

# Paradoxical Behavior of Organodiselenides: Pro-Oxidant to Antioxidant <sup>†</sup>

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<sup>†</sup> Presented at the 1st International Electronic Conference on Catalysis Sciences, 10–30 November 2020; Available online: <https://eccs2020.sciforum.net>.

Published: 9 November 2020

**Abstract:** Over the years, organodiselenides have emerged as the biologically relevant class of molecules. On the one hand, such compounds are known for pro-oxidant effects leading to toxicity in biological systems. On the other hand, there are growing evidences about their bio-mimetic activities as catalysts such as glutathione peroxidase (GPx)-like activity. Our recent work has explored this paradoxical behavior of diselenides in developing antioxidants and/or anticancer agents. For this purpose, a number of alkyl and aryl diselenides have been evaluated in different biological models. The results have shown that aryl diselenides, in particular pyridinediselenides, altered the ratio of the intracellular thiol redox pairs of glutathione (GSH) and glutathione disulfide (GSSG) towards reduction (antioxidant) rather than oxidation (pro-oxidant) to protect normal cells against radiation damage and to induce cytotoxicity in tumor cells. Further, these studies have also postulated that the intracellular redox state, the level of thioredoxin reductase (TrxR), and reductive intermediates (e.g., selenol and/or selone) might play a very important role in the manifestation of the toxicities of aryl diselenides in cells.

**Keywords:** organodiselenides; glutathione peroxidase; thioredoxin reductase; reductive stress; cytotoxicity

## 1. Introduction

Glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) are the two most important selenoenzymes present in mammalian cells for maintaining redox homeostasis [1]. GPx catalyzes the reduction of toxic hydro/lipid peroxides into nontoxic water molecules by using glutathione (GSH) as a cofactor. Similarly, TrxR catalyzes the reduction of oxidized thioredoxin (Trx) into a reduced form by using dihydronicotinamide-adenine dinucleotide phosphate (NADPH) as a cofactor. The catalytic activities of GPx and TrxR are governed by selenocysteine, a selenium containing amino acid present in their active sites [1]. The natural existence of such selenoenzymes has been the driving force for the design, synthesis, and evaluation of organoselenium compounds as pharmacological agents [2]. In the last one or few decades, several reports have appeared in the literature on the synthesis as well as the biological activities of both alkyl and aryl diselenides [3,4]. These studies have together confirmed the ability of diselenides to mimic GPx-like activity and to act as substrates for TrxR under cell-free conditions. However, under cellular conditions, diselenides can manifest antioxidant or pro-oxidant activity depending on their chemical forms, concentration, and redox state of the host cells [3,4]. Pyridine is an important chemical form present in several biological molecules like vitamin and nucleic acid. Additionally, it has been shown that diselenides containing heterocyclic

rings (e.g., pyridine) are better catalysts than compounds containing simple aryl groups (e.g., diphenyl diselenide ( $\text{Ph}_2\text{Se}_2$ )) [5,6]. On similar lines, we have recently reported the synthesis, purification, structure, and biological activities of dipyridinediselenide ( $\text{Py}_2\text{Se}_2$ ) and its amide derivative diniotinamidediselenide ( $\text{Nic}_2\text{Se}_2$ ) [5–9]. The present report discusses the salient findings of the biological activities of these two molecules so as to improve our understanding towards the toxicology of organodiselenides. The chemical structures of  $\text{Py}_2\text{Se}_2$  and  $\text{Nic}_2\text{Se}_2$  are given in Scheme 1.

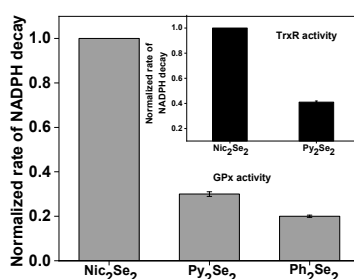


**Scheme 1.** Chemical structures of dipyridinediselenide ( $\text{Py}_2\text{Se}_2$ ) and its amide derivative diniotinamidediselenide ( $\text{Nic}_2\text{Se}_2$ ).

