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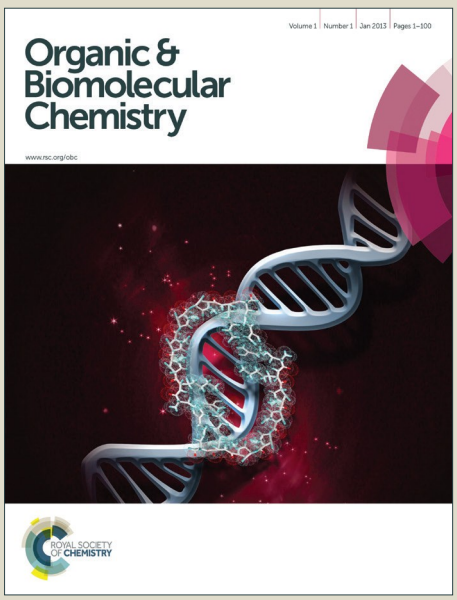

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ARTICLE

Insights into the Catalytic Mechanism of Synthetic Glutathione Peroxidase Mimetics

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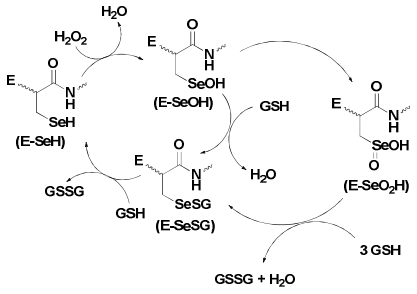
Glutathione Peroxidase (GPx) is a key selenoenzyme that protects biomolecules from oxidative damage. An extensive research has been carried out to design and synthesize small organoselenium compounds as functional mimics of GPx. While the catalytic mechanism of the native enzyme itself is poorly understood, the synthetic mimics follow different catalytic pathways depending upon the structures and reactivity of various intermediates formed in the catalytic cycle. The steric as well as electronic environments around the selenium atom not only modulate the reactivity of these synthetic mimics towards peroxides and thiols, but also the catalytic mechanisms. The catalytic cycle of small GPx mimics also dependent on the nature of peroxides and thiols used in the study. In this review, we discuss how the catalytic mechanism varies with the substituents attached to the selenium atom.

Introduction

Selenoenzymes are a class of proteins that contain selenocysteine (Sec, U) residue in their active sites. The biological activities of these enzymes are interesting due to the unique redox properties of the selenium atom.[1] Selenoenzymes act as antioxidants by maintaining the cellular redox balance or they can control the thyroid hormone levels. Till date, around 30 selenoproteins are known and few of these selenoproteins are isolated and characterized biochemically.[2] The major selenoenzymes discovered to date include formate dehydrogenases,[3] hydrogenases,[4] glycine reductase[5] iodothyronine deiodinases (ID),[6] thioredoxin reductases (TrxR),[7] selenophosphate synthetase,[8] and glutathione peroxidase (GPx).[9] Significant research efforts have been directed toward the synthesis of small molecule mimics of these selenoenzymes for better understanding of their catalytic activities and mechanisms. Although synthetic mimics are not known for many of these enzymes, synthetic compounds that mimic the function of GPx have been studied extensively.

The mammalian GPx is an important antioxidant enzyme, which protects various biomolecules such as proteins, amino acids, lipids, DNA etc from oxidative damage. GPx catalyzes the reduction of hydroperoxides using glutathione (GSH) as cofactor.[10] The GPx superfamily consists of four enzymes, cytosolic GPx (cGPx), phospholipid hydroperoxide GPx (PHGPx), plasma GPx (pGPx) and gastrointestinal GPx (giGPx).[11] Although all of these enzymes contain Sec residue in their active sites, their reactivity is highly dependent on the nature of peroxides and thiols.[12]

The proposed catalytic cycle of GPx contains three steps involving three active states of the enzyme. In the first step, the selenolate state (E-SeH) of Sec residue reduces hydroperoxides to water (or alcohol) to form oxidized selenenic acid (E-SeOH) state,[9a,13] which subsequently reacts with one equivalent of GSH to generate the selenenyl sulfide (E-SeSG) state. Nucleophilic attack of a second equivalent of cellular GSH regenerates the active selenol species with elimination of the oxidized GSH (GSSG). Cleavage of the -Se-S- bond by GSH is the rate determining step in the overall process (Scheme 1).[11]



Scheme 1 Proposed mechanism for the GPx-catalyzed reduction of H_2O_2.

At higher concentration of hydroperoxides, the selenium centre may undergo overoxidation to produce the

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corresponding seleninic acid (E-SeO_2H), which may be converted to the selenenyl sulfide by reaction with excess GSH. However, the formation of overoxidized seleninic acid derivative may reduce the catalytic activity, although it has not been demonstrated *in vivo*. The crystal structure of GPx (Fig. 1a) indicates that the Sec residue (Sec 45) remains very close to two other amino acid residues glutamine (Gln80) and tryptophan (Trp158) (Fig. 1b) forming a catalytic triad.[14] These two amino acid residues are very important for the catalytic activity as they stabilize the selenolate moiety by hydrogen bonding interaction. Such interactions may also prevent the cleavage of C-Se bond, which generally leads to toxicity of organoselenium compounds.

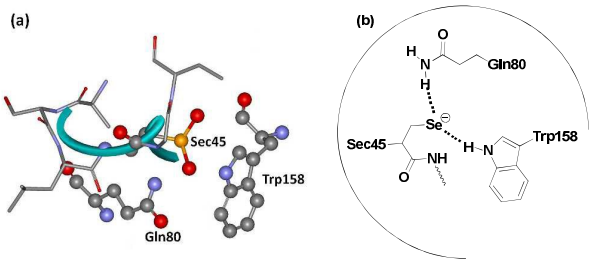


Fig. 1 (a) Active site of glutathione peroxidase in seleninic acid form (PDB Code 1GP1 Figure) determined by X-ray crystallography. (b) Catalytic triad at the active site of GPx.

