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Highly Efficient Glutathione Peroxidase and Peroxiredoxin Mimetics Protect Mammalian Cells against Oxidative Damage**

Debasish Bhowmick, Shubhi Srivastava, Patrick D'Silva, and Govindasamy Mugesh**

Abstract: *Novel isoselenazoles with high glutathione peroxidase (GPx) and peroxiredoxin (Prx) activities provide remarkable cytoprotection to human cells, mainly by exhibiting antioxidant activities in the presence of cellular thiols. The cytotoxicity of the isoselenazoles is found to be significantly lower than that of ebselen, which is being clinically evaluated by several groups for the treatment of reperfusion injuries and stroke, hearing loss, and bipolar disorder. The compounds reported in this paper have the potential to be used as therapeutic agents for disorders mediated by reactive oxygen species.*

Reactive oxygen species (ROS), which are normally generated inside the cell as byproducts of various metabolic processes, contribute to different intracellular functions, including gene expression. However, dysregulation of this redox homeostasis results in elevated ROS levels, which cause oxidative damage to the cellular building blocks, namely nucleic acids, proteins, and lipids, ultimately leading to cell death.[1] Under normal physiological conditions, the cellular redox balance is maintained by several antioxidant systems, among which the glutathione (GSH), thioredoxin (Trx), and peroxiredoxin (Prx) cycles play central roles.[2] However, under pathological conditions, the cellular antioxidant machinery is insufficiently able to counterbalance the elevated ROS levels resulting from the aberrant metabolic pathways, which thus lead to disorders such as neurodegeneration, cancer, diabetes, atherosclerosis, arthritis, kidney failure, and aging.[1–3] In this regard, selenium compounds have been studied for their ability to reduce hydrogen peroxide by mimicking the function of glutathione peroxidase (GPx), a selenoenzyme, using thiol cofactors.[4] Whereas the GPx-like activity of synthetic organoselenium compounds and the chemical mechanism underlying this activity have been studied extensively in the past,[4,5] there is limited information available on the antioxidant activity of these compounds in human cells. Herein, we show that the diselenides **3a–3d** undergo a rapid cyclization to produce the corresponding isoselenazoles **4a–4d**, which exhibit high GPx-like activity. We also describe the remarkable cytoprotective effects of these compounds on human cell lines.

Diselenide **1**, which was required for this study, was synthesized by the low-temperature lithiation of 3-methoxybenzaldehyde with *n*BuLi in the presence of *N*,*N*,*N*′-trimethylethylenediamine, followed by selenium insertion and oxidative workup.[6] Treatment of **1** with primary amines ($R-NH_2$) in dry acetonitrile provided the Schiff bases **2a–2d**, which afforded diselenides **3a–3d** upon reduction with $NaBH_4$ and oxidation. These diselenides, however, were found to be quite unstable and underwent rapid cyclization to produce the cyclic isoselenazoles **4a–4d** during purification (Figure 1A). It should be noted that such a facile cyclization

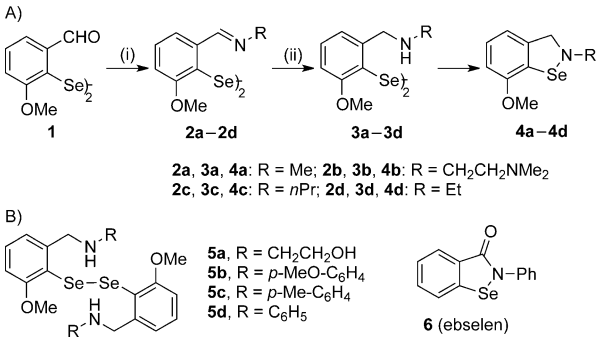

Figure 1. A) Synthesis of diselenides **3a–3d** and their spontaneous conversion into the isoselenazoles **4a–4d**. Reagents and Conditions: i) $R-NH_2$, MeCN, HCl, 4 h; ii) $NaBH_4$, MeOH, 30 min, O_2. B) Chemical structures of the secondary-amine-based diselenides **5a–5d** and ebselen.

