactions (Schemes 1 and 2). It is important to note that on using other coupling reagents, such as N,N' -Dicyclohexylcarbodiimide (DCC) and 1,1'-Carbonyldiimidazole (CDI), the reactions were not successful, resulting in a complex mixture of byproducts and the starting materials.

The target molecules were obtained in good to excellent yields ranging from 55 to 96%. As an example, compound **2a** was obtained in 61% yield (Scheme 1). When the amine used was 1-phenylpropan-2-amine ($R = \text{Me}$), the yields of the compounds were slightly lower in comparison to those obtained using phenylethylamine, as can be seen by comparing **2a** and **2b** (Table 1, entries 1–2). Not surprisingly, exchanging aromatic for aliphatic acid-diselenides led to a pronounced increase in the yields, as shown in Table 1, entries 3–6. Additionally, we found that a change in the chain length between the selenium atom and the amide group did not produce a strong effect on the product yields (Table 1, entries 3 and 5 or 4 and 6). Overall yields of the synthetic routes from the bromo carboxylic acids or anthranilic acid ranged from 44 to 96%.

By applying this strategy many different biologically active amines can be easily transformed into organoselenium compounds and the biological activities of these amide-diselenides can be explored. Furthermore, the modification of the chain length or conformation of the diselenides gives us some evidences of the influence of the Se-heteroatom interaction between selenium and the amide group on the antioxidant activity of the molecules synthesized.

The structure and purity of all products obtained were satisfactorily confirmed by ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and HRMS spectrometry. Their molecular structures are all reported for the first time and selected physical properties are shown in Table 1.



Scheme 1. Reagents and conditions: i. 1 M HCl, NaNO_2 , H_2O , 20 min, r.t. ii. Se^0 , NaOH, NaBH_4 , H_2O , 2 h, r.t. iii. CH_2Cl_2 , amine ($\text{N}_2\text{HCH(R)CH}_2\text{Ph}$), DMAP, Et_3N , 0°C , EDC, 24 h, r.t.

2.2. Biological evaluation

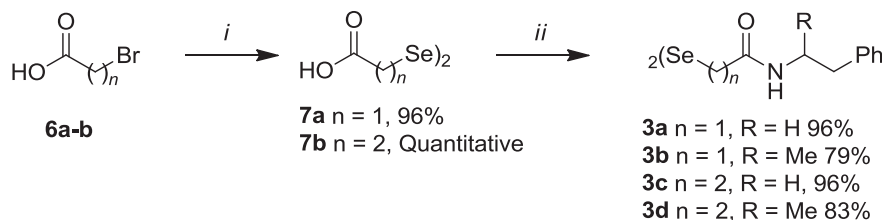
Reactive oxygen species (ROS), such as superoxides ($\text{O}_2^{\cdot-}$), hydroxyl radicals ($\cdot\text{OH}$) and hydrogen peroxide (H_2O_2) are produced in small quantities during cell metabolism. These species are known for their high reactivity and their tendency to initiate and participate in chain reactions, causing extensive damage to cells [62]. Organism defense against ROS may occur through antioxidant enzymes and naturally occurring antioxidants [63]. The aim of the study reported herein was to investigate the antioxidant properties of the synthesized compounds and shed light on their mechanisms of action. This was performed by carrying out three different antioxidant *in vitro* assays, aiming to show that the compounds can act as antioxidants via multiple mechanisms, increasing their final antioxidant activity. From these studies we can show that some factors, such as the size of the chain, steric hindrance and strength of the Se-heteroatom interaction, may be of overriding importance in relation to one mechanism but not to another.

2.2.1. Glutathione peroxidase-like activity

After the synthesis, we explored the potential antioxidant activity of all of the novel diselenide derivatives by measuring the time required to reduce the concentration of the thiol by 50% (T_{50}), determined according to the Tomoda method [64,65] using benzenethiol (PhSH) as a glutathione alternative. In this method, the reduction of H_2O_2 was monitored through the ultraviolet (UV) absorption increase at 305 nm, due to diphenyl disulfide formation. The literature contains reports of some compounds with chelating groups, such as amines, amides or alcohols, close to selenium that showed a positive influence on the GPx-like activity [66–74] and we anticipated that our proposed structures would show the same influence due to the presence of the amide group.

Thus, we examined the catalytic activities of our compounds (**2a–b** and **3a–d**) and the results are outlined in Table 2. Linear increases in absorbance (Fig. 2) were observed by mixing MeOH, the catalyst (**2a–b** and **3a–d**), PhSH and H_2O_2 . The reduction of H_2O_2 was observed at catalyst concentrations of 25, 50, 100 and 200 μM , and the concentration of 100 μM was selected for further studies. The control reaction was performed in the absence of catalyst and did not show notable activity. On slightly modifying the method, that is, replacing the methanol solvent with ethanol for reasons of solubility, once again no appreciable thiophenol oxidation in the absence of the catalysts or hydrogen peroxide was observed, as well as for both references (ebselen and diphenyl diselenide). In addition, in the first 120 s, the reactions were carried out in the absence of H_2O_2 and in all the cases the oxidation of PhSH (via an exchange reaction with diselenide compounds) was not detected and there was no appreciable formation of PhSSPh . The addition of H_2O_2 produced significant increase in the absorbance at 305 nm (PhSSPh formation), characterizing the GPx mimetic activity (For details, see the Supporting Information).

For comparison purposes, ebselen and $(\text{PhSe})_2$, well-known GPx-like mimetics [1,75], were used as controls in our study and their activities were ascribed the value of 1.0, in each case (Entries 1–2). Most of the tested compounds showed catalytic activity in this screening which was at least comparable to that shown by the controls, confirming that the nitrogen-containing diselenide is a viable group in relation to obtaining GPx-mimetic agents. The aromatic derivatives **2a** and **2b** provided the best results (Entries 3–4), with compound **2b** being capable of reducing the concentration of thiol to half its value (T_{50}) in only 16.87 min (Entry 4), approximately 9 times faster than ebselen ($T_{50} = 154.26$ min) (Entry 1) and 3 times than $(\text{PhSe})_2$. In addition, for compound **2a** it was found that $T_{50} = 29.73$ min, which is around 5 and 2 times



Scheme 2. Reagents and conditions: i. EtOH, NaBH₄, SeO, 18 h ii. CH₂Cl₂, amine (N₂HCH(R)CH₂Ph), DMAP, Et₃N, 0 °C, EDC, 24 h, r.t.

Table 1
Physical properties of the synthesized compounds **2a–b** and **3a–d**.

Entry	Product	Overall yield (%) ^a	Melting point (m.p.) (°C) ^b	HRMS ^c found/calculated ([M ⁺])
1		2a 48	199–201	609.0561/609.0560
2		2b 44	200–201	637.0879/637.0873
3		3a 92	93–94	485.0249/485.0245
4		3b 76	Oil	513.0558/513.0558
5		3c 96	114–115	513.0558/513.0565
6		3d 83	95–96	541.0885/541.0871

^a Yields of compounds isolated in two steps from the corresponding carboxylic acids.

^b Melting points are uncorrected.