## 2. Bio-Chemical Activities under Cell-Free Conditions

### 2.1. GPx-Like Activity

The GPx-like activities of organoselenium compounds were evaluated by coupled NADPH assay. The principle of this assay is the reduction of peroxide (e.g., cumene hydroperoxide or  $\text{H}_2\text{O}_2$ ) by organoselenium compounds using GSH and NADPH as cofactors. Accordingly, GPx-like activity of  $\text{Py}_2\text{Se}_2$  and  $\text{Nic}_2\text{Se}_2$  was measured by quantitatively estimating the decay of NADPH or GSH in presence of peroxides through sensitive analytical techniques like spectrophotometer tests, HPLC, or NMR under cell-free conditions and compared with a standard diselenide-like  $\text{Ph}_2\text{Se}_2$  [5–9]. The results indicated that their activities followed the order of  $\text{Nic}_2\text{Se}_2 > \text{Py}_2\text{Se}_2 > \text{Ph}_2\text{Se}_2$  (Figure 1). The higher GPx-like activity of  $\text{Nic}_2\text{Se}_2$  was attributed to the presence of the nonbonding interaction between Se with N in this molecule [9]. As expected, the GPx-like activity of a diselenide can be initiated, either through its reduction into selenol ( $\text{RSeH}$ ) by GSH or its oxidation into selenenic acid ( $\text{RSeOH}$ ) by peroxide [9]. The enzyme kinetic analysis of  $\text{Nic}_2\text{Se}_2$  revealed that the GPx cycle of  $\text{Nic}_2\text{Se}_2$  was initiated predominately by its reduction with GSH. Further, it was also shown that, unlike simple aryl diselenide ( $\text{Ph}_2\text{Se}_2$ ), the reduction of  $\text{Nic}_2\text{Se}_2$  having a pyridine ring first formed selenol ( $\text{RSeH}$ ) which was immediately converted into a stable selone ( $\text{RC=Se}$ ), and this intermediate (i.e., the stable selone) can further take part in scavenging of reactive oxygen species (ROS) directly or indirectly through entry into a GPx cycle [9].



**Figure 1.** Plot showing the glutathione peroxidase (GPx) activity of different organodiselenides in terms of the rate (change in absorbance ( $\Delta\text{OD}$ )/min) of decay of NADPH under cell-free conditions. Inset of the figure shows the thioredoxin reductase (TrxR) activity in terms of the rate ( $\Delta\text{OD}/\text{min}$ ) of decay of NADPH using different organodiselenides as substrates under cell-free conditions. In both figures, the rate of NADPH decay is normalized with respect to that of  $\text{Nic}_2\text{Se}_2$ , the most active derivative. The catalyst concentrations (organoselenium compound) used for GPx and TrxR assay were 10  $\mu\text{M}$  and 25  $\mu\text{M}$ , respectively.

## 2.2. Substrate of TrxR

Several studies have shown that TrxR can reduce organodiselenides into the corresponding selenols and/or selones [3,4,10–12]. Accordingly, Nic<sub>2</sub>Se<sub>2</sub> and Py<sub>2</sub>Se<sub>2</sub> were evaluated for their abilities to undergo TrxR-mediated reduction. This possibility can be checked by simply following the decay of NADPH in the presence of the test compound and the enzyme TrxR. Alternatively, the test compound was also evaluated for its competitive inhibitory effect on the TrxR-mediated reduction of dithionitrobenzoic acid (DTNB) into thionitrobenzoic acid (TNB). Interestingly, at an equimolar concentration, both Nic<sub>2</sub>Se<sub>2</sub> and Py<sub>2</sub>Se<sub>2</sub> were found to increase the decay of NADPH or to inhibit the TNB formation in the TrxR activity assay reactions, suggesting that these molecules can undergo TrxR-mediated reduction [10,11]. Notably, Nic<sub>2</sub>Se<sub>2</sub> appeared to be a more potent substrate than Py<sub>2</sub>Se<sub>2</sub> for the mammalian TrxR (Figure 1).