was only observed for amide-based diaryl diselenides.[5f] Earlier studies had shown that diaryl diselenides with secondary amine moieties do not undergo any cyclization,[7] indicating that the presence of the methoxy group in **3a–3d** is probably responsible for the cyclization. However, the stability of the diselenides depends on the nature of the substituent attached to the nitrogen atom. For example, the secondary-amine-based diselenides **5a–5d**, which feature $-CH_2CH_2OH$, phenyl, or other aryl substituents (Figure 1B), are quite stable and do not undergo cyclization under identical conditions.

[*] Dr. D. Bhowmick,[+] Prof. Dr. G. Mugesh
Department of Inorganic and Physical Chemistry
Indian Institute of Science
Bangalore-560012 (India)
E-mail: mugesh@ipc.iisc.ernet.in

S. Srivastava,[+] Prof. Dr. P. D'Silva
Department of Biochemistry, Indian Institute of Science
Bangalore-560012 (India)
E-mail: patrick@biochem.iisc.ernet.in

[+] These authors contributed equally to this work.

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The GPx-like activity of compounds **4a–4d** was studied using GSH as the thiol cofactor and three different peroxides as the substrates. The initial rates (v_0) were determined by using a GSH–GSSG coupled assay.[8] The activity of the new compounds was compared to that of ebselen (**6**), which is a known scavenger of ROS in the presence of GSH[9] and Trx,[10] and an efficient substrate for the human thioredoxin reductase (TrxR).[11] Compounds **4a–4d** were found to be two to three times more active than ebselen in all three peroxide systems (Figure 2). The diselenides **5a–5d** exhibited much

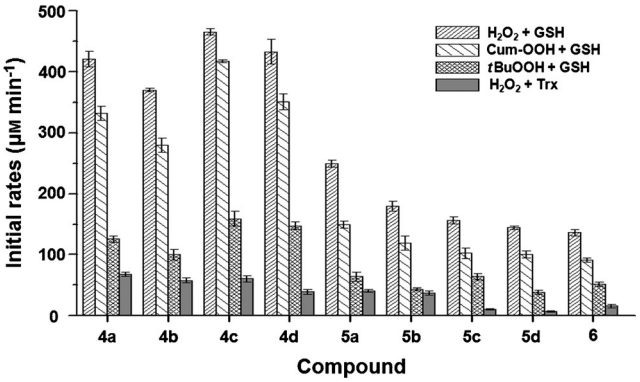
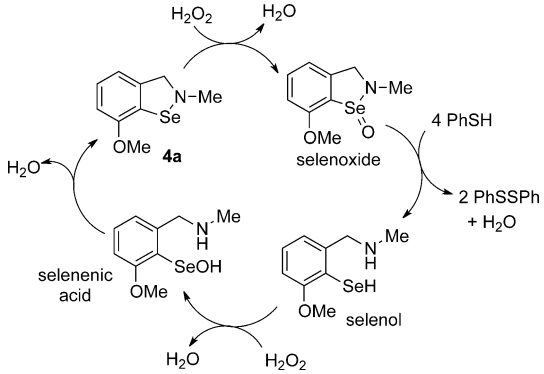


Figure 2. Initial rates for the reduction of peroxides by GSH or Trx in the presence of selenium compounds. GSH–GSSG assay: phosphate buffer (0.1 M, pH 7.5), catalyst (80.0 μM), GSH (2.0 mM), NADPH (0.4 mM), EDTA (1.0 mM), glutathione reductase (1.7 units/mL), peroxides (1.6 mM), 22 °C. Trx assay: phosphate buffer (50 mM, pH 7.5), catalyst (10.0 μM), Trx (10.0 μM), NADPH (250 μM), EDTA (1.0 mM), TrxR (50 nM), H_2O_2 (200 μM), 22 °C. The control reactions were performed under identical conditions in the absence of selenium compounds.