^c HRMS: High Resolution Mass Spectrometry.

Table 2
Glutathione peroxidase-like activity of organoselenium catalysts **2a–b**, **3a–d**, **ebbselen** and **(PhSe)₂**.

Entry ^{a,b}	Catalyst ^c	T ₅₀ (min) ^{d,e}	Efficiency relative to ebbselen	Efficiency relative to (PhSe)₂
1 ^f	Ebselen	154.26 (±6.35)	1.00	—
2 ^f	(PhSe)₂	55.04 (±3.50)	2.80	1.00
3 ^g	2a	29.73 (±0.16)	5.20	1.85
4 ^g	2b	16.87 (±0.33)	9.14	3.26
5	3a	44.90 (±2.15)	3.43	1.23
6	3b	299.63 (±8.00)	—	—
7	3c	81.43 (±1.77)	1.89	—
8	3d	76.00 (±2.57)	2.03	—

^a Under this condition addition of H₂O₂ in the absence of the organoselenium compound did not produce any significant oxidation of PhSH for both solvents (MeOH and EtOH).

^b Under this condition in the absence of the H₂O₂ (MeOH or EtOH + catalyst + PhSH) did not observe any significant oxidation of PhSH.

^c MeOH (1 mL); catalyst (0.1 mM); PhSH (2 mM); H₂O₂ (5 mM).

^d T₅₀ is the time required, in min, to reduce the thiol concentration by 50% after the addition of H₂O₂.

^e Data in parentheses: experimental error.

^f Both solvents are used (MeOH and EtOH) and it was not observe any appreciable difference in the T₅₀ values.

^g EtOH was used in place of MeOH.

faster than our references, ebselen and (PhSe)₂ respectively (Entry 3). Compounds **3a–d** were tested under the same conditions.

The phenylethylamine derivative **3a**, characterized by a shorter chain length, also showed good catalytic activity (T₅₀ = 44.90 min) (Entry 5) while diselenide **3b** (Entry 6) was not able to promote the reduction of H₂O₂ at an appreciable rate (T₅₀ = 299.63 min). It should be noted that when the carbon chain length between the amide and selenium is increased ($n = 2$, derivative **3c** and **3d**), only a moderate activity in the reduction of hydrogen peroxide (Entries 7–8) was observed, such activity is however 2 times better than that of ebselen, but slower than diphenyl diselenide. The length of the carbon chain is thus important with regard to the interaction between the selenium atom and the amidic nitrogen, with two carbons being the ideal distance, forming a five membered ring. Aromatization of this side chain restricts the possible conformations forcing the interaction to occur. The presence or absence of the methyl group within the phenylethylamine fragment seems to be less important in terms of the GPx-like activity.

2.2.2. Inhibition of thiobarbituric acid reactive substances (TBARS) production in brain homogenates

Lipid peroxidation is an important mechanism involved in cellular toxicity and it can be either a primary response to peroxide attack to biomembranes via Fenton or Haber–Weiss reactions or can be secondary toxic response caused by oxidative stress induced by electrophiles agents [76]. The presence of lipid peroxidation indicates the potential disruption of biomembrane integrity and is an important biomarker of oxidative stress. Consequently, the protective effect against lipid peroxidation has a fundamental

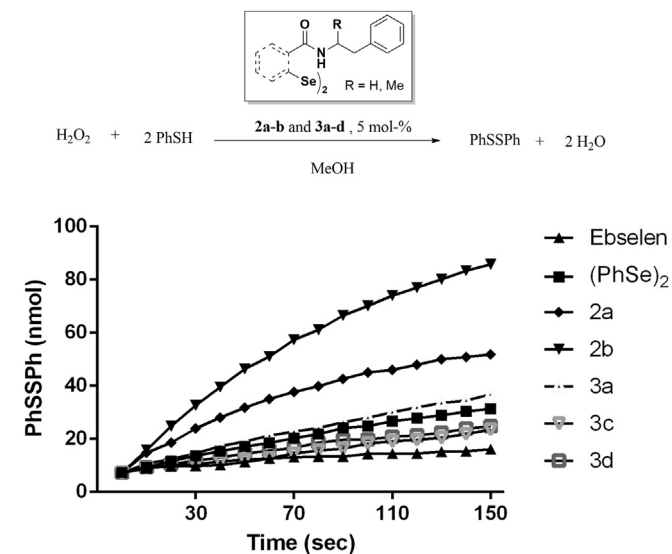


Fig. 2. Glutathione peroxidase-like behavior of catalysts **2a–b**, **3a**, **3c–d**, **ebbselen** and **(PhSe)₂**.

importance for the design of new clinically effective antioxidant drugs and it is an active field of drug research [45]. TBARS are formed by the reaction of thiobarbituric acid (TBA) with byproducts of lipid peroxidation, mainly malondialdehyde. Thus, the stable colored product of TBA with malondialdehyde (TBARS) can be used to quantify lipid peroxidation in biological samples [77]. Brain homogenates (S1) can be used as a source of biomembranes and we have been using this model for some time as an index of lipid peroxidation (for details see references in 43 and 28). In this study, we used Fe(II) as an inducer of brain lipid peroxidation as it caused a considerable increase in the TBARS production. All of the new compounds inhibited Fe(II)-induced TBARS formation. However, compounds **2a** and **2b** were more effective than ebselen and they inhibited TBARS formation at lower concentration than did ebselen. Compounds **3a–d** were also inhibitors of lipid peroxidation; however, compounds **3a–d** were less effective than ebselen as inhibitors of Fe(II)-induced TBARS formation in brain homogenates (Fig. 3). Notably, the results obtained here indicated that the aromatic derivatives **2a** and **2b** were more effective than ebselen confirming the trend already observed during the GPx-mimic test. Although the exact molecular mechanism involved in the inhibition of lipid peroxidation by diselenide compounds is still unknown, we have proposed that their transformation to selenol intermediates is involved in the degradation of organic peroxides formed in biological membranes [35,78]. Consequently, in brain homogenates, diselenide compounds can react with both low molecular thiol molecules and with thiol-containing proteins, such as lactate dehydrogenase (LDH) and aminolevulinic acid dehydratase (ALA-D) [43,79] resulting in the formation of selenol intermediates.

2.2.3. Substrate for rat liver thioredoxin reductase (TrxR)

Ebselen, its diselenide and various diselenide compounds act as substrates for mammalian TrxR [80–82]. In this study the potential reduction of new diselenides by partially purified rat liver enzyme was investigated by determining the consumption of Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) in the presence and absence of new synthesized compounds (for details see Ref. [81]). In contrast to the GPx-like assay, compounds **2a** and **2b** were not reduced by hepatic TrxR (Table 3). Compounds **3a**, **3c** and **3d** were good substrates for TrxR and their relative activity was higher than that of ebselen (Table 3). Indeed, the consumption of NADPH by partially purified hepatic TrxR was around 4–5 times higher in the presence of compounds **3a**, **3c** and **3d** than in the presence of ebselen. The structural requirements for a selenium

Table 3

NADPH oxidation by partially purified hepatic TrxR obtained from rats in the presence of organoselenium catalysts **2a–b**, **3a–d** and ebselen.^a

Entry	Catalyst	Substrate of TrxR (nmolNADPH/min/mg)	Efficiency relative to ebselen
1	Ebselen ^b	3.4 (±1.0)	1.0
2	2a nd	0	—
3	2b nd	0	—
4	3a	19.1 (±2.2)	5.6
5	3b nd	0	—
6	3c	16.1 (±3.7)	4.7
7	3d	13.7 (±7.1)	4.0

nd Not detected during 10 min of assay.