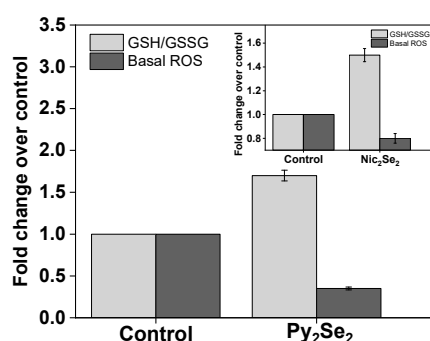
## 3. Comparative Cytotoxicity and Redox Modulation in Cells

Having understood the catalytic activities of Py<sub>2</sub>Se<sub>2</sub> and Nic<sub>2</sub>Se<sub>2</sub>, these molecules were investigated for cytotoxicity in cellular models [7,8,10,11]. Table 1 shows the half maximal inhibitory concentration (IC<sub>50</sub>) values of Py<sub>2</sub>Se<sub>2</sub> and Nic<sub>2</sub>Se<sub>2</sub> to induce cytotoxicity in various cell types by MTT assay. It can be seen that Py<sub>2</sub>Se<sub>2</sub> was more toxic than Nic<sub>2</sub>Se<sub>2</sub> in all the cell types investigated.

**Table 1.** Half maximal inhibitory concentration (IC<sub>50</sub>) of organodiselenides in cell lines [8,10,11].

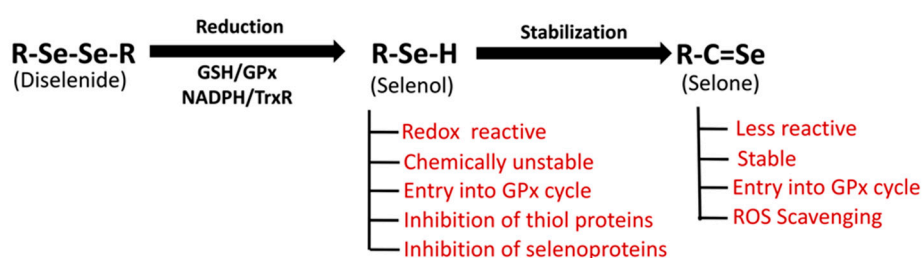
Compounds	CHO	WI38	A549	MCF7
	Normal ovary epithelium	Normal lung fibroblast	Lung carcinoma	Breast carcinoma
2,2'-dipyridyl diselenide (Py <sub>2</sub> Se <sub>2</sub> )	~6 µM	~8 µM	~5 µM	~5 µM
2,2'-diselenobis-[3-amidopyridine] (Nic <sub>2</sub> Se <sub>2</sub> )	>100 µM			~70 µM

In general, the cytotoxic effects of alkyl and aryl diselenides are correlated with their pro-oxidant effects or abilities to modulate the intracellular redox environment (GSH/GSSH) towards oxidation [3,4]. Accordingly, Py<sub>2</sub>Se<sub>2</sub> and Nic<sub>2</sub>Se<sub>2</sub> were expected to show similar effects in cellular systems. However, the results have shown that the treatments of CHO and A549 cells with Nic<sub>2</sub>Se<sub>2</sub> and Py<sub>2</sub>Se<sub>2</sub>, respectively, in a nearly identical concentration range (10–25 µM) resulted in a significant decrease in basal ROS level and a concurrent increase in total thiol content as well as an increase in the ratio of the glutathione couple (GSH/GSSH) (Figure 2) [10,11]. Additionally, the treatment with Py<sub>2</sub>Se<sub>2</sub> or Nic<sub>2</sub>Se<sub>2</sub> led to a significant increase in the transcript level of γ-GCL, a rate-limiting enzyme involved in GSH biosynthesis [10,11]. Thus, these results have suggested that Py<sub>2</sub>Se<sub>2</sub> or Nic<sub>2</sub>Se<sub>2</sub> induced reductive environment in cells. However, the extent of the reductive state in terms of the fold change in the ratio of GSH/GSSG was significantly higher in Py<sub>2</sub>Se<sub>2</sub>-treated cells as compared to Nic<sub>2</sub>Se<sub>2</sub>-treated cells [10,11]. Further reductive stress in Py<sub>2</sub>Se<sub>2</sub>-treated A549 cells was found to be associated with DNA damage, cell cycle deregulation, and apoptosis [11]. On the other hand, the pretreatment of CHO cells with Nic<sub>2</sub>Se<sub>2</sub> showed significant protection from γ-radiation-induced DNA damage and cell death [10]. Both Py<sub>2</sub>Se<sub>2</sub> and Nic<sub>2</sub>Se<sub>2</sub> are expected to undergo reduction within cellular environments via two pathways: (1) futile cycle involving GSH; (2) GPx cycle involving GSH; and (3) working as a TrxR substrate involving NADPH. Of these three pathways, the futile cycle is believed to be responsible for the pro-oxidant effects of diselenides through generation of ROS, whereas the rest two pathways can contribute to the reductive environment through ROS scavenging, either directly or via intermediates like selone [3,4,9,10]. Since the treatments of cells with Py<sub>2</sub>Se<sub>2</sub> and Nic<sub>2</sub>Se<sub>2</sub> exhibited elevation rather than decrease in GSH/GSSG ratio, the entry of Py<sub>2</sub>Se<sub>2</sub> and Nic<sub>2</sub>Se<sub>2</sub> into GPx and TrxR appeared to be the predominant pathways for their reduction within cells. At this stage, it is also important to understand the factors contributing to differential toxicity of Nic<sub>2</sub>Se<sub>2</sub> versus Py<sub>2</sub>Se<sub>2</sub>. In this context, one of the probable factors could be the formation of reaction intermediates. As shown in Scheme 2, the reductive pathway of Py<sub>2</sub>Se<sub>2</sub> and Nic<sub>2</sub>Se<sub>2</sub> forms selenol, which is further, converted into selone [9].