lower activities than the isoselenazoles **4a–4d**, but were still found to be marginally more active than ebselen. In particular, compounds **5b–5d**, which feature larger substituents on the nitrogen atoms, exhibited lower activities than **5a**. The bulkier substituents in **5b–5d** may prevent interactions with GSH as cleavage of the Se–Se bond by GSH is important for their GPx activity. Interestingly, compounds **4a–4d** and **5a–5d** also exhibited Prx-type activities by catalyzing the reduction of H_2O_2 in the presence of Trx at comparatively low concentrations (Figure 2). In fact, the Prx-mimetic activity appeared to be the most prominent one for the novel compounds. For example, the Prx-mimetic activity of the two representative compounds **4a** and **4c** was found to be almost three orders of magnitude larger than their GPx activity (see the Supporting Information, Table S1). Ebselen also exhibited a higher activity in the presence of Trx, which is in agreement with the previous observations of Zhao and Holmgren.[10]

Thiols such as PhSH or GSH can readily cleave the Se–N bond in ebselen (**6**), which is essential for its biological activity.[5f] Interestingly, the reactivity of the Se–N bond in **4a–4d** is remarkably different from that of ebselen. When compounds **4a–4d** were treated with an equimolar amount of PhSH, no reaction was observed under ambient conditions. With an excess amount of PhSH (5 equiv), these compounds underwent very slow reactions with the thiol over a period of five days to produce the corresponding selenenyl sulfides and diselenides (Figure S33). In contrast, the diselenides **5a–5d** reacted readily with one equivalent of PhSH to quantitatively produce the corresponding selenenyl sulfides and selenols (Figure S34). On the other hand, **4a–4d** reacted rapidly with H_2O_2 to produce the corresponding selenoxides, which further reacted with PhSH to produce the respective selenols. The reactions of selenols with H_2O_2 regenerated **4a–4d** via the formation of selenenic acids (Scheme 1) for compound **4a** (Figure S35–37).



Scheme 1. Catalytic cycle of compound **4a** involving selenoxide, selenol, and selenenic acid species.

To understand the antioxidant potential of these compounds in human cell lines, human-kidney-derived HEK293T cells that had been pretreated with H_2O_2 were incubated with the selenium compounds (Figure 3 A). The cellular ROS levels were measured using a DCFDA fluorescence probe (Figure S38; DCFDA = 2′,7′-dichlorodihydrofluorescein diacetate). When the HEK293T cells were treated with 500 μM of H_2O_2, there was an enhancement in the peroxide level as evidenced by an increase in the fluorescence intensity (Figure 3 A). Treatment of the cells with 40 μM of compounds **4a–4d** led to a decrease in the fluorescence intensity (Figure 3 A), indicating the ROS scavenging effect of the selenium compounds. The activities of **4a** and **4c** were found to be higher than that of ebselen (**6**). To understand the effect of these compounds on elevated H_2O_2 levels inside the cells, the antioxidant enzyme catalase, which converts H_2O_2 into water and molecular oxygen, was inhibited using 3-amino-1,2,4-triazole (3-AT). At this elevated H_2O_2 level, the ROS scavenging activities of **4a–4d** were much higher than that of ebselen (Figure 3 B). Similar results were obtained when the H_2O_2 specific dye Amplex Red was used for the experiments (Figure S39 A), indicating that compound **4a–4d** exert their antioxidant effect by reducing the amount of H_2O_2 in the cells. To test the ability of ebselen and **4a–4d** to combat oxidative stress in other mammalian cell types, we carried out experiments with HeLa cell lines. An increase in the ROS level either upon treatment of cells with H_2O_2 or by inhibiting the catalase activity was observed by fluorescence microscopy. As shown in Figure 3 C, the ROS scavenging activity of **4a** was significantly higher than that of ebselen under oxidative

