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Small Organoselenium Compounds: More than just Glutathione Peroxidase Mimics

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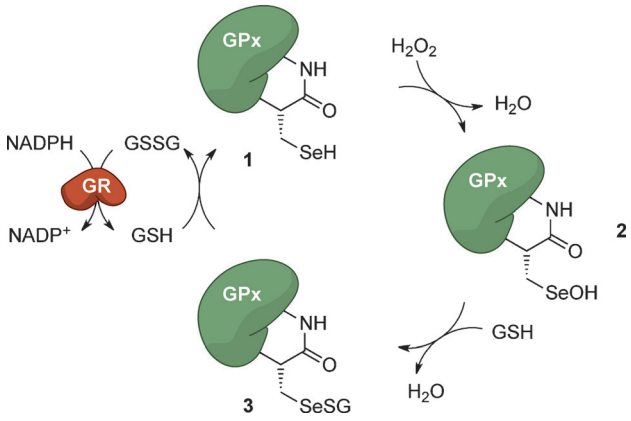
Reactive oxygen species (ROS), which are formed in biological systems as byproducts from metabolic processes or through external influence, are usually tamed in normal operating cells as redox balances are maintained by several antioxidant systems. Although ROS contribute to cellular functions, such as gene expression, and the formation of superoxide radical anions from oxygen is essential for the catabolic mechanism, any imbalance can cause severe problems such as neurodegeneration, cancer, diabetes, aging, and other disorders through lipid peroxidation or oxidative damage to the living cell.

Glutathione peroxidase (GPx) is one of the mammalian antioxidant enzymes, which is able to decompose ROS, such as peroxides, in the cell to water or alcohols by using glutathione (GSH) or other related thiols as cofactors. The human GPx enzymes have a selenocysteine residue in their active site which is responsible for the catalytic activity. Their first isolation was performed in 1973 by Flohé and co-workers confirming the enzyme contains selenium atoms.[1] The X-ray structural analysis showed the selenium atom positioned in its active site.[2] Thioredoxin (Trx) and peroxiredoxin (Prx) are similarly important proteins with antioxidant activities. Their function is based on cysteine residues with a sulfur atom in their active sites with different mechanisms of action for the detoxification of ROS.

The discovery that GPx contains a selenium moiety that is responsible for its redox properties established selenium as an essential trace element. About 70 μg selenium are recommended by the World Health Organization (WHO) as an average daily intake in human. Even before that finding it was observed that selenium has a beneficial effect on living organisms.[3] A typical catalytic cycle for GPx is shown in Scheme 1, in which hydrogen peroxide is shown as an oxidant. After the reaction of hydrogen peroxide with the selenol moiety (Enz-SeH) **1** of the selenocysteine residue, a selenenic acid **2** (Enz-SeOH) is formed. This selenenic acid then reacts with cellular thiols, such as GSH, to initially form a selenenyl sulfide **3** (E-Se-SG), which requires a second thiol equivalent



Scheme 1. Proposed catalytic cycle of glutathione peroxidase (GPx) for the reduction of hydrogen peroxide and regeneration of glutathione (GSH) using glutathione reductase (GR).

for the cleavage of the selenium–sulfur bond and regenerates selenol **1**. In this cleavage process, one equivalent of glutathione disulfide (GSSG) is produced, which can be reduced again to GSH by the NADPH-dependent glutathione reductase (GR) to maintain the GSH level within the cell. Large amounts of ROS could lead to the formation of seleninic acid (Enz-SeO_2H), a reaction that is reversible in the presence of GSH. However, further oxidation to a selenonic acid (Enz-SeO_3H) will inactivate the enzyme.

Subsequently it was also shown that small, selenium-containing molecules have an effect on biological systems. The compound ebselen **4** (Figure 1) was amongst the first

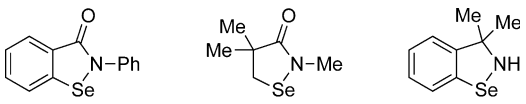


Figure 1. Ebselen **4** and related GPx mimics.

molecules systematically studied and shown to mimic GPx activity,[4] but also related aliphatic compounds **5**[5] and aromatic cyclic compounds **6**[6] were evaluated. Many other compounds have also been investigated as potential GPx mimics.[7]