^a NADPH oxidation was monitored at 340 nm for 10 min, in the absence or presence of organoselenium compounds.

^b The oxidation of NADPH determined in the presence of ebselen was inhibited by more than 95% by the addition of 5 µmol/L of AuCl₃.

compound to act as a substrate for TrxR are unknown, and this issue is very complex. In fact, we have previously observed that small modifications to simple diselenides can decrease or completely inhibit the ability of a given compound to act as a substrate for TrxR [81]. From this study, it clearly emerges that the aromatic substituents are detrimental to the diselenide functionality, at least with regard to the affinity for TrxR.

In contrast, three out of four aliphatic derivatives (compounds **3a**, **3c** and **3d**) are able to properly interact with the enzyme, being fully converted into their selenol counterparts. In this case we can speculate that the less hindered and more flexible structure allows the diselenide moiety to be accessible and easily transformed.

However, further studies are clearly needed to better understand the structural determinants indispensable for the interaction of selenium compounds with TrxR. In fact, different selenium compounds can be reduced by TrxR, including inorganic compounds [83].

3. Conclusions

In summary, in this paper, we have described the synthesis, in good to excellent yields, of novel diselenide derivatives. The compounds were fully characterized by ¹H, ¹³C NMR and by HRMS. All diselenides were evaluated for their antioxidant capacity in order to better understand the structural determinants essential for the antioxidant properties and shed light on the SAR of this class of compounds. The new derivatives showed antioxidant activity

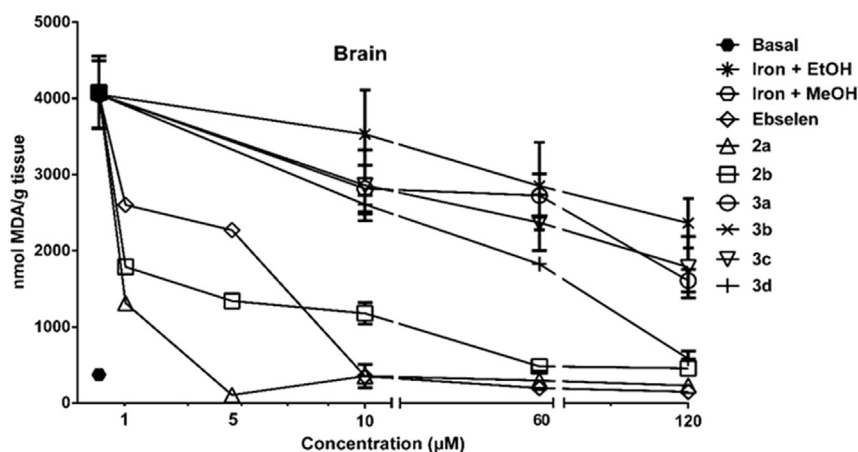


Fig. 3. Effect of amphetamine diselenide derivatives (**2a–b** and **3a–d**) and ebselen on TBARS production in rat brain homogenates (S1). All compounds were tested at final concentrations ranging from 1 to 120 µmol/L. Results are expressed as nmol of malondialdehyde (MDA) per g of brain.

through a GPx-like mechanism, they provide protection against lipid peroxidation and, some of them can act as substrates for TrxR. The aromatic derivatives **2a** and **2b** show a high potential to catalyze the reduction of H_2O_2 to water at the expense of thiophenol, with diselenide **2b** exhibiting a T_{50} value of 16.87 min, whereas for the known GPx mimetic ebselen a T_{50} value of 154.26 min was observed. The improved ability to act as a GPx-mimic may be due to the non-covalent interaction between selenium and amidic-nitrogen, this being particularly evident on comparing the activity of aliphatic and aromatic diselenides. In particular, in the aromatic derivative the structural constraint forces such interactions to occur and enhances the GPx-like activity.

The inhibition of lipid peroxidation can be in part explained by the transformation of diselenides into selenol intermediates through the reaction with thiol groups present in brain homogenate. However, under the conditions of the TrxR assay, the absence of appreciable concentrations of NADPH may have hampered the reduction of some of the diselenides by TrxR. Nevertheless, the results obtained in this study indicate that all of the new compounds can exhibit important antioxidant properties, which are mostly superior to those displayed by ebselen, evidencing that these preliminary results are encouraging and indicate that these new nitrogen-containing diselenides merit further investigation, aimed at the development of new therapeutic drugs for the treatment of oxidative stress-related disease.

4. Experimental protocols

4.1. General methods and materials

NMR spectras (^1H NMR and ^{13}C NMR) were recorded on a Varian AS-400 or Bruker Avance 200 spectrometer. Chemical shifts (δ) are reported (in ppm) relative to the TMS (^1H NMR) and the solvent (^{13}C NMR). ESI-microTOF-Q II measurements were performed with a microTOF Q-II (Bruker Daltonics) mass spectrometer equipped with an automatic syringe pump (KD Scientific) for sample injection. The mass spectrometer was operated in the positive ion mode. The sample was injected using a constant flow (3 $\mu\text{L}/\text{min}$). The solvent was a chloroform/methanol mixture. The ESI-microTOF-Q II instrument was calibrated in the mass range of 50–3000 m/z using an internal calibration standard (low concentration tuning mix solution) supplied by Agilent Technologies. Data were processed employing Bruker Compass Data Analysis software version 4.0. Column chromatography was carried using Merck Silica Gel (230–400 mesh). Thin layer chromatography (TLC) was conducted using Merck Silica Gel GF₂₅₄, 0.25 mm thickness. For visualization, the TLC plates were either placed under ultraviolet light, or stained with iodine vapor or acidic vanillin. The melting points were determined using a microscopy coverslip on a Micro Chemical MQA PF digital apparatus and are uncorrected. All common reagents and solvents were used as purchased unless otherwise noted. The product yields included in all tables refer to isolated yields. Ebselen was prepared as described in a previous report [84].

4.2. General procedure for the synthesis of diselenide **5**

Sodium nitrite (0.456 g, 6.61 mmol) in 6 mL of water was added dropwise to a stirred solution of anthranilic acid (0.856 g, 6.24 mmol) in 1 mL of 37% hydrochloric acid and 7 mL of water cooled in an ice bath. The solution of the resulting diazonium salt was stirred for 20 min while a solution of sodium diselenide was prepared in the following fashion. To a suspension of selenium (1 g, 12.6 mmol) and sodium hydroxide (0.5 g, 14.67 mmol), a 0 °C solution of sodium borohydride (0.06 g, 1.57 mmol) and sodium hydroxide (0.083 g, 2.07 mmol) in 1.2 mL water was added. The

reaction was kept under argon atmosphere and stirred at room temperature for 1 h. The mixture was then slowly warmed to 110 °C and left at this temperature for an additional 30 min. The resulting dark red solution was used without further treatment. The diazonium salt solution was added dropwise to the sodium diselenide solution. The resulting mixture was stirred for 0.5 h at room temperature, then slowly warmed to 100 °C, cooled to room temperature and filtered over Celite. The red/orange solution was acidified with 10% hydrochloric acid and the precipitate so formed was collected, resuspended in methanol and refluxed. The suspension was filtered off, and the filtrate was evaporated *in vacuo* yielding 0.9 g (80% yield) of the target acid.