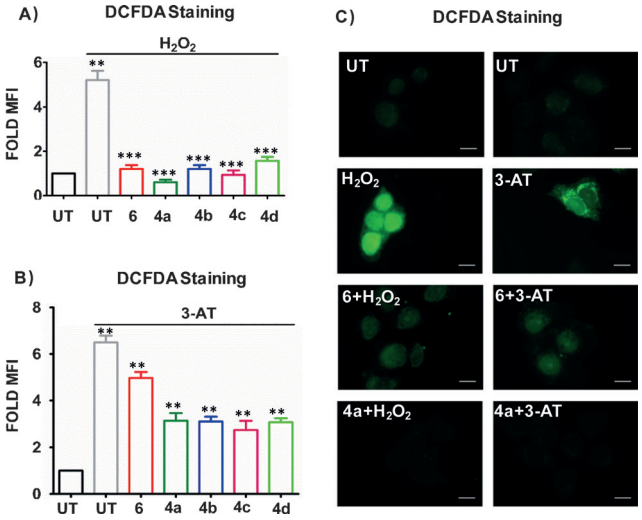


Figure 3. ROS scavenging activities of the selenium compounds in human cells. A, B) Relative mean fluorescence intensities of HEK293T cells left untreated (UT) or treated with selenium compounds after exposure to H_2O_2 or 3-AT. Data is represented as the FOLD mean fluorescence intensity (MFI) compared to that of untreated cells. Bars denote mean values (±SEM; $n=3$, P^{**} (t-test) <0.001, P^{***} (t-test) <0.0001). C) HeLa cells were treated with ebselen or **4a**, and the level of H_2O_2 was measured in the presence of DCFDA by fluorescence microscopy. Scale bars: 10 nm.
SEM = standard error of the mean.

stress conditions. The other isoselenazoles (**4b**–**4d**) also exhibited good activities in HeLa cells, and the ROS scavenging activities of these compounds were found to be similar to that of ebselen (Figure S39E). The antioxidant activities of diselenides **5a**–**5d** were found to be similar to or lower than that of ebselen (Figure S39B–E), which is in agreement with their GPx activity (Figure 2).

To understand the participation of various thiol cofactors,[12] we inhibited three major components of the antioxidant machinery, namely GSH, Trx, and glutaredoxin (Grx), by using specific inhibitors. The cellular level of GSH was reduced by treating the cells with buthionine sulfoximine (BSO), which is known to block GSH biosynthesis.[13] The cells were treated with BSO (50 μM) prior to exposure to H_2O_2 and selenium compounds, and a significant decrease in the GSH level was observed (Figure S46). In a control experiment, the HEK293T cells were treated only with BSO in the absence of H_2O_2 (Figure S40A). Upon DCFDA staining, we observed that the ROS scavenging activities of ebselen and **4a**–**4d** marginally decreased with a significant deleterious effect on the antioxidant effect of **4a** (Figure 4A). However, compounds **4a**–**4d** exhibited considerable antioxidant activity, probably owing to the availability of other cofactors in the cells. On the other hand, an almost complete loss of activity was observed for the diselenides **5a**–**5d** upon depletion of GSH (Figures S40B and S41A), indicating that the diselenides primarily use GSH as a cofactor for their GPx-like activity. We also depleted the intracellular level of Trx by treating the cells with suberoylanilide hydroxamic acid (SAHA).[14] The decrease in the Trx1 level was confirmed by Western blot analysis (Figure S46). The cells were incubated with SAHA (7.5 μM) for five hours prior to the treatment with H_2O_2. A significant decrease in the ROS scavenging activity was observed for ebselen and compounds **4a**–**4d** (Figures 4B and S40C), indicating that these compounds also use the Trx system for their antioxidant activity. In particular, the antioxidant activity of **4b** is mainly due to the presence of Trx as this compound lost almost its entire activity upon treatment with SAHA. Interestingly, very high levels of oxidative stress were observed in cells treated with the diselenides **5a**–**5d**, which is probably due to the poor permeability of these compounds compared to that of the cyclic isoselenazoles. In fact, an enhancement in the ROS level over the control level was observed for these compounds upon depletion of Trx (Figures S40D and S41B).