A considerable attention has been directed toward design and synthesis of small molecule organoselenium compounds that functionally mimic GPx enzyme.[15] Synthetic mimics were primarily synthesized to understand the chemistry at the active site of GPx. However, they may also be very useful as drugs for diseases related to oxidative stress. The synthetic mimics were designed with a heteroatom near the selenium centre considering the GPx catalytic triad in the active site. Majority of the synthetic mimics reported so far can be classified into two major categories on the basis of their structures. The first category of compounds contains heteroatom directly bonded to selenium centre. In the second category, the compounds do not have direct selenium-heteroatom bond, but a heteroatom is placed near the selenium centre leading to weak selenium-heteroatom non-covalent interactions.[15] Although these compounds mimic the function of the natural GPx enzyme, they may not necessarily follow a similar catalytic cycle, which is highly dependent on their structure and reactivity. Natural enzymes mostly use GSH as the thiol cofactor. However, synthetic mimics may use any thiols such as GSH or small aromatic/aliphatic thiols for their catalytic activities. Selenium atom in all the organoselenium compounds is responsible for the reduction of peroxides, but its reactivity towards thiol and peroxides depends on the electronic and steric environment around the selenium centre. Their catalytic mechanism is also dependent on the nature of peroxides and thiols employed in the study. Therefore, in this review, we provide an overview of the catalytic mechanism of different synthetic mimics.

Ebselen and Related Cyclic Compounds

Ebselen (2-phenyl-1,2-benzisoselenazole-3)-(2*H*)-one (**1**) containing a direct Se-N bond (Fig. 2) was the earliest example of a synthetic GPx mimic. Ebselen exerts many biological functions both *in vitro* and *in vivo* systems.[16] It is associated with many therapeutic properties such as reduction of hydrogen peroxide (H_2O_2) and lipid peroxides, effective scavenging of highly reactive peroxynitrite and inhibition of a variety of free radical generating enzymes and enzymes involved in inflammatory diseases such as lipoxygenase and cyclooxygenase.[17] Ebselen was found to be less toxic because of its stable isoselenazole moiety and it is under phase III clinical trial for the treatment of hearing loss, inflammation, stroke, neurodegenerative diseases, reperfusion injury, bipolar disorder etc.[18]

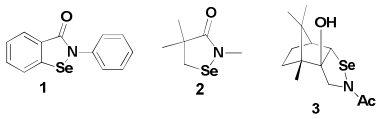
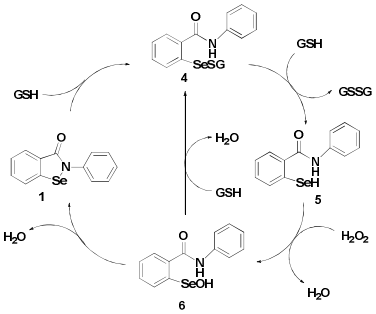


Fig. 2 Chemical structures of the cyclic selenenyl amides **1-3**.

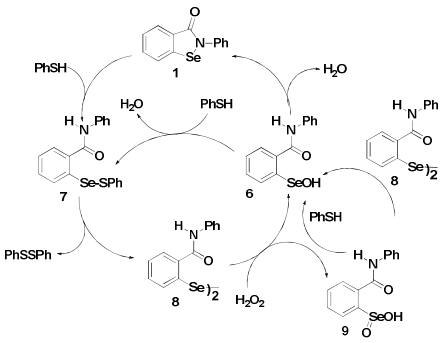
Although ebselen is studied extensively as GPx mimic, its catalytic cycle for the reduction of hydroperoxides by thiol has been controversial for many years.[19] The first proposed mechanism, shown in Scheme 2, involves selenol **5**, selenenyl sulfide **4** and selenenic acid **6** as the intermediates. Although Ebselen exhibits moderate catalytic activity using GSH as the cofactor, several studies showed that it is relatively inefficient catalyst in the presence of aromatic thiols such as thiophenol, or other related thiols such as benzyl thiol.[20]



Scheme 2. Initially proposed catalytic mechanism of ebselen.

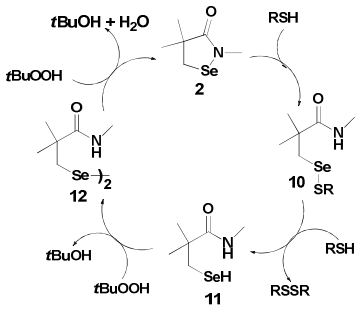
Detailed experimental and theoretical studies revealed that the poor activity of ebselen and related analogues is due to an extensive thiol exchange reaction, which is initiated by the strong Se···O non-covalent interaction in the selenenyl sulfide intermediate.[20b] As the thiol exchange reaction prevents the formation of the active selenol species, Mugesh and co-workers proposed a revised catalytic mechanism for the GPx-like activity of ebselen using PhSH based on the ^{77}Se NMR experiments.[20] According to this mechanism, the selenenyl sulfide **7** produces a diselenide intermediate **8** by disproportionation reaction instead of generating the corresponding selenol. The diselenide **8**, which acts as the active catalyst in this mechanism, reduces peroxides and is

oxidized to the corresponding selenenic acid **6** (Scheme 3).[20d] The disproportionation reaction of the selenenyl sulfide **7** to diselenide **8** was found to be the rate limiting step in the overall catalytic reaction. Therefore, the extent of thiol exchange reaction and the stability of the selenenyl sulfide intermediate in solution are highly dependent on the nature of the thiol used as cofactor.