4.2.1. 2,2'-diselenanediyl dibenzoic acid (**5**)

Pale yellow solid. M.p. 289–292 °C, (lit. [85] m.p. 292–293 °C) 80% Yield; ^1H NMR (DMSO- d_6 , 400 MHz) δ = 8.48 (d, J = 4 Hz, 1H), 8.14 (d, J = 8 Hz, 1H), 7.91 (t, J = 8 Hz, 1H), 7.84–7.77 (m, 1H); ^{13}C (DMSO- d_6 , 100 MHz) δ = 168.63, 133.96, 133.59, 131.65, 129.57, 128.83, 126.59.

4.3. General procedure for the synthesis of diselenides **7a–b**

The literature procedure [59] was slightly modified. To a two-necked round-bottom flask, under argon atmosphere, Se^0 (1 equivalent) and ethanol (5 mL/mmol) were added followed by the addition of NaBH_4 (2 equivalents). The mixture was left under stirring until the solution became colorless. After this time, Se^0 (1 equivalent) was added and the mixture was warmed briefly using a hot air gun until boiling started. After 30 min, the bromo acid (2 equivalents) dissolved in ethanol (1 mL/mmol) was added to the brownish-red ethanolic solution of Na_2Se_2 and the mixture was stirred for 12 h. The mixture was then diluted in water and extracted with ethyl acetate (3 \times 20 mL). The organic layer was dried over MgSO_4 and concentrated under vacuum to give the products as solids. The crude diselenides **7a–b** were used without further purification.

4.3.1. 2,2'-diselenanediyl diacetic acid (**7a**)

Yellow solid. M.p. 100–103 °C, (lit. [59] m.p. 102–105 °C) 96% Yield; ^1H NMR (DMSO- d_6 , 400 MHz) δ = 13.60 (sl, 2H), 3.76 (s, 4H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ = 172.14, 23.92.

4.3.2. 3,3'-diselenanediyl dipropanoic acid (**7b**)

Pale yellow solid. M.p. 127–130 °C, (lit. [59] m.p. 130–132 °C) Quantitative yield; ^1H NMR (DMSO- d_6 , 400 MHz) δ = 3.05 (t, J = 8 Hz, 4H), 2.71 (t, J = 8 Hz, 4H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ = 173.17, 35.50, 24.01.

4.4. General procedure for the synthesis of nitrogen-containing diselenides **2a–b** and **3a–d**

The literature procedure was modified [86]. The mixture of diselenide acid (5 or 7a–b) (1 equivalent), amine (1.1 equivalent) and 4-dimethylaminopyridine (DMAP) (0.2 equivalent) in dry CH_2Cl_2 (5 mL) was cooled to 0 °C, triethylamine (1.1 equivalent), and 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) (1.1 equivalent) were then added. The solution was stirred for 24 h. The reaction mixture was diluted with CH_2Cl_2 and washed with 0.5 N HCl (3 \times 20 mL), 1% NaOH solution (3 \times 20 mL) and brine. After drying over MgSO_4 , the solvent was evaporated off under reduced pressure and the crude product was purified by flash chromatography (acetate:hexane).

4.4.1. 2,2'-diselanediylbis(*N*-phenethylbenzamide) (**2a**)

White solid. M.p. 199–200 °C, (25:75 acetate:hexane) 61% Yield; ¹H NMR (CDCl₃, 200 MHz) δ = 7.87 (d, *J* = 8 Hz, 2H), 7.38–7.13 (m, 16H), 6.23 (t, *J* = 6 Hz, 2H), 3.76 (q, *J* = 6 Hz, 4H), 2.98 (t, *J* = 8 Hz, 4H); ¹³C NMR (CDCl₃, 50 MHz) δ = 168.16, 138.73, 133.10, 131.73, 128.87, 128.78, 126.69, 126.46, 126.08, 41.32, 35.62. HRMS (ESI) *m/z* calculated for C₃₀H₂₈N₂O₂Se₂ [M⁺]: 609.0560, found 609.0561.

4.4.2. 2,2'-diselanediylbis(*N*-(1-phenylpropan-2-yl)benzamide) (**2b**)

White solid. M.p. 200–201 °C, (15:85 acetate:hexane) 55% Yield; ¹H NMR (CDCl₃, 400 MHz) δ = 7.87 (d, *J* = 8 Hz, 2H), 7.35–7.17 (m, 16H), 5.99 (d, *J* = 4 Hz, 2H), 4.55–4.48 (m, 2H), 2.99 (dd, *J*¹ = 12 Hz, *J*² = 4 Hz, 2H), 2.91 (dd, *J*¹ = 12 Hz, *J*² = 8 Hz, 2H), 1.27 (d, *J* = 8 Hz, 6H); ¹³C NMR (CDCl₃, 100 MHz) δ = 167.47, 137.59, 131.66, 129.58, 128.48, 126.61, 126.38, 126.02, 46.76, 42.25, 19.95. HRMS (ESI) *m/z* calculated for C₃₂H₃₂N₂O₂Se₂ [M⁺]: 637.0873, found 637.0879.

4.4.3. 2,2'-diselanediylbis(*N*-phenethylacetamide) (**3a**)

Yellow solid. M.p. 93–94 °C, (35:65 acetate:hexane) 96% Yield; ¹H NMR (CDCl₃, 400 MHz) δ = 7.30–7.27 (m, 4H), 7.21–7.19 (m, 6H), 7.00 (t, *J* = 4 Hz, 2H), 3.58–3.46 (m, 8H), 2.87–2.79 (m, 4H); ¹³C NMR (CDCl₃, 100 MHz) δ = 170.09, 138.66, 128.68, 128.49, 126.42, 41.18, 35.46, 31.66. HRMS (ESI) *m/z* calculated for C₂₀H₂₄N₂O₂Se₂ [M⁺]: 485.0245, found 485.0249.

4.4.4. 2,2'-diselanediylbis(*N*-(1-phenylpropan-2-yl)acetamide) (**3b**)

Dark yellow oil, (30:70 acetate:hexane) 79% Yield; ¹H NMR (CDCl₃, 200 MHz) δ = 7.28–7.19 (m, 10H), 6.82 (d, *J* = 6 Hz, 2H), 4.30–4.17 (m, 2H), 3.53–3.52 (m, 2H), 2.98–2.97 (m, 2H), 2.94–2.61 (m, 4H), 1.17 (d, *J* = 8 Hz, 6H); ¹³C NMR (CDCl₃, 100 MHz), δ = 169.42, 138.12, 129.28, 128.28, 126.33, 47.20, 42.50, 31.86, 20.08. HRMS (ESI) *m/z* calculated for C₂₂H₂₈N₂O₂Se₂ [M⁺]: 513.0558, found 513.0558.