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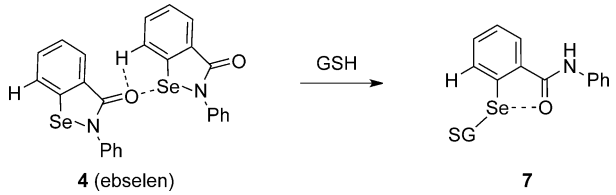
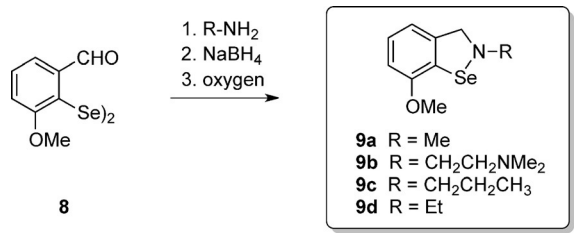
The very close proximity of the nitrogen moiety to the selenium atom in compounds **4**–**6** resembles the structural features of the active site around the selenocysteine residue in GPx. In GPx, nitrogen moieties of a tryptophane (Trp158) and of a glutamine (Gln80) residue stabilize the selenium atom during the redox process. Recent investigations on the mechanism of action of ebselen and related compounds revealed that the initial reaction of ebselen with thiols, such as GSH, leads to the formation of selenenyl sulfides, which are further stabilized by non-covalent selenium–oxygen interactions as shown in compound **7** in Scheme 2. The strength of



Scheme 2. Reaction of ebselen **4** with GSH.

this non-covalent Se···O interaction actually prevents the formation of the catalytically active selenol and explains the relatively poor GPx activity of these compounds. A recent publication from Mugesh, Row and co-workers revealed that these Se···O interactions are unusually short in the solid state of ebselen and derivatives and favor the cleavage of their N–Se bond hence contributing to the facile reaction with GSH.[8] This is shown in the structure of **4** in Scheme 2 with average Se···O distances of 2.5 Å and C–H···O distances of 2.35 Å.

Based on the above considerations, the search for novel GPx mimics with the potential of protecting mammalian cells against oxidative damage has recently had a major breakthrough. D'Silva and Mugesh et al. synthesized highly efficient isoselenazoles of type **9** (Scheme 3).[9] These compounds



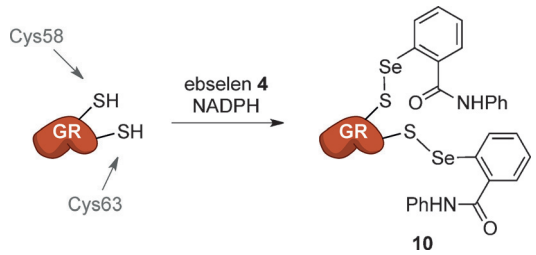
Scheme 3. Synthesis of novel GPx mimics **9a**–**d**.

are available in a few synthetic steps from the easy accessible diselenide **8**[10] through imine formation and reduction leading to spontaneous cyclization.[11] While compounds **9** with aromatic substituents R do not cyclize spontaneously and remain stable as diselenides, the compounds presented herein (**9a**–**d**) can only be isolated in the form of the isoselenazoles. Related diselenides[12] and other selenium-containing compounds[13] have also been investigated as potential GPx mimetics.

Compounds **9a**–**d** are remarkably active as GPx and Prx mimics with the activities being about two- to three-times more than that of ebselen **4**. Different published test systems (various hydrogen peroxides and different thiol substrates) have been investigated, but D'Silva, Mugesh and co-workers also investigated their antioxidant potential in human cell lines. By inhibiting the antioxidant enzyme catalase in the cell, they were able to measure cellular ROS levels using a fluorescent probe. They could differentiate between the three major components of the antioxidant system by selective inhibition of GSH, Trx, and glutaredoxin (Grx). Treatment of the cells with compounds **9** showed a reduced fluorescence indicating their ability to scavenge ROS. It was found that GSH or Trx are essential for the antioxidant activity of isoselenazoles **9** with Trx playing a major role in promoting antioxidant activity.

Furthermore, the damage to major cellular components, such as DNA, proteins, or lipids caused by the ROS was investigated in the absence and presence of compounds **9**. The extent of DNA damage was measured using an antibody essay. While ebselen **4** provided about 70% protection, compounds **9a** and **9c** showed remarkable greater than 95% prevention of DNA damage.

Of major concern in the development of compounds with antioxidant properties is always their toxicity. The effect of the compounds on cell death caused by oxidative stress was also examined. It was found that the toxicity of compounds **9** was several times lower than that of ebselen **4**. The lethality, however, increased upon depletion of the cellular levels of GSH and Trx. The toxicity of ebselen **4** is probably due to the inhibition of GR caused by ebselen, an idea supported by the spectrometric confirmation of the corresponding selenenyl-sulfide **10** (Scheme 4). This seems to be the first experimental evidence for GR inhibition in mammalian cells caused by selenium compounds such as **4**.



Scheme 4. Proposed inhibition of GR by ebselen **4**.

The novel isoselenazoles **9** with excellent antioxidant activities are able to scavenge ROS efficiently in the presence of GSH and Trx and prevent DNA damage. Their very low toxicity profile suggests that these compounds have the potential for further development into therapeutic agents to tackle diseases associated with oxidative stress as they supplement the antioxidant machinery in mammalian cells.

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