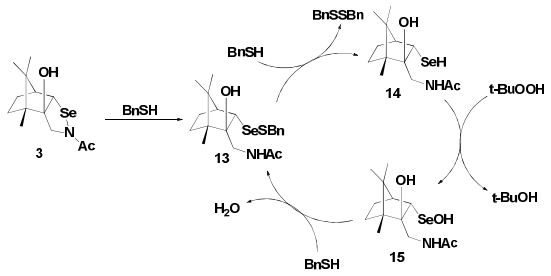
Scheme 3 A revised catalytic mechanism of ebselen (**1**).

Riech *et al*. have reported the antioxidant activity of cyclic selenenyl amide **2** (Fig. 2).[21] Based on the reactions of compound **2** with *t*-BuOOH (TBHP) and thiols, they have proposed a catalytic mechanism shown in Scheme 4. Similar to ebselen, initial reaction of compound **2** with thiol leads to the cleavage of the Se-N bond generating the corresponding selenenyl sulfide **10**, which in the presence of excess thiol produces the active selenol species **11**. Interestingly, selenol **11** reacts with peroxides to generate the diselenide **12** instead of producing the corresponding selenenic acid, which is observed for the native GPx enzymes (Scheme 1).[22] It is noteworthy that both the catalytic cycles of ebselen and compound **2** involve the corresponding diselenide as the intermediates. However, the mechanism of formation of the diselenides is entirely different.



Scheme 4 The proposed catalytic cycle of selenenyl amide **2**.

Back and Dyck have synthesized a camphor-based selenenyl amide **3** (Fig. 2)**,** which exhibits GPx-like activity by reducing *t*-BuOOH using benzyl thiol (BnSH) as cofactor.[23] The catalytic mechanism that is followed by **3** is significantly different from that of **1** and **2**. The first nucleophilic attack of thiol at the selenium centre cleaves the Se-N bond, generating the corresponding selenenyl sulfide **13**, which form a catalytic cycle with selenol **14** and selenenic acid **15** (Scheme 5). Thus, unlike other cyclic selenenyl amides, the catalytic mechanism of **3** resembles the catalytic cycle of native GPx enzymes. Compound **3** acts a pro-catalyst as it is not directly involved in the catalytic cycle. In the entire cycle, the conversion of selenenyl sulfide to selenol is the rate determining step.



Scheme 5 Proposed mechanism for the GPx activity of the camphor–derived selenenamide **3.**

Mugesh *et al*. reported a series of peptide-containing ebselen analogues (Fig. 3),[24] whose activities and catalytic mechanisms are highly dependent on the nature of the peptide chains attached to the nitrogen atom. For example, the catalytic cycle for compound **16** is different from that of compounds **17-18**. A catalytic mechanism for compound **16** was proposed using ^{77}Se NMR. Interestingly, it was observed that the mechanism is significantly different from that of ebselen. In contrast to ebselen, compound **16** reacts with thiol to generate the selenol intermediate, which is the active species in the catalytic cycle. Thus, the reactivity of selenenyl sulfide derived from **16** with thiol is different from that of compound **7**, which does not produce any selenol in the cycle.[20d] In contrast, the peptide-based ebselen analogues having Phe residue such as compounds **17-18** follow the mechanism identical to that of ebselen (Scheme 3), involving the diselenide intermediates. Therefore, the catalytic mechanism of peptide containing ebselen analogues was found to be dependent on the nature of the peptide chains.

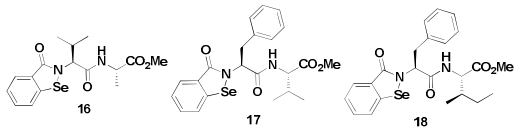


Fig. 3 Chemical structures of peptide containing ebselen analogues **16-18**.

Singh and co-workers reported novel isoselenazolines **19-20** and isoselenazoline *Se*-oxides **21-22** (Fig. 4),[25] which are stabilized by intramolecular Se···O interactions. These compounds exhibited higher GPx-like activities compared to that of ebselen that contain a C=O group. Particularly, the selenoxides **21-22** are almost 3-4 times more active than ebselen. Based on the experimental studies, they have proposed a catalytic mechanism for compound **21**.[25]

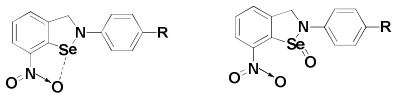
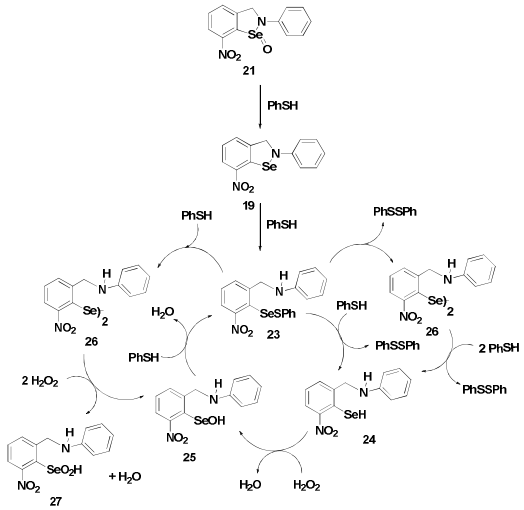


Fig. 4 Chemical structures of isoselenazoline **19-20** and their oxides **21-22**.

Scheme 6 indicates that the selenenyl amide bond is cleaved by thiol (PhSH) to produce the selenenyl sulfide intermediate **23**, which acts as a true catalyst in the catalytic mechanism involving compounds **24** and **25**. Although this pathway is different from that of ebselen, the formation of the diselenide **26** from the corresponding selenenyl sulfide by disproportionation reaction is quite similar to that observed during the reduction of peroxides by ebselen.