4.4.5. 3,3'-diselanediylbis(*N*-phenethylpropanamide) (**3c**)

Dark yellow solid. M.p. 114–115 °C (40:60 acetate:hexane) 96% Yield; ¹H NMR (CDCl₃, 400 MHz) δ = 7.32–7.19 (m, 10H), 6.06 (sl, 2H), 3.52 (q, *J* = 8 Hz, 4H), 3.10 (t, *J* = 8 Hz, 4H), 2.83 (t, *J* = 8 Hz, 4H), 2.59 (t, *J* = 8 Hz, 4H); ¹³C NMR (CDCl₃, 100 MHz), δ = 171.32, 138.74, 128.73, 128.60, 126.49, 40.72, 37.37, 35.58, 24.38. HRMS (ESI) *m/z* calculated for C₂₂H₂₈N₂O₂Se₂ [M⁺]: 513.0565, found 513.0558.

4.4.6. 3,3'-diselanediylbis(*N*-(1-phenylpropan-2-yl)propanamide) (**3d**)

Pale yellow solid. M.p. 94–95 °C, (30:70 acetate:hexane) 83% Yield; ¹H NMR (CDCl₃, 400 MHz) δ = 7.30–7.17 (m, 10H), 6.03 (d, *J* = 8 Hz, 2H), 4.32–4.21 (m, 2H), 3.09 (t, *J* = 8 Hz, 4H), 2.86 (dd, *J*¹ = 12 Hz, *J*² = 4 Hz, 2H), 2.71 (dd, *J*¹ = 16 Hz, *J*² = 8 Hz, 2H), 2.59–2.56 (m, 4H), 1.13 (d, *J* = 8 Hz, 6H); ¹³C NMR (CDCl₃, 100 MHz), δ = 170.57, 137.94, 129.34, 128.32, 126.37, 46.30, 42.40, 37.43, 24.47, 19.97. HRMS (ESI) *m/z* calculated for C₂₄H₃₂N₂O₂Se₂ [M⁺]: 541.0871, found 541.0885.

4.5. Pharmacological evaluation

4.5.1. Glutathione-peroxidase-like activity assay

The catalytic activity of the nitrogen-containing diselenides as a GPx model enzyme was evaluated according to the Tomoda method [64,65]. Initially, were mixed the selenium catalyst (final concentration: 0.1 mM) and a methanol (or ethanol when was necessary) solution of thiophenol (PhSH) (final concentration 2 mM) at 25 (±3) °C. After around 120 s, the catalytic GPx model reaction (H₂O₂ + 2PhSH → 2H₂O + PhSSPh) was initiated by the addition of

H₂O₂ (final concentration: 5 mM). The reduction of H₂O₂ was monitored by UV spectrophotometry, at 305 nm, via the formation of PhSSPh. The reaction was monitored for more 150 s, more than three times under the same conditions. Activities of the compounds were compared with the activity of ebselen and (PhSe)₂ (positives controls).

4.5.1.1. Calculations for the T₅₀ values. The T₅₀ value is done by mean of extrapolation or a rule of 3. The velocity of PhSSPh formation is determined from the linear portion of peroxidase-like activity spectrophotometric quantification. Thus, using a generic example, for a compound that exhibited a rate of PhSSPh formation of 0.1 μmol per min or per 60 s (in 1 mL, this means 0.1 mmol and indicates the consumption of 0.2 mmol of PhSH/min or 60 s), knowing that the total concentration of PhSH used was 5 mmol/L, it is possible calculate that at 10 min or 600 s the expected consumption will be 2 mmol, and that specifically 1.25 mmol of PhSSPh (i.e., 2.5 mmol consumption of PhSH, which is 50% of total PhSH) will occur at 12.5 min or 750 s. Thus, the linear portion of PhSSPh formation was used to calculate the velocity of reaction and then extrapolate it to 50% consumption of PhSH.

4.5.2. Thiobarbituric acid reactive substances (TBARS) assay

TBARS was quantified by the method of Ohkawa et al. [87] as modified by Puntel et al. [88]. In short, brain homogenates (1/5, g/v) were prepared in 10 mmol/L Tris buffer, pH 7.4 and centrifuged for 10 min at 3.6000 rpm. The supernatant (S1) was used as a source of lipids in the assay. An aliquot of 20 μL of S1 was incubated for 60 min at 37 °C with freshly prepared Fe₂SO₄ (final concentration of 10 μM in a final volume of 30 μL) in 10 mmol/L Tris buffer (pH 7.4) in the presence or absence of compounds 2a–b, 3a–d or ebselen. In the next step, 40 μL of 8.1% sodium dodecyl sulfate (SDS), 100 μL of buffered acetic acid (pH 3.4) and 100 μL of 0.8% thiobarbituric acid (TBA) were added sequentially. The mixture was incubated at 100 °C for 1 h. The samples were then centrifuged at 6.000 rpm for 3 min and the color developed was measured in 200 μL of the supernatant using an Elisa plate reader at 532 nm. TBARS levels are expressed as nmol of MDA/g of tissue calculated against a standard curve of MDA.

4.5.3. Thioredoxin reductase (TrxR) assay

TrxR from rat liver was purified essentially as described by Hill et al. [22]. TrxR activity was determined according to Zhao and Holmgren [80]. The activity was investigated in a buffer containing 30 mM Tris–HCl, 1.2 mM EDTA, pH 7.4, 100 μL of partially purified TrxR and 120 μM of NADPH.

Acknowledgments

The authors thank CEBIME, CNPq, INCT-Catálise, CAPES, and FAPESC-Pronex for financial support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2014.09.022>.

References

- [1] E.E. Alberto, V. Nascimento, A.L. Braga, Catalytic application of selenium and tellurium compounds as glutathione peroxidase enzyme mimetics, *J. Braz. Chem. Soc.* 21 (2010) 2032–2041.
- [2] C.T. Santi, C. Scalera, M. Piroddi, F. Galli, Selenium containing compounds from poison to drug candidates: a review on the GPx-like activity, *Curr. Chem. Biol.* 7 (2013) 25–36.

