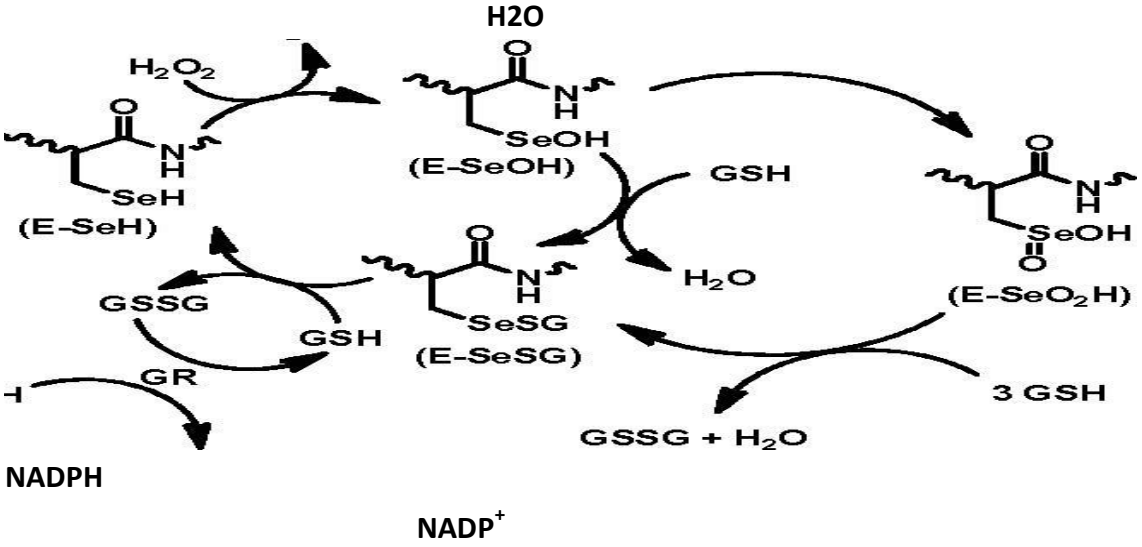
ABSTRACT

Hydroperoxides are very harmful and they can oxidize several biomolecules, causing different disease states. Glutathione peroxidase (GPx) is a mammalian selenoenzyme which protects human body from oxidative damage by catalyzing the reduction of harmful peroxides using glutathione (GSH) as a cofactor. GPx contains selenocysteine in its active site. The catalytic cycle of GPx enzymes is believed to involve three steps. In the first step, the reduced selenolate moiety (E-SeH) of Sec residue reduces hydroperoxides to water (or alcohol) to form oxidized selenenic acid (E-SeOH), which upon reaction with one equivalent of GSH generates selenenyl sulfide (E-SeSG) intermediate. A second equivalent of cellular GSH attacks at the –Se-S-bond to regenerate the active selenol species with elimination of the oxidized GSH (GSSG) and thus completes the catalytic cycle (Scheme 1). Therefore, the formation of the selenol species from the selenenyl sulfide intermediate is a crucial step for the catalytic activity. Cleavage of the -Se-S-bond is the rate determining step in the overall process. The GSH concentration in the cellular level is maintained by an enzyme glutathione reductase



Scheme 1. Proposed catalytic mechanism of GPx. The rapid reaction of the selenenic and seleninic acids with GSH ensures that the selenium moiety in the enzyme is not irreversibly inactivated.

(GR), which reduces GSSG to GSH using NADPH as cofactor. Thus, in the overall process, 2 equivalents of GSH are used to reduce one equivalent of hydroperoxide to corresponding water or alcohol (Scheme 2). At high hydroperoxide concentration, selenium center may undergo overoxidation to produce corresponding seleninic acid ($ESeO_2H$) and selenonic acid ($E\text{-}SeO_3H$). Seleninic acid may rapidly converts to the selenenyl sulfide by reaction with GSH, whereas, overoxidized selenonic acid causes decrease in the catalytic activity.

$$2\,GSH + ROOH \xrightarrow{GPx} ROH + H_2O + GSSG$$

Scheme 2. The overall reaction catalyzed by GPx enzyme.