Scheme6 Proposed catalytic mechanism for the GPx-like activity of compound **21**.

Recently, Mugesh and coworkers have synthesized similar type of isoselenazole compounds **28-31** (Fig. 5),[26] which contain a methoxy substituent in the *ortho*-position and alkyl substituents on the nitrogen atom. Interestingly, it was observed that compounds **28-31** display excellent glutathione peroxidase activity both *in vitro* and inside human cells. A comparison of the catalytic activity shows that all the isoselenazole compounds exert very high activities as compared to ebselen. Interestingly, these compounds also mimic the peroxiredoxins in human cells by using cellular thioredoxin as reducing agents.

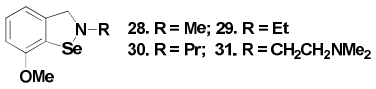
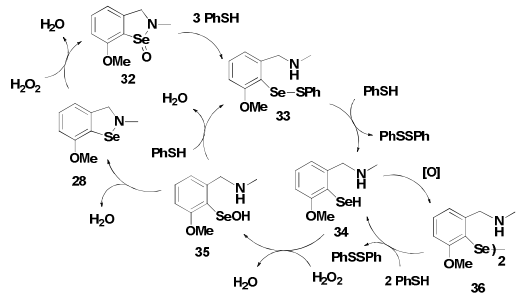


Fig. 5 Chemical structures of isoselenazoles **28-31**.

Although these isoselenazoles are structurally similar to compounds **19-20**, the reactivity towards thiol is significantly different. It is previously observed that the selenium centre in cyclic selenenyl amide compounds readily undergoes reductive cleavage by the thiol. The amide moiety makes the selenium centre highly electrophilic facilitating the nucleophilic attack of the thiol. On the other hand, compounds **19-20**, which lack the carbonyl group, also react with thiol to generate the corresponding selenenyl sulfides. It has been shown that the presence of intramolecular secondary Se···O interaction with the nitro group enhances the reactivity of the Se–N bond towards cleavage by thiol.[25] In contrast, isoselenazoles **28-31** reacted very slowly with thiol to cleave the Se-N bond as the selenium centre is not activated. Thus, the catalytic mechanism of **31** involves the oxidation of selenium with peroxides generating the selenoxide intermediates **32**, which subsequently undergoes rapid reaction with an excess amount of PhSH to produce the corresponding selenenyl sulfide **33**. Compound **33** then follows a catalytic cycle similar to the native enzyme involving the selenol **34** and the selenenic acid **35** (Scheme 7). It is noticed that, unlike compound **26,** the diselenide **36** is produced from the selenol intermediate after auto-oxidation. Although isoselenazoles **19** and **28** maintain a similar catalytic cycle which involves selenenyl sufide, selenol and selenenic acids as the intermediates, the reactivity towards thiol and peroxides is significantly different due to the different electronic environment around the selenium atom.



Scheme 7 Catalytic mechanism for the GPx-like activity of compound **28**.

Selenides and Seleninate Esters

Back and Moussa showed that a number of allyl selenides **37-40** (Fig. 6) exhibit very good GPx-like activity in the presence of *t*-BuOOH as the substrate and benzyl thiol as the cofactor.[27] These selenides act as pro-catalysts. Compound **37** having three spacer carbon atoms between selenium and the alcohol moiety was found to exhibits very high antioxidant activity. However, Compound **43** having an amide moiety also shows good catalytic activity.

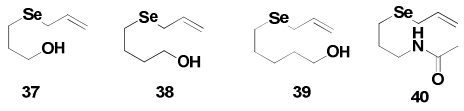
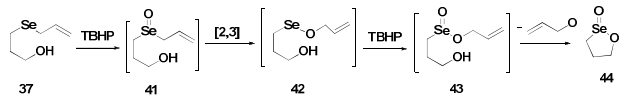


Fig. 6 Chemical structures of allyl selenides **37-40**.

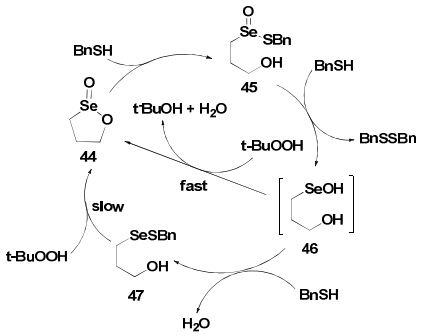
The allyl selenide **37** reacts readily with TBHP to produce the selenoxide **41,** which undergoes a [2,3] sigmatropic rearrangement to generate the seleninate ester **42**. In the presence of excess peroxide, the selenium undergoes overoxidation to produce an unstable intermediate **43,** which

after rapid cyclization generates the cyclic seleninate ester **44**. (Scheme 8).



Scheme 8 Reaction of compound **37** with peroxide to produce cyclic compound **44**.

The catalytic mechanism (Scheme 9) suggests that compound **44** first reacts with BnSH to produce the thioseleninate **45,** which undergoes further thiolysis reaction to afford the selenenic acid **46** and disulfide BnSSBn. The selenenic acid **46** reconverts to the seleninate ester **44** by oxidative cyclization in the presence of TBHP. However, in the presence of an excess thiol, compound **46** reacts with another BnSH molecule to produce the selenenyl sulfide **47**, which after prolonged reaction with peroxide regenerates the seleninate ester **44**.



Scheme 9 Proposed catalytic mechanism of the cyclic seleninate ester **44**.