- [3] M. Iwaoka, K. Arai, From sulfur to selenium. A new research arena in chemical biology and biological chemistry, *Curr. Chem. Biol.* 7 (2013) 2–24.
- [4] A.L. Braga, J. Rafique, Synthesis of biologically relevant small molecules containing selenium. Part A. Antioxidant compounds, in: Z. Rappoport (Ed.), *The Chemistry of Organic Selenium and Tellurium Compounds*, John Wiley & Sons Ltd, Chichester, 2013.
- [5] G. Roy, B.K. Sarma, P.P. Phadnis, G. Mughesh, Selenium-containing enzymes in mammals: chemical perspectives, *J. Chem. Sci.* 117 (2005) 287–303.
- [6] D. Bhowmick, G. Mughesh, Tertiary amine-based glutathione peroxidase mimics: some insights into the role of steric and electronic effects on anti-oxidant activity, *Tetrahedron* 68 (2012) 10550–10560.
- [7] C. Jacob, G.L. Giles, N.M. Giles, H. Sies, Sulfur and selenium: the role of oxidation state in protein structure and function, *Angew. Chem. Int. Ed.* 42 (2003) 4742–4758.
- [8] D. Bhowmick, G. Mughesh, Enzyme mimetic chemistry of organoselenium compounds, in: *PATAI's Chemistry of Functional Groups*, John Wiley & Sons, Ltd, 2013.
- [9] U.H. Gandhil, T.P. Nagaraja, K.S. Prabhu, Selenoproteins and their role in oxidative stress and inflammation, *Curr. Chem. Biol.* 7 (2013) 65–73.
- [10] W.A. Pryor, *Free Radicals in Biology*, Academic Press, New York, 1976.
- [11] D.S. Armstrong, R.S. Soal, R.G. Cutler, T.F. Slater, *Free Radicals in Molecular Biology, Aging and Disease*, Raven Press, New York, 1984.
- [12] M.J. Parnham, E. Graf, Selenoorganic compounds and the therapy of hydroperoxide-linked pathological conditions, *Biochem. Pharmacol.* 36 (1987) 3095–3102.
- [13] C.W. Nogueira, J.B.T. Rocha, in: Z. Rappoport (Ed.), *Organic Selenium and Tellurium Compound*, vol. 3, Wiley, Chichester, UK, 2012 (Part II, Chapter 21).
- [14] F. Galli, A. Battistoni, R. Gambari, A. Pompella, F. Braganzzi, F. Pilolli, L. Iuliano, M. Piroddi, M.C. Dechechi, G. Cabrini, Oxidative stress and antioxidant therapy in cystic fibrosis, *Bioch. et Bioph. Acta* 1822 (2012) 690–713.
- [15] J.M. Mccord, I. Fridovic, Superoxide dismutase an enzymic function for erythrocyte (hemocytin), *J. Biol. Chem.* 244 (1969) 6049–6055.
- [16] J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, W.G. Hoekstra, Selenium - biochemical role as a component of glutathione peroxidase, *Science* 179 (1973) 588–590.
- [17] K.R. Maddipati, L.J. Marnett, Characterization of the major hydroperoxide-reducing activity of human-plasma - purification and properties of a selenium-dependent glutathione-peroxidase, *J. Biol. Chem.* 262 (1987) 17398–17403.
- [18] L. Flohé, W.A. Gunzler, H.H. Sock, Glutathione peroxidase: a selenoenzyme, *FEBS Lett.* 32 (1973) 132–134.
- [19] S. Toppo, L. Flohé, F. Ursini, S. Vanin, M. Maiorino, Catalytic mechanisms and specificities of glutathione peroxidases: variations of a basic scheme, *Biochim. Biophys. Acta Gen. Subj.* 1790 (2009) 1486–1500.
- [20] A. Holmgren, J. Lu, Thioredoxin and thioredoxin reductase: current research with special reference to human disease, *Biochem. Biophys. Res. Commun.* 396 (2010) 120–124.
- [21] P.N. Stefanie, H.H. Huang, D.L. Williams, Thioredoxin glutathione reductase: its a role in redox biology and potential as a target for drugs against neglected disease, *Biochim. Biophys. Acta Gen. Subj.* 1810 (2011) 1262–1271.
- [22] K.E. Hill, G.W. McCollum, M.E. Boeglin, R.F. Burk, Thioredoxin reductase activity is decreased by selenium deficiency, *Biochem. Biophys. Res. Commun.* 234 (1997) 293–295.
- [23] L. Orian, S. Toppo, Organochalcogen peroxidase mimetics as potential drugs: a long story of a promise still unfulfilled, *Free Radic. Biol. Med.* 66 (2014) 65–74.
- [24] G. Mughesh, W.W. du Mont, H. Sies, Chemistry of biologically important synthetic organoselenium compounds, *Chem. Rev.* 101 (2001) 2125–2179.
- [25] J.D. Brian, Catalase and glutathione peroxidase mimics, *Biochem. Pharmacol.* 77 (2009) 285–296.
- [26] G. Mughesh, H.B. Singh, Synthetic organoselenium compounds as antioxidants: glutathione peroxidase activity, *Chem. Soc. Rev.* 29 (2000) 347–357.
- [27] K.I. Priyadarsini, B.G. Singh, A. Kunwar, Current developments on synthesis, redox reactions and biochemical studies of selenium antioxidants, *Curr. Chem. Biol.* 7 (2013) 37–46.
- [28] C.W. Nogueira, G. Zeni, J.B.T. Rocha, Organoselenium and organotellurium compounds: toxicology and pharmacology, *Chem. Rev.* 104 (2004) 6255–6285.
- [29] T.G. Back, Z. Moussa, Remarkable activity of a novel cyclic seleninate ester as a glutathione peroxidase mimetic and its facile in situ generation from Allyl 3-hydroxypropyl selenide, *J. Am. Chem. Soc.* 124 (2002) 12104–12105.
- [30] N.M.R. McNeil, M.C. Matz, T.G. Back, Fluxional cyclic seleninate ester: NMR and computational studies, glutathione peroxidase-like behavior, and unexpected rearrangement, *J. Org. Chem.* 78 (2013) 10369–10382.
- [31] M.C. Fong, C.H. Schiesser, Intramolecular homolytic substitution with amidyl radicals: A free-radical synthesis of ebselen and related analogues, *J. Org. Chem.* 62 (1997) 3103–3108.
- [32] A. Ruwet, M. Renson, Synthesis of substituted aromatic aldehydes and ketones with ortho-thioether or ortho-selenoether functions in synthesis of sulfated and selenated heterocycles, *B. Soc. Chim. Belg.* 78 (1969) 571.
- [33] T. Schewe, Molecular actions of ebselen - an antiinflammatory antioxidant, *Gen. Pharmacol.* 26 (1995) 1153–1169.
- [34] S. Shaaban, R. Diestel, B. Hinkelmann, Y. Muthukumar, R.P. Verma, F. Sasse, C. Jacob, Novel peptidomimetic compounds containing redox active chalcogens and quinones as potential anticancer agents, *Eur. J. Med. Chem.* 58 (2012) 192–205.