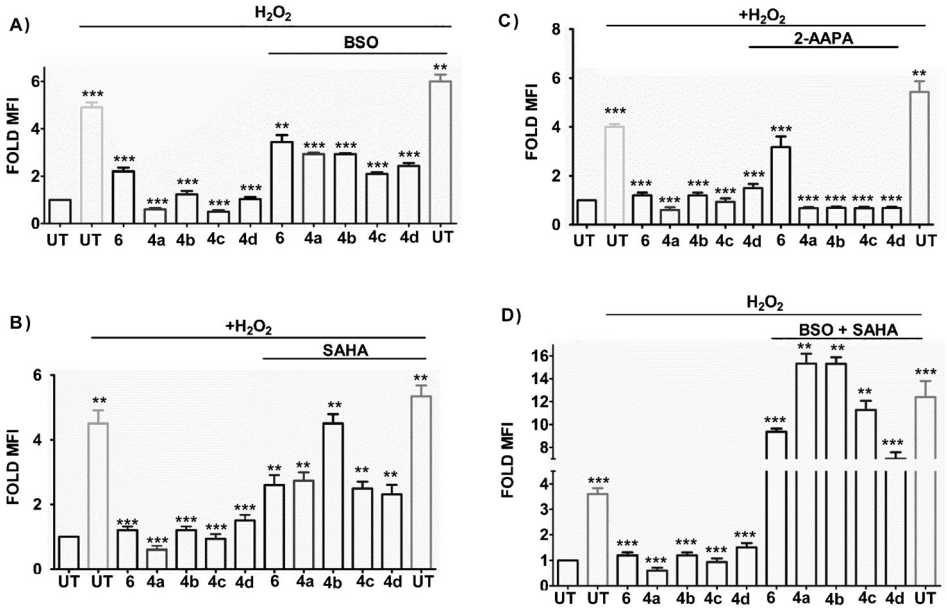


Figure 4. A–D) ROS scavenging activities of selenium compounds in HEK293T cells. Relative mean fluorescence intensities of cells that were left untreated (UT) or treated with the compounds with prior exposure to BSO (A), SAHA (B), 2-AAPA (C), or BSO and SAHA (D) followed by treatment with H_2O_2. Bars denote mean values (±SEM; $n=3$, P^{**} (t-test) <0.001, P^{***} (t-test) <0.0001).

To determined whether Grx is involved in the antioxidant function of the compounds, we depleted the cellular level of Grx by treating the cells with 2-AAPA (2-AAPA = 2-acetylamino-3-[4-(2-acetylamino-2-carboxyethyl-sulfanyl-thiocarbonylamino)phenylthiocarbamoylsulfanyl]propionic acid),[15] which inhibits Grx1, for 20 minutes (Figure S46).

Interestingly, the ROS scavenging activities of **4a**–**4d** (Figures 4C and S40E) and **5a**–**5d** (Figures S40F and S41C) were almost unaffected by the decrease in Grx concentration, indicating that these compounds do not use the Grx system for their activity. In contrast, ebselen appeared to use Grx as well for its activity as there was a significant enhancement in the ROS level when the cells were treated with 2-AAPA (Figure 4C). When a combination of BSO and SAHA was used, the amount of ROS produced in the presence of **4a**–**4d** was similar to that in the control experiment (Figure 4D). In the case of diselenides **5a**–**5d**, an enhancement in the ROS level was observed upon treatment of the cells with BSO and SAHA (Figure S42). These observations indicate that the presence of GSH or Trx is essential for the activity of both isoselenazoles (**4a**–**4d**) and diselenides (**5a**–**5d**).