Subsequently, Back *et al*. and Singh *et al*. have synthesized a series of aromatic analogues **48-51** (Fig. 7) of cyclic seleninate ester **44**. Back *et al* showed that the aromatic analogue **48** exhibits less catalytic activity compared to that of **44**.[28] However, introduction of electron donating substituents such as methoxy group (**49**) can enhance the activity considerably.[29] Although the catalytic activities differ significantly, Back *et al.* observed that the catalytic cycles remain unaltered involving the cyclic ester, thioseleninate and the selenenic acid intermediates.[28-30] Formation of selenenyl sulfide was observed in excess thiol concentration as a deactivation pathway. However, the cyclic seleninate ester is regenerated from the selenenyl sulfide in the presence of TBHP either via the thioseleninate intermediate or the corresponding diselenide. Interestingly, Back and co-workers could not observe any diselenide for the reduction of TBHP with BnSH, which suggests that disproportionation of selenenyl sulfide is not the major pathway in the regeneration of seleninate esters.

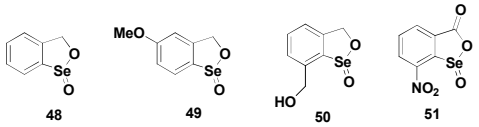
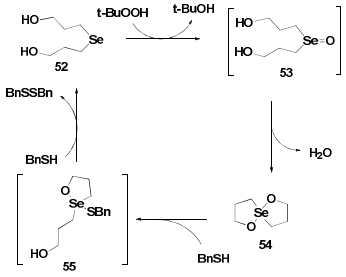


Fig. 7 Chemical structures of cyclic seleninate esters **48-51**.

In contrast, Singh *et al.* have shown that the catalytic mechanism of the cyclic seleninate ester **51** for the reduction of TBHP using PhSH is different when the thiol concentration is high.[31] Compound **51** follows a catalytic mechanism similar to that of **48**. However, in the presence of an excess thiol, the selenenyl sulfide, generated from the corresponding selenenic acid in the deactivation pathway, regenerates the seleninate ester **51** via formation of the diselenide intermediate, which is not observed by Back and co-workers.[31b] This might be due to the *ortho*-nitro substituent that stabilizes the selenenyl sulfide intermediate by Se···O non-covalent interaction leading to the formation of the diselenide by disproportionation reaction.

Back and co-workers have also reported a highly efficient GPx mimic di-(3-hydroxy-propyl)selenide **52,** which exhibits almost 15 times higher activity than ebselen.[32] It follows an interesting catalytic mechanism that involves a novel spirodioxaselenanonane **54** as an intermediate. Unlike the monoselenide **37**, compound **52** directly takes part in the catalytic cycle and reduces peroxides. Initial oxidation by *t*-BuOOH produces a transient selenoxide **53,** which undergoes spontaneous cyclization to generate the cyclic spirodioxaselenanonane **54**. Selenide **52** is subsequently regenerated upon reaction with two equivalents of BnSH (Scheme 10). This was the first report of a catalytic mechanism involving a cyclic selenanonane as the intermediate.



Scheme 10 Catalytic cycle of selenide **52** for the reduction of TBHP.

Singh and co-workers have reported a stable cyclic selenenate ester **56** (Fig. 8) that exhibits almost 300 times higher GPx-like activity as compared to that of diphenyl diselenide (PhSeSePh) in the presence of H_2O_2 as substrate and PhSH as the cofactor.[33] Unlike compound **44**, the selenenate ester **56** first reacts with peroxides, generating the corresponding cyclic seleninate and thereby acting as the active intermediate in the catalytic cycle.

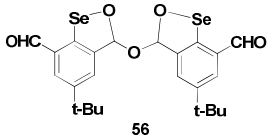


Fig. 8 Chemical structure of the selenenate ester **56**.

Excellent catalytic activities of compounds **44** and **56** also indicate that compounds containing intramolecular Se-O bonds are equally effective GPx mimics as the commonly

studied Se-N derivatives. This interaction is also very important for the stabilization and isolation of catalytic intermediates.

Back and co-workers have shown the catalytic activity of some monoselenides **57-58** (Fig. 9).[30] These monoselenides generally exhibit their antioxidant properties by Se(II)/Se(IV) redox cycle in the presence of peroxides and thiol. However, Braga, Detty and their co-workers studied the GPx-like activities of a series of monoselenides **59-60** and their oxides **61-62** (Fig. 10) that follow a mechanism involving a hydroxy perhydroxy selenane intermediate.[34]

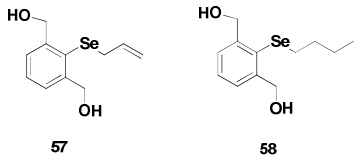


Fig. 9 Chemical structures of the monoselenides **57-58**.

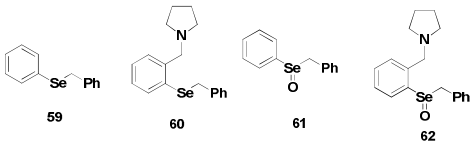
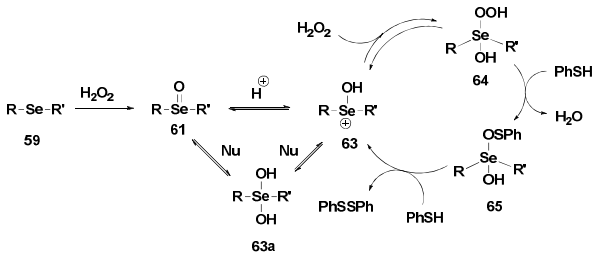


Fig. 10 Chemical structures of the monoselenides **59-60** and their oxides **61-62**.