- [35] I. Saito, T. Asano, K. Sano, K. Takakura, H. Abe, T. Yoshimoto, H. Kikuchi, T. Ohta, S. Ishibashi, Neuroprotective effect of an antioxidant, ebselen, in patients with delayed neurological deficits after aneurysmal subarachnoid hemorrhage, *Neurosurgery* 42 (1998) 269–277.
- [36] C. Santi, S. Santoro, B. Battistelli, Organoselenium compounds as catalysts in nature and laboratory, *Curr. Org. Chem.* 14 (2010) 2442–2462.
- [37] D.M. Freudendahl, S. Santoro, S.A. Shahzad, C. Santi, T. Wirth, Green chemistry with selenium reagents: development of efficient catalytic reactions, *Angew. Chem. Int. Ed.* 48 (2009) 8409–8411.
- [38] C. Tidei, M. Piroddi, F. Galli, C. Santi, Oxidation of thiols promoted by PhSeZnCl, *Tetrahedron Lett.* 53 (2012) 232–234.
- [39] E. Moreno, D. Plano, I. Lamberto, M. Font, I. Encio, J.A. Palop, C. Sanmartin, Sulfur and selenium derivatives of quinazoline and pyrido[2,3-d]pyrimidine: synthesis and study of their potential cytotoxic activity in vitro, *Eur. J. Med. Chem.* 47 (2012) 283–298.
- [40] A.L. Braga, J. Rafique, Synthesis of biologically relevant small molecules containing selenium. Part B. Anti-infective and anticancer compounds, in: Z. Rappoport (Ed.), *The Chemistry of Organic Selenium and Tellurium Compounds*, vol. 4, John Wiley & Sons, Ltd, Chichester, UK, 2013, pp. 1053–1117.
- [41] K.P. Bhabak, A.A. Vernekar, S.R. Jakka, G. Roy, G. Mughesh, Mechanistic investigations on the efficient catalytic decomposition of peroxynitrite by ebselen analogues, *Org. Biomol. Chem.* 9 (2011) 5193–5200.
- [42] M.R. Stralio, J. de Oliveira, G. Mancini, A.C.D. Baily, A. Latini, A.M. Deobald, J.B.T. Rocha, A.F. de Bem, Disubstituted diaryl diselenides as potential antiperoxidative compounds: involvement of TrxR and GPx-like systems, *Eur. J. Pharm. Sci.* 48 (2013) 717–725.
- [43] C.W. Nogueira, J.B.T. Rocha, Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds, *Arch. Toxicol.* 85 (2011) 1313–1359.
- [44] G.E. Arteel, H. Sies, The biochemistry of selenium and the glutathione system, *Environ. Toxicol. Phar.* 10 (2001) 153–158.
- [45] F.C. Meotti, E.C. Stangherlin, G. Zeni, C.W. Nogueira, J.B.T. Rocha, Protective role of aryl and alkyl diselenides on lipid peroxidation, *Environ. Res.* 94 (2004) 276–282.
- [46] K. Satheeshkumar, G. Mughesh, Synthesis and antioxidant activity of peptide-based ebselen analogues, *Chem. Eur. J.* 17 (2011) 4849–4857.
- [47] A.D. Prestes, S.T. Stefanello, S.M. Salman, A.M. Pazini, R.S. Schwab, A.L. Braga, N.B.D. Barbosa, J.B.T. Rocha, Antioxidant activity of beta-selenoamines and their capacity to mimic different enzymes, *Mol. Cell. Biochem.* 365 (2012) 85–92.
- [48] F. Kumakura, B. Mishra, Priyadarsini, M. Iwaoka, A water-soluble cyclic selenide with enhanced glutathione peroxidase-like catalytic activities, *Eur. J. Org. Chem.* (2010) 440–445.
- [49] S. Kumar, H. Johansson, L. Engman, L. Valgimigli, R. Amorati, M.G. Fumo, G.F. Pedulli, Regenerable chain-breaking 2,3-dihydrobenzo[b]selenophene-5-ol antioxidants, *J. Org. Chem.* 72 (2007) 2583–2595.
- [50] S.T. Stefanello, A.S. Prestes, T. Ogunmoyole, S.M. Salman, R.S. Schwab, C.R. Brender, L. Dornelles, J.B.T. Rocha, F.A.A. Soares, Evaluation of in vitro antioxidant effect of new mono and diselenides, *Toxicol. In Vitro* 27 (2013) 1433–1439.
- [51] L.C. Soares, E.E. Alberto, R.S. Schwab, P.S. Taube, V. Nascimento, O.E.D. Rodrigues, A.L. Braga, Ephedrine-based diselenide: a promiscuous catalyst suitable to mimic the enzyme glutathione peroxidase (GPx) and to promote enantioselective C-C coupling reactions, *Org. Biomol. Chem.* 10 (2012) 6595–6599.
- [52] A.J. Mukherjee, S.S. Zade, H.B. Singh, R.B. Sunoj, Organoselenium chemistry: role of intramolecular interactions, *Chem. Rev.* 110 (2010) 4357–4416.
- [53] K.P. Bhabak, G. Mughesh, Amide-based glutathione peroxidase mimics: effect of secondary and tertiary amide substituents on antioxidant activity, *Chem. Asian J.* 4 (2009) 974–983.
- [54] E.E. Alberto, L.C. Soares, J.H. Sudati, A.C.A. Borges, J.B.T. Rocha, A.L. Braga, Efficient synthesis of modular amino acid derivatives containing selenium with pronounced GPx-like activity, *Eur. J. Org. Chem.* (2009) 4211–4214.
- [55] K. Selvakumar, P. Shah, H.B. Singh, R.J. Butcher, Synthesis, structure, and glutathione peroxidase-like activity of amino acid containing ebselen analogues and diaryl diselenides, *Chem. Eur. J.* 17 (2011) 12741–12755.
- [56] T. Takei, Y. Urabe, Y. Asahina, H. Hojo, T. Nomura, K. Dedachi, K. Arai, M. Iwaoka, Model study using designed selenopeptides on the importance of the catalytic triad for the antioxidative functions of glutathione peroxidase, *J. Phys. Chem. B.* 118 (2014) 492–500.
- [57] P.C. Trippier, K.J. Labby, D.D. Hawker, J.J. Mataka, R.B. Silverman, Target- and mechanism-based therapeutics for neurodegenerative diseases: strength in numbers, *J. Med. Chem.* 56 (2013) 3121–3147.
- [58] X. Yang, Q. Wang, Y. Tao, H. Xu, A modified method to prepare diselenides by the reaction of selenium with sodium borohydride, *J. Chem. Res. (S)* 4 (2002) 160–161.
- [59] T.G. Back, Z. Moussa, Diselenides and allyl selenides as glutathione peroxidase mimetics. Remarkable activity of cyclic seleninates produced in situ by the oxidation of allyl omega-hydroxyalkyl selenides, *J. Am. Chem. Soc.* 125 (2003) 13455–13460.
- [60] K. Omura, D. Swern, Oxidation of alcohols by activated dimethyl-sulfoxide - preparative, steric and mechanistic study, *Tetrahedron* 34 (1978) 1651–1660.