This thesis consists of **five chapters**. The **first chapter** provides a general introduction of the selenium and its biological importance. This chapter also explains the oxidant and antioxidant systems present in mammalian system and followed by a brief

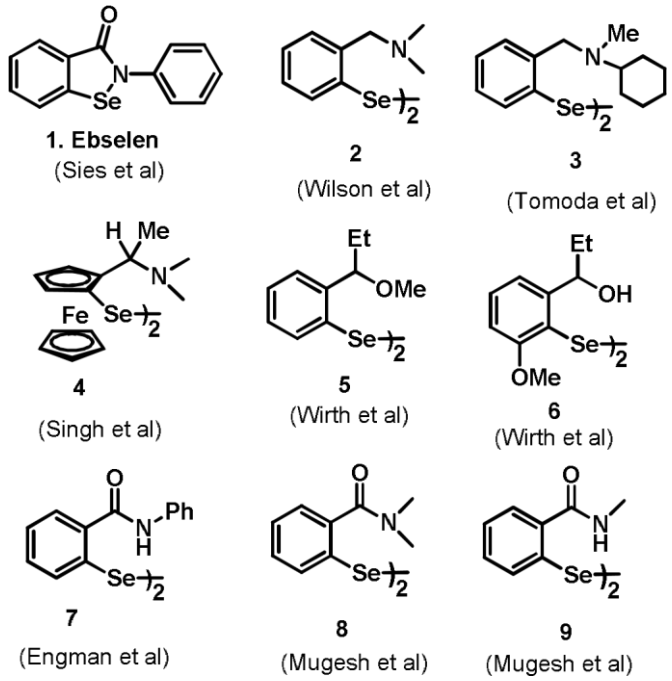


Figure 1. Chemical structures of the synthetic GPx mimics **1-9**.

introduction of GPx enzyme. Apart from this, this chapter also provides a brief overview on the synthetic mimics of GPx. Due to many therapeutic application, different research groups have synthesized small molecule organ selenium compounds as the functional mimics of the GPx. These mimics includes ebselen (**1**) and related selenenyl amides, *tret*amine based diselenides (**2-4**), diselenides **5-6** having Se···O interactions, amide-based diselenides **7-9** etc. (Figure 1).

Although, *tert*-amine-based diselenides exhibit higher catalytic activities compared to that of the ebselen and amide-based diselenides, most of these compounds show poor catalytic activities in the presence of aromatic thiols due to the undesired thiol exchange reactions. The nucleophilic attack of the thiol predominantly takes place at the selenium center, due to the strong Se···N/O non-covalent interactions, instead of at the sulfer center in the selenenyl sulfide intermediates inhibiting the effective regeneration of the selenols. Regeneration of selenol is very important as it is responsible for the reduction of peroxides. However, Tomoda and coworkers showed that the Se···N non-covalent interaction in the *tert*-amine-based diselenide is also very important for the catalytic activity as this interaction has certain positive roles in other intermediates in the catalytic cycle. Therefore, synthesis of GPx mimics containing an amino group near to the selenium center without Se···N non-covalent interactions in the selenenyl sulfide intermediates was of interest for many researchers since few decades.

In 2008, Bhabak and Mugesh reported compounds **13-15** by replacing the aromatic hydrogen of compounds **10-12** with methoxy substituent in the *ortho*-position and they showed that compounds **13-15** exhibit almost one order of magnitude higher catalytic activities compared

to that of the parent diselenides **10-12**. It has been suggested that the electronic and steric effects of the 6-methoxy groups play key roles in the enhancement of the catalytic activity. However, it is not clear whether it is the electronic effect or the steric effect that is responsible for the increase in the GPx-like activity. Therefore, compounds **16-18**, containing methoxy group in the *para*-position, were synthesized and their catalytic activities were compared with that of the compounds **1012** and **13-15** which is discussed in **chapter two** (Figure 2). Comparison of the GPx-like activities using different peroxides as the substrate and PhSH or GSH as the cofactor show that compounds **16-18** are slightly higher active than compounds **10-12**, whereas,

13. R = Me
14. R = Et
15. R = Pr

10. R = Me
11. R = Et
12. R = Pr

16. R = Me
17. R = Et
18. R = Pr

Figure 2. Substitution of aromatic hydrogens by methoxy group in the *ortho*-and *para*-positions to synthesize compound **13-15** and **16-18**, respectively.

very less active than the corresponding *ortho*-methoxy substituted compounds **13-15**, strongly suggesting that methoxy group has very little effect in the catalytic activity of *para*-methoxy compounds **16-18**. Detailed mechanistic studies using ^{77}Se NMR and theoretical studies show that methoxy group in the *ortho*-position has the following effects

1 Methoxy group reduces the Se···N non-covalent interaction energy significantly inhibiting the thiol exchange reaction in the selenenyl sulfide intermediates, whereas, an extensive thiol exchange reaction was observed in the selenenyl sulfide intermediates derived from the corresponding *para*-methoxy compounds.