In the presence of peroxides, selenoxide **61** is converted to the hydroxy perhydroxy selenane **64** via hydroxyselenonium **63.** Compound **64** is kinetically better oxidizing agent than the selenoxide **61**. Compound **64** then reacts with two equivalents of PhSH to produces PhSSPh and **63a** via an intermediate **65** restarting the catalytic cycle with H_2O_2 (Scheme 11). The catalytic activity of the selenoxide is little affected by electronic demands at the Se centre, however, selenoxides containing chelating groups, especially amino groups, were found to be better catalysts.



Scheme 11 Proposed catalytic mechanism for the GPx-like activity of **59** and **61**.

Catalytic mechanism of diaryl diselenides

Diaryl diselenides are another class of GPx mimics that exhibit excellent catalytic activities for the reduction of peroxides. It was observed that diphenyl diselenide (PhSeSePh) exhibits two times higher activity than that of ebselen. The reductive cleavage of the diselenide bond by thiol generates the corresponding selenol intermediate (PhSeH) that reduces peroxides. Subsequently, Spector and co-workers[35] have reported diaryl diselenides **66-67** (Fig. 11), which contain a *tert*-amino moiety near to the selenium centre. These diselenides were found to be more active than ebselen. They proposed that the selenium and the nitrogen atoms are involved in an intramolecular non-covalent interaction, which modulates the catalytic activity of diaryl diselenides.

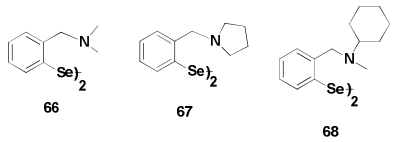
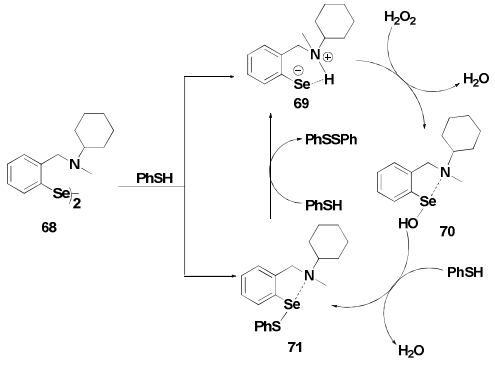


Fig. 11 Chemical structures of the amine-based diselenides **66-68**.

Iwaoka and Tomoda have studied the catalytic mechanism of diselenide **68** for the reduction of H_2O_2 by PhSH using ^{77}Se NMR spectroscopy.[36] They have proposed a catalytic cycle (Scheme 12) based on the experimental findings. It is observed that the non-bonded Se···N interaction plays important roles in the catalytic cycle. The initial nucleophilic attack of the PhSH produces a mixture of selenenyl sulfide **71** and selenolate ion **69**, which is generated from the corresponding selenol after deprotonation by the amino moiety. **69** and **71** form a catalytic cycle with selenenic acid **70**. The diselenides actually act as pro-catalysts as they do not directly take part in the catalytic cycle. However, they follow a catalytic cycle similar to that of the native GPx enzyme for reducing peroxides.

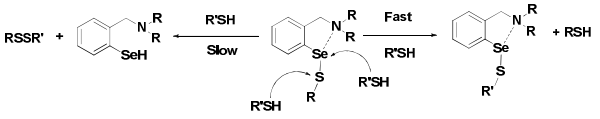
Based on the mechanistic observations, Iwaoka and Tomoda[36] proposed the following possible roles of the amino group in the catalytic cycle: (i) The amino group can generate kinetically more reactive and more nucleophilic selenolate anion (E-Se$^-$) from the selenol intermediate (E-SeH) after deprotonation. (ii) The non-covalent Se···N interaction prevents the selenenic acid moiety from overoxidation in the presence of excess peroxides. (iii) The Se···N interaction facilitates the nucleophilic attack of thiol at the sulfur center of selenenyl sulfide intermediate allowing effective regeneration of the selenol species.



Scheme 12 Catalytic cycle of diselenide **75** in the presence of H_2O_2 and PhSH.

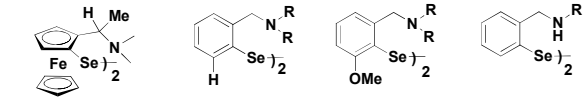
Although the *tert*-amine-based diselenides are significantly more active than ebselen, detailed experimental investigations

indicate that their catalytic efficiency may reduce considerably by other deactivation pathways. In the selenenic acid intermediate, the selenium centre undergoes overoxidation to produce the corresponding seleninic or selenonic acids, which reduces the catalytic activity significantly at lower concentrations of the thiol. On the other hand, in the selenenyl sulfide intermediate, the nucleophilic attack of thiol predominantly takes place at the selenium centre due to the thiol exchange reaction (Scheme 13), which prevents the regeneration of active selenol species. Although the Se···N interactions in selenenyl sulfides derived from *tert*-amine-based diselenide are much less than Se···O interaction in **7**, this Se···N interaction is sufficient enough to cause significant thiol exchange reaction.[35] In this context, Singh and co-workers reported redox-active diferrocenyl diselenides **72-73** (Fig. 12), which exert higher catalytic activity than that of diselenides **66-67**.[37]



Scheme 13 Thiol exchange reaction in selenenyl sulfide intermediate.