- [61] M. Collins, H. Salouros, A.T. Cawley, J. Robertson, A.C. Heagney, A. Arenas-Queral, Delta C-13 and delta H-2 isotope ratios in amphetamine synthesized from benzaldehyde and nitroethane, *Rapid Commun. Mass Sp.* 24 (2010) 1653–1658.
- [62] W.G. Salgueiro, M.C.D.F. Xavier, L.F.B. Duarte, D.F. Câmara, D.A. Fagundes, A.T.G. Soares, G. Perin, D. Alves, D.S. Avila, Direct synthesis of 4-Organysulfenyl-7-chloro quinolines and their toxicological and pharmacological activities in *Caenorhabditis elegans*, *Eur. J. Med. Chem.* 75 (2014) 448–459.
- [63] A.S.D. Hadjipavlou-Litina, M. Unzeta, J. Marco-Contelles, Analysis of the antioxidant properties of differently substituted 2- and 3-indolyl carbonyl derivatives and related derivatives, *Eur. J. Med. Chem.* 63 (2013) 670–674.
- [64] M. Iwaoka, S. Tomoda, A model study on the effect of an amino group on the antioxidant activity of glutathione-peroxidase, *J. Am. Chem. Soc.* 116 (1994) 2557–2561.
- [65] V. Nascimento, E.E. Alberto, D.W. Tondo, D. Dambrowski, M.R. Detty, F. Nome, A.L. Braga, GPx-Like activity of selenides and selenoxides: experimental evidence for the involvement of hydroxy perhydroxy selenane as the active species, *J. Am. Chem. Soc.* 134 (2012) 138–141.
- [66] V. Galet, J.L. Bernier, J.P. Henichart, D. Lesieur, C. Abadie, L. Rochette, A. Lindenbaum, J. Chalas, J.F.R. Delafaverie, B. Pfeiffer, P. Renard, Benzosele-nazolinone derivatives designed to be glutathione-peroxidase mimetics feature inhibition of cyclooxygenase/5-lipoxygenase pathways and anti-inflammatory activity, *J. Med. Chem.* 37 (1994) 2903–2911.
- [67] G. Mughesh, A. Panda, H.B. Singh, N.S. Puneekar, R.J. Butcher, Glutathione peroxidase-like antioxidant activity of diaryl diselenides: a mechanistic study, *J. Am. Chem. Soc.* 123 (2001) 839–850.
- [68] S.K. Tripathi, U. Patel, D. Roy, R.B. Sunoj, H.B. Singh, G. Wolmershauser, R.J. Butcher, Synthesis, intramolecular nonbonded chalcogen center dot center dot center dot OH interactions, and glutathione peroxidase-like activity, *J. Org. Chem.* 70 (2005) 9237–9247.
- [69] K.P. Bhabak, G. Mughesh, Functional mimics of glutathione peroxidase: bio-inspired synthetic antioxidants, *Acc Chem. Res.* 43 (2010) 1408–1419.
- [70] C.A. Bayse, R.A. Baker, K.N. Ortwin, Relative strengths of Se···N, O interactions: Implications for glutathione peroxidase activity, *Inorg. Chim. Acta* 358 (2005) 3849–3854.
- [71] W. Hassan, S. Narayanaperumal, K. Gul, A.U. Rahman, A.L. Braga, O.E.D. Rodrigues, J.B.T. Rocha, Modulation of diorganoyl dichalcogenides reactivity by non-bonded nitrogen interactions, *Chem. Biol. Interact.* 199 (2012) 96–105.
- [72] T. Wirth, Glutathione peroxidase-like activities of oxygen-containing diselenides, *Molecules* 3 (1998) 164–166.
- [73] A.S. Hodage, P.P. Phadnis, A. Wadawale, K.I. Priyadarsini, V. Jain, Synthesis, characterization and structures of 2-(3,5-dimethylpyrazol-1-yl)ethylseleno derivatives and their probable glutathione peroxidase (GPx) like activity, *Org. Biomol. Chem.* 9 (2011) 2992–2998.
- [74] S. Kumar, S.K. Tripathi, H.B. Singh, G. Wolmershauser, Synthesis, reactivity, electrochemical and crystallographic studies of diferrocenoyl diselenide and ferrocenoyl selenides, *J. Organomet. Chem.* 689 (2004) 3046–3055.
- [75] S.R. Wilson, P.A. Zucker, R.R.C. Huang, A. Spector, Development of synthetic compounds with glutathione-peroxidase activity, *J. Am. Chem. Soc.* 111 (1989) 5936–5939.
- [76] J.A. Timbrel, Principles of Biochemical Toxicology, fourth ed., Publisher Informa Healthcare, USA, 2009.
- [77] D. Grotto, L.S. Maria, J. Valentini, C. Paniz, G. Schmitt, S.C. Garcia, J.B.T. Rocha, M. Farina, Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification, *Quim. Nova* 32 (2009) 169–174.
- [78] C.W. Nogueira, J.B.T. Rocha, Diphenyl diselenide a janus-faced molecule, *J. Braz. Chem. Soc.* 21 (2010) 2055–2071.
- [79] I.J. Kade, J.B.T. Rocha, Gallic acid modulates cerebral oxidative stress conditions and activities of enzyme-dependent signaling systems in streptozotocin-treated rats, *Neurochem. Res.* 38 (2013) 761–771.
- [80] R. Zhao, A. Holmgren, A novel antioxidant mechanism of ebselen involving ebselen diselenide, a substrate of mammalian thioredoxin and thioredoxin reductase, *J. Biol. Chem.* 277 (2002) 39456–39462.
- [81] A.S. de Freitas, A.S. Prestes, C. Wagner, J.S. Haigert, D. Alves, L.P. Oliveira, I.J. Kade, J.B.T. Rocha, Reduction of diphenyl diselenide and analogs by mammalian thioredoxin reductase is independent of their glutathione peroxidase-like activity: a possible novel pathway for their antioxidant activity, *Molecules* 15 (2010) 7699–7714.
- [82] A.S. de Freitas, J.B.T. Rocha, Diphenyl diselenide and analogs are substrates of cerebral rat thioredoxin reductase: a pathway for their neuroprotective effects, *Neurosci. Lett.* 503 (2011) 1–5.
- [83] S. Kumar, M. Björnstedt, A. Holmgren, Selenite is a substrate for calf thymus thioredoxin reductase and thioredoxin and elicits a large non-stoichiometric oxidation of NADPH in the presence of oxygen, *Eur. J. Biochem.* 207 (1992) 435–439.
- [84] S.J. Balkrishna, B.S. Bhakuni, D. Chopra, S. Kumar, Cu-catalyzed efficient synthetic methodology for ebselen and related Se-N heterocycles, *Org. Lett.* 12 (2010) 5394–5397.
- [85] A. Ruwet, M. Renson, Mise au point d'une synthèse du chlorure d'ortho méthylselenobenzoyl. Etude de son comportement vis-à-vis des dérivés organocadmiques, *B. Soc. Chim. Belg.* 75 (1966) 157.
- [86] N.L. Moller, K.E. Dietzel, H.T. Schneider, K. Brune, Novel 5-aminosalicylic acid NSAID conjugates - synthesis - pharmacological and toxicological properties, *Eur. J. Med. Chem.* 24 (1989) 463–469.
- [87] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal. Biochem.* 95 (1979) 351–358.
- [88] R.L. Puntel, D.H. Roos, D. Grotto, S.C. Garcia, C.W. Nogueira, J.B.T. Rocha, Antioxidant properties of Krebs cycle intermediates against malonate pro-oxidant activity in vitro: a comparative study using the colorimetric method and HPLC analysis to determine malondialdehyde in rat brain homogenates, *Life Sci.* 81 (2001) 51–62.