Oxidative stress damages major cellular components, such as the DNA, proteins, and lipids.[2a,16,17] To understand the protective effect of the selenium compounds against DNA damage, we measured the amount of γH2AX foci formation. HEK293T cells were treated with H_2O_2 followed by test compounds and the extent of DNA damage was probed with the anti-γH2AX antibody. Whereas ebselen provided about 70% protection, **4a** and **4c** exhibited remarkable activities with more than 95% protection (Figure S43). The diselenides **5a** and **5b** also prevented DNA damage with a protection of approximately 92% and 85%, respectively (Figure S43). As the toxicity of selenium compounds is a major concern in the drug development process, we studied the effect of ebselen, **4a**, **4c**, **5a**, and **5b** on cell death caused by oxidative stress. When HEK293T cells were stained with fluorescence-active propidium iodide (PI) after treatment with the selenium compounds,[18] a decrease in cell death was observed (Figures 5A and S44A,B). At a concentration of 10 μM, approximately 15% cell death was observed with ebselen, whereas the toxicities of **4a**, **4c**, **5a**, and **5b** were found to be much lower. For these compounds, the lethality was only approximately 3–4%, and there was no significant increase in the lethality when the concentration of these compounds was increased from 10 μM to 80 μM (Figure 5A). Depletion of cellular GSH levels led to a significant increase in cell death, and the toxicity increased further when Trx was depleted by treatment with SAHA (Figure S44C–F). In contrast, no increase in the lethality was observed when the Grx level was reduced by treating the cells with 2-AAPA (Figure S44G,H). These results confirm that the selenium compounds rely on GSH and Trx for their ROS scavenging activities, with Trx playing a major role in promoting the antioxidant activity.

The higher toxicity of ebselen compared to those of **4a**, **4c**, **5a**, and **5b** is probably due to the inhibition of glutathione reductase (GR), an enzyme that regenerates GSH from GSSG by using NADPH. To analyze GR inhibition, we used cell lysates obtained after treatment with the selenium compounds. Interestingly, ebselen almost entirely inhibited the GR activity. Compounds **4a** and **4c** weakly inhibited GR,

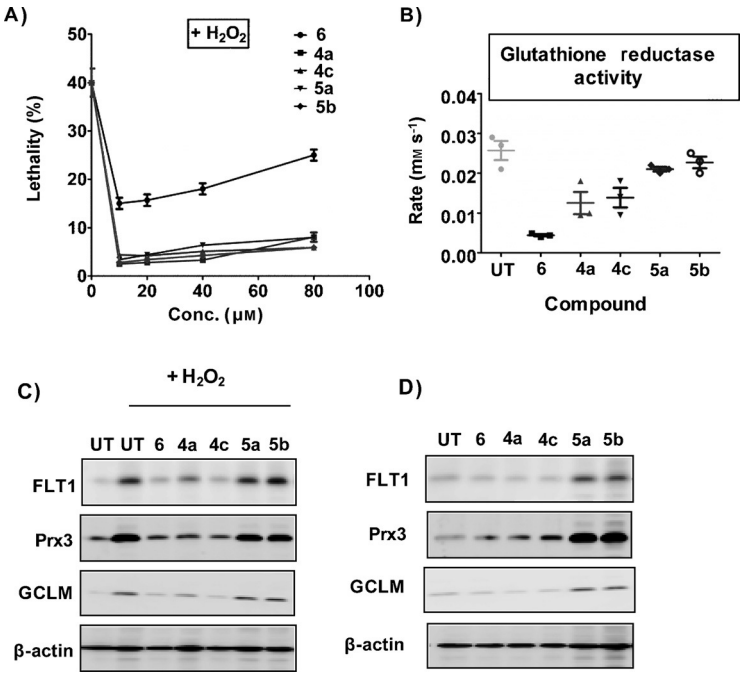


Figure 5. A) Toxicities of ebselen and **4a**–**4d** in HEK293T cells as determined by PI staining and represented in terms of the percentage lethality. B) Inhibition of GR activity in cell lysate. C, D) The effect of selenium compounds on the antioxidant machinery and Nrf2-responsive genes. Blots were probed with anti-β-actin antibodies as a loading control.