**Figure 2.** Plot showing the fold changes in the levels of the basal glutathione (GSH)/glutathione disulfide (GSSG) ratio and reactive oxygen species (ROS) in A549 cells after treatment with Py<sub>2</sub>Se<sub>2</sub> (10 μM) for 24 h [11]. The inset shows fold changes in the levels of the basal GSH/GSSG ratio and ROS in CHO cells after treatment with Nic<sub>2</sub>Se<sub>2</sub> (10 μM) for 16 h [10]. The levels of the GSH/GSSG ratio and ROS in the treatment groups are normalized with respect to that of the control group.

#### R - Aryl group with pyridine ring



**Scheme 2.** Scheme depicting the reductive pathway of aryl (with a pyridine ring) diselenide forming intermediates with their probable functions.

Of these two intermediates, selenol is considered to be highly reactive and can react with cellular thiol or thiol containing proteins, which inhibit their activities and thus can cause cytotoxicity. On the other hand, selone is more stable as well as less reactive species. Therefore, the relative proportions of selenol and selone as well as the stability of the latter can dictate the toxicity of aryl diselenides containing a pyridine ring. Notably, our studies have shown that selone is the predominant intermediate formed during the reduction of Nic<sub>2</sub>Se<sub>2</sub> due to availability of selenoamide moiety providing extra stabilization to the reduced species [9]. However, in case of Py<sub>2</sub>Se<sub>2</sub>, there is a possibility of the degradation of selone species, explaining its higher toxicity in both normal and tumor cells. These assumptions were also justified considering the fact that cells treated with Py<sub>2</sub>Se<sub>2</sub> showed inhibition or decrease in the activity of selenium or thiol containing proteins like GPx, TrxR, glutathione S transferase (GST), and glutathione reductase (GR) [11]. On the contrary, the Nic<sub>2</sub>Se<sub>2</sub> treatment of cells did not show much inhibition of these proteins [10]. In addition to the above discussed factors, tumor versus normal cells differ in terms of the intracellular redox state as well as the expression levels of TrxR, which is generally over-expressed in tumor cells, and therefore, these may also contribute to differential toxicity of aryl diselenides containing a pyridine ring; however, this needs to be validated in future studies.

## 4. Conclusions

In conclusion, our results, for the first time, provided evidence that low concentrations of aryl diselenides containing a pyridine ring modulate the intracellular redox state towards reduction

(antioxidant) rather than the oxidation (pro-oxidant) side in both normal and cancer cells. The reductive stress mediated by such compounds led to cytotoxic or apoptotic effects in cancer cells. Additionally, the cellular redox state, the level of TrxR, and reductive intermediates (selenol and selenone) appeared to be the major determinants of the toxicity of aryl diselenides with a pyridine ring. Accordingly, our future interest is to correlate the cellular speciation of diselenides with their biological activities in cells and *in vivo* model systems.

**Funding:** This research received no external funding.

**Acknowledgments:** The authors thank V.K.J., UM-DAE-CEBS, B.G.S., BARC, P.P.P., BARC and students whose names appeared as co-authors in the publications. The authors also acknowledge A.K. and A.K.T., for their support and encouragement. K.I.P. thanks DAE for the Raja Ramanna Fellowship.

**Conflicts of Interest:** The authors declare no conflict of interest.

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