Experimental evidences show that the catalytic cycle is identical to that of compound **68**. However, the higher activity is ascribed due to the negligible interaction between the nitrogen and selenium atoms in selenenyl sulfide intermediate in the presence of ferrocenyl moiety. Subsequently Mugesh and coworkers have reported a novel amine-based diselenides **76-78** by substituting the *ortho*-hydrogen in compounds **66, 74-75** with methoxy substituent (Fig. 12).[38] Interestingly, a dramatic increase in the activity for the reduction of H_2O_2 by PhSH was observed. Particularly, compound **76** showed almost one order of magnitude higher catalytic activity than that of parent diselenide **66**. They proposed a catalytic mechanism for compounds **76-78** based on the experimental and theoretical investigations. The catalytic mechanism was unaltered involving selenol, selenenic acid and selenenyl sulfide intermediates. However, it was noticed that the methoxy group protects the selenium centre in selenenic acid from overoxidation. Moreover, it reduces the thiol exchange



72. [R, S; R, S] (+)
73. [S, R; S, R] (-)

66. R=Me
74. R=Et
75. R=Pr

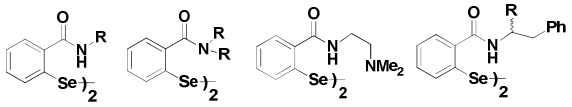
76. R=Me
77. R=Et
78. R=Pr

79. R=Me
80. R=Et
81. R=Pr

Fig. 12 Chemical structures of the amine-based diselenides **66, 72-81**.

reaction significantly in selenenyl sulfide intermediate by reducing the Se···N interaction. ^{77}Se NMR studies show that selenenyl sulfide derived from the diselenide **66** requires a large excess of PhSH to overcome the thiol exchange reaction.[38,39] However, one equivalent of PhSH is sufficient for the complete conversion of selenenyl sulfide derived from **76** to the corresponding selenol. Rapid formation of the active senenol species is responsible for the very high catalytic activities of diselenides **76-78**. Mugesh and co-workers also reported diselenides **79-81** by substituting the *tert*-amino moiety in compounds **66, 74-75** with *sec*-amine (Fig. 12).[40] Introduction of the *sec*-amino group increases the activity considerably than that of compounds **66, 74-75** due to their higher basicity and solubility. However, the catalytic cycle for the reduction of H_2O_2 using PhSH was unaffected.

As the catalytic cycle of ebselen involves the diselenide **8** as the key intermediate that reduces peroxides, Mugesh and co-workers synthesized and explored the catalytic activity of a series of diaryl diselenides **82-89** containing amide moiety (Fig. 13).[41] *Sec*-amide-based diselenides **82-85** exhibit almost similar activities compared to that of ebselen, however, the activity is increased 10-20 times in the presence of *tert*-amide moiety.



82. R=Me
83. R=Et
84. R=Pr
85. R=iPr

86. R=Me
87. R=Et
88. R=Pr
89. R=iPr

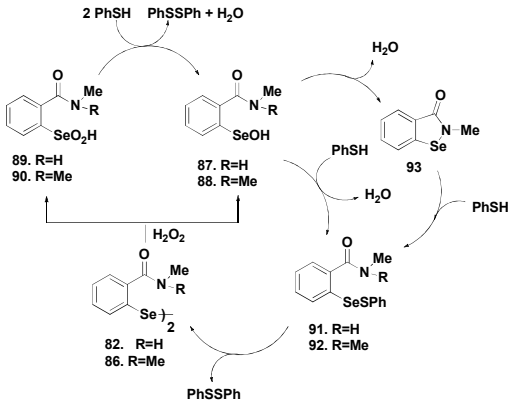
90

91. R=H
92. R=Me

Fig. 13 Chemical structures of the amide-based diselenides **82-92**.

Amide-based diselenides were found to be much lower active than the corresponding amine-based diselenides. Strong Se···O interaction in selenenyl sulfide intermediate induces extensive thiol exchange reaction that reduces the catalytic activity. Moreover, experimental studies show that the reactivity of these diselenides towards thiol is completely different than that of ebselen, which reacts rapidly with thiol to cleave the Se-N bond. In contrast, diselenides **82-89** undergo very slow reaction with thiol to cleave the –Se-Se- bond. Therefore, the oxidative cleavage of the –Se-Se- bond by peroxides was found to be the first step in the catalytic cycle of the amide-based diselenides for the reduction of peroxides. The catalytic mechanisms were similar for both the *sec*- and *tert*-amide-based diselenides. Scheme 14 indicates that **82** and **86** first react with H_2O_2 to produce a mixture of the corresponding selenenic acids **87-88** and seleninic acids **89-90**, which are readily converted to the respective selenenyl sulfides **91-92** by excess thiol (Scheme 14). Formation of **93** was observed from **87** after water elimination, which was not possible for **88** due to absence of the hydrogen atom. Regeneration of diselenides by disproportionation is the rate determining step in the overall catalytic cycle of amide-based diselenides. Extensive thiol exchange reaction in the selenenyl sulfide intermediate prevents the formation of selenol species. Interestingly, introduction of an additional amino moiety (**90**) changes the catalytic mechanism completely. The thiol cofactor is deprotonated by the additional amino group generating more nucleophilic thiolate anion, which leads to the rapid cleavage of the diselenide bond. Therefore, compound **90** follows a catalytic cycle that involves selenol as the active intermediate.[42] Braga and co-workers also reported

sec-amide based diselenides **91-92**, which exhibit higher catalytic activities than ebselen.[43]



Scheme 14 Catalytic mechanism of amide-based diselenides **89** and **93**.

The excellent antioxidant activities of amine-based diaryl diselenides led many researchers to design and synthesis of diaryl diselenides that contain oxygen as the heteroatom near the selenium centre. Wirth and co-workers reported a series of oxygen containing diaryl diselenides **94-98** (Fig. 14).[44] Compound **94** showed the highest catalytic activity compared to that of compounds **95-97**. However, these diselenides were found to be much less active than the corresponding amine-based diselenides despite of having non-covalent Se···O interaction. However, they follow similar catalytic cycle for peroxide reduction.