whereas the diselenides **5a** and **5b** did not inhibit the GR activity (Figure 5B). The inhibition of GR by the selenium compounds was confirmed independently by using pure GR enzyme in a GSH/GSSG coupled assay (Figure S45). The nucleophilic attack of the active-site cysteines (Cys58 and Cys63) at the selenium center of ebselen and the subsequent formation of two stable Se–S bonds are responsible for the inhibition (Figure 6). The formation of selenenyl sulfides with GR was confirmed by MALDI-TOF mass spectrometry (Figure S47), and its effect on electron transfer was studied by UV/Vis spectroscopy[19] (Figure S48). To the best of our knowledge, this study provides the first experimental evidence that the toxicity of such selenium compounds in mammalian cells is associated with the inhibition of GR by these compounds.

High ROS levels and the resulting oxidative stress lead to the activation of several cellular stress pathways. Nrf2 is the major transcription factor that regulates the responses against

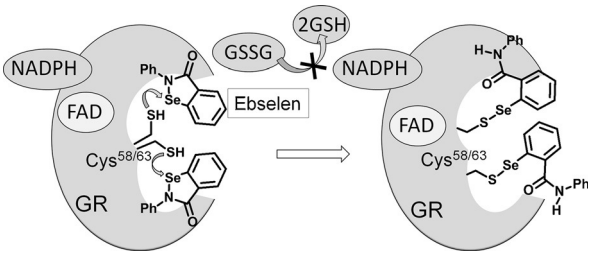


Figure 6. The Se–N bond cleavage by the active-site cysteine residues (Cys58 and Cys63) produces enzyme-bound selenenyl sulfides.

oxidative stress, including the expression of antioxidant enzymes.[20] To understand whether the selenium compounds alter the Nrf2 response, we tested the activation of downstream genes regulated by Nrf2. We observed an enhancement in the expression levels of Nrf2-regulated genes (FLT1, peroxiredoxin-3, glutamate–cysteine ligase modifier subunit)[21] upon treatment with H_2O_2. These expression levels of the regulatory genes reverted back to normal levels when the cells were treated with ebselen, **4a**, or **4c** (Figure 5C,D), suggesting that these compounds do not alter the Nrf2 signaling. However, an increased expression of Nrf2-responsive genes was detected in cells treated with **5a** or **5b**, suggesting that the ROS scavenging activity of the diselenides is partly mediated by an enhanced cellular antioxidant response (Figure 5C,D).

In summary, we have described the synthesis and the GPx- and Prx-like activities of novel isoselenazoles and diselenides. The isoselenazoles were readily internalized into cells and displayed excellent GPx and Prx activities. Interestingly, the toxicity of the selenium compounds reported in this paper towards cells was remarkably low. These compounds are able to effectively scavenge ROS to prevent DNA damage in the presence of GSH and Trx and provide an important cytoprotection method to the cell. We believe that the selenium compounds, particularly the isoselenazoles, reported here have the potential to be used as therapeutic agents for diseases associated with oxidative stress. Importantly, the compounds do not down-regulate the endogenous antioxidant response, but rather supplement the antioxidant system in mammalian cells.

Keywords: antioxidants · DNA damage · glutathione peroxidase · reactive oxygen species · selenium

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[1] a) C. C. Winterbourn, *Nat. Chem. Biol.* **2008**, *4*, 278–286; b) P. D. Ray, B.-W. Huang, Y. Tsuji, *Cell. Signalling* **2012**, *24*, 981–990, and references therein.

[2] a) H. Sies, *Free Radical Biol. Med.* **1999**, *27*, 916–921; b) E. S. Arnér, A. Holmgren, *Eur. J. Biochem.* **2000**, *267*, 6102–6109; c) S. Rhee, H. Chae, K. Kim, *Free Radical Biol. Med.* **2005**, *38*, 1543–1552; d) Z. A. Wood, E. Schröder, H. J. Robin, L. B. Poole, *Trends Biochem. Sci.* **2003**, *28*, 32–40; e) H. Z. Chae, H. J. Kim, S. W. Kang, S. G. Rhee, *Diabetes Res. Clin. Pract.* **1999**, *45*, 101–112.