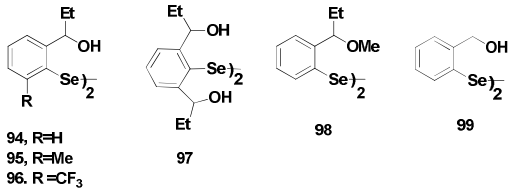


Fig. 14 Chemical structures of the oxygen containing diselenides **94-99**.

Singh and co-workers have studied the catalytic activity of diselenide **99** in the presence of TBHP and PhSH.[31a] Diselenide **99** reacts with two equivalent of PhSH to produce the corresponding selenol, which forms a catalytic cycle with the corresponding selenenic acid and selenenyl sulfide intermediates. However, the selenenic acid or other overoxidized compounds could not be detected due to rapid conversion of the selenol to the selenenyl sulfide even at higher peroxide concentration. The selenenyl sulfide was found to be the most stable intermediate in the catalytic cycle.

Spiroselenuranes

Spirodioxyselenurane as the GPx mimic was first described by Back and co-workers. Compound **54** exhibits excellent GPx-like activity for the reduction of TBHP using BnSH as the cofactor.[32] In contrast, the corresponding aromatic analogues **100** exhibits lower antioxidant activity than **54** and the activity is further decreased in the presence of carbonyl moieties in compound

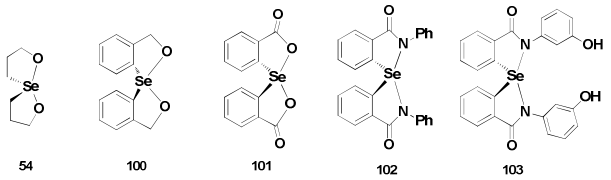
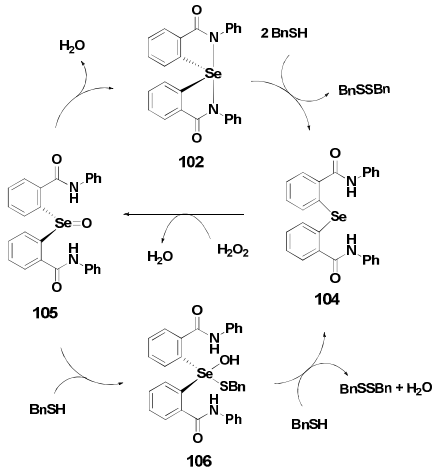


Fig. 15 Chemical structures of the spiro compounds **54, 100-103**.

101 (Fig. 15).[28,29] However, the activity of compound **100** is enhanced considerably in the presence of electron-donating substituents such as methoxy group on the aromatic ring.[29] The catalytic cycle of spirodioxyselenurane **54** for the reduction of peroxides is shown in Scheme 10. The aromatic derivatives **100-101** also follow a similar mechanism for the reduction of TBHP in the presence of BnSH. Although the GPx-like activities of spirodioxyselenuranes are studied extensively, corresponding diazaselenuranes as GPx mimics are less explored. Mugesh and co-workers synthesized the first stable spirodiazaselenurane **102** (Fig. 15) and studied its antioxidant activity in the presence of H_2O_2 and BnSH.[45] Compound **102** was found to be less active as catalyst than ebselen. However, the activity is enhanced when an electron donating substituent is attached to the aromatic ring (**103**).[46] Based on ^{77}Se NMR studies, they proposed a catalytic cycle for compound **102** that is shown in scheme 15. It is observed that cleavage of the Se-N bonds by BnSH produces the selenide **104**, which is oxidized to the selenoxide **105** by peroxides forming a catalytic cycle. However, in excess thiol concentration, the selenoxide **105** may react with BnSH to afford the intermediate **106**, which is converted to the selenide **104** upon reaction with a second equivalent of BnSH. Formation of the selenide is a crucial step as it is the active intermediate in the cycle. On the other hand, stability of the Se-N bonds highly depends on the nature of substituents on the nitrogen atoms. Phenyl substituents stabilize the Se-N bonds significantly, however, the stability is decreased considerably in the presence of heterocyclic, benzylic or aliphatic substituents leading to the rapid formation of the selenide intermediates.[47]



Scheme 15 Catalytic mechanism of spirodiazaselenurane **102**.

Conclusion

In this review, we have discussed how the catalytic mechanism of GPx mimics is altered depending on the structures of organoselenium compounds and the reactivity of various intermediates toward thiols and peroxides. A detailed analysis of the catalytic mechanisms reveal that synthetic GPx mimics can be classified into different categories based on their structures and compounds from each category follow a characteristic catalytic mechanism for the reduction of peroxides by thiol. However, the mechanisms are also altered between same types of compounds having different substituents. Furthermore, the nature of thiol cofactor can alter the catalytic mechanism.

Acknowledgements

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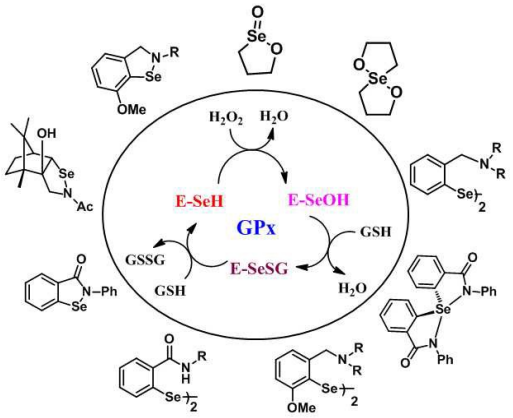

The review focuses on the variation of catalytic mechanisms of synthetic glutathione peroxidise (GPx) mimics depending on their structures and reactivities towards thiols and peroxides. Compounds of different categories follow a characteristic mechanism for the reduction of peroxides.