[3] a) H. Sies, *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 1058–1071; *Angew. Chem.* **1986**, *98*, 1061–1075; b) R. A. Cairns, I. S. Harris, T. W. Mak, *Nat. Rev. Cancer* **2011**, *11*, 85–95; c) J. W. Baynes, *Diabetes* **1991**, *40*, 405–412; d) M. Schieber, N. S. Chandel, *Curr. Biol.* **2014**, *24*, R453–R462.

[4] a) G. Mugesh, H. B. Singh, *Chem. Soc. Rev.* **2000**, *29*, 347–357; b) G. Mugesh, W. W. du Mont, H. Sies, *Chem. Rev.* **2001**, *101*, 2125–2179.

[5] a) A. Müller, E. Cadenas, P. Graf, H. Sies, *Biochem. Pharmacol.* **1984**, *33*, 3235–3240; b) T. G. Back, B. P. Dyck, *J. Am. Chem. Soc.* **1997**, *119*, 2079–2083; c) M. Iwaoka, S. Tomoda, *J. Am. Chem. Soc.* **1994**, *116*, 2557–2561; d) T. Wirth, *Molecules* **1998**, *3*, 164–166; e) T. G. Back, Z. Moussa, M. Parvez, *Angew. Chem. Int. Ed.* **2004**, *43*, 1268–1270; *Angew. Chem.* **2004**, *116*, 1288–1290; f) K. P. Bhabak, G. Mugesh, *Acc. Chem. Res.* **2010**, *43*, 1408–1419.

[6] a) D. L. Comins, J. D. Brown, *J. Org. Chem.* **1984**, *49*, 1078–1083; b) D. J. Press, N. M. R. McNeil, M. Hambrook, T. G. Back, *J. Org. Chem.* **2014**, *79*, 9394–9401.

[7] K. P. Bhabak, G. Mugesh, *Chem. Eur. J.* **2009**, *15*, 9846–9854.

[8] F. Ursini, M. Maiorino, R. Brigelius-Flohe, K. D. Aumann, A. Roveri, D. Schomburg, L. Flohe, *Methods Enzymol.* **1995**, *252*, 38–53.

[9] H. Sies, H. Masumoto, *Adv. Pharmacol.* **1997**, *38*, 229–246.

[10] R. Zhao, A. Holmgren, *J. Biol. Chem.* **2002**, *277*, 39456–39462.

[11] R. Zhao, H. Masayasu, A. Holmgren, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 8579–8584.

[12] a) L. A. Sena, N. S. Chandel, *Mol. Cell* **2012**, *48*, 158–167; b) T. Finkel, *J. Cell Biol.* **2011**, *194*, 7–15.

[13] J. Martensson, A. Jain, E. Stole, W. Frayer, P. A. Auld, A. Meister, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 9360–9364.

[14] L. M. Butler, X. Zhou, W. S. Xu, H. I. Scher, R. A. Rifkind, P. A. Marks, V. M. Richon, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11700–11705.

[15] S. S. Sadhu, E. Callegari, Y. Zhao, X. Guan, T. Seefeldt, *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 456–462.

[16] A. Vernekar, D. Sinha, S. Srivastava, P. U. Paramasivam, P. D'Silva, G. Mugesh, *Nat. Commun.* **2014**, *5*:5301, 1–13.

[17] M. K. Shigenaga, C. J. Gimeno, B. N. Ames, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 9697–9701.

[18] Z. Darzynkiewicz, X. Li, J. Gong, *Methods Cell Biol.* **1994**, *41*, 15–38.

[19] K. Becker, M. Gui, R. H. Schirmer, *Eur. J. Biochem.* **1995**, *234*, 472–478.

[20] Q. Ma, *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 401–426.

[21] D. A. Dickinson, A. L. Levonen, D. R. Moellering, E. K. Arnold, H. Zhang, V. M. Darley-Usmar, H. J. Forman, *Free Radical Biol. Med.* **2005**, *37*, 1152–1159.

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