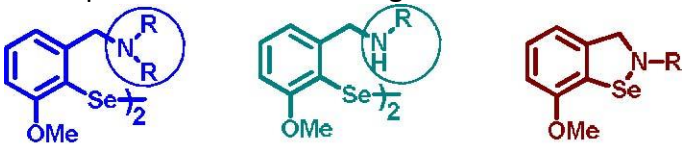
2 Zwitterionic characters of the selenol intermediates derived from compounds **1315** are higher, generating more nucleophilic selenolate anions, compared to that of the compounds **10-12** and **16-18**.

3 Methoxy group in the *ortho*-position protects the selenium moiety from overoxidation in the selenenic acid intermediate, however, selenenic acids generated from compounds **10-12** and **16-18** readily undergo overoxidation in the presence of excess peroxide to produce the corresponding seleninic acids and selenonic acids.

Therefore, the steric effect of the methoxy group in the ortho-position was found to be more important than its electronic effect to increase the catalytic activities of compounds **13-15** significantly.

It is known earlier that, *tert*-amino group can generate the selenolate moiety after deprotonation of the selenol, which is further enhanced in the presence of methoxy group in the *ortho*-position. Increase in the selenolate character leads to the higher catalytic activity, as selenol is the active intermediate in the catalytic cycle. Therefore, replacement of the *tert*-amino moiety by *sec*-amino group in compounds **13-15** may increase the activity significantly, as *sec*-amino group can deprotonates the selenol more effectively due to its higher basicity than the corresponding *tert*-amine, which is discussed in the **third chapter**. An

attempt to synthesize compounds **19-21** ended up with compounds **22-24**, which are formed by rapid cyclization of compounds **19-21** (Figure 3). Interestingly, for the first time, it was observed than amine-based diselenides undergo cyclization. Comparison of the catalytic activities of compounds **22-24** with that of compounds **13-15** reveals that *sec*-amine substituted compounds exhibit significantly higher activity compared to that of the *tert*-amine-based diselenides except compound **13**, which shows exceptionally high activity in the presence of PhSH as the cofactor. Unlike compounds **13-15**, compounds **22-24** react very slowly with the thiol, suggesting that reaction with the peroxide is the first step for these compounds generating the corresponding selenoxides, which subsequently react with thiol to produce the selenenyl sulfides. Catalytic cycles of these compounds were proposed based on the experimental studies using ^{77}Se NMR and mass spectrometry.



13. R = Me
14. R = Et
15. R = Pr

19. R = Me
20. R = Et
21. R = Pr

22. R = Me
23. R = Et
24. R = Pr

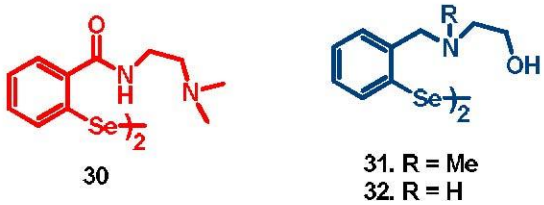
Figure 3. Chemical structures of the *tert*-amine-based diselenides **13-15**, *sec*-aminebased diselenides **19-21** and cyclic compounds **22-24**.

It is clear from the previous chapter that the higher basicity of the *sec*-amine enhances the catalytic activity significantly compared to that of the corresponding *tert*amine based compounds. The higher activity is due to the higher selenolate character of the selenol intermediate; however, it is not known whether the basic amino group deprotonates the thiol also

25. R = Me
26. R = H

27. R = Me
28. R = H

29



30

31. R = Me
32. R = H

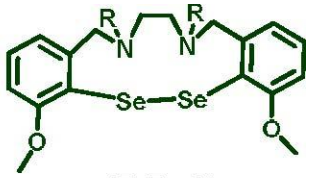
Figure 4. Chemical structures of the diselenides **25-30** containing additional amino groups and compounds **31-32** with alcoholic moieties.

to generate a more nucleophilic thiolate anion. As the amino groups of the amine-based diselenides are already interacting with the selenium center, the availability of these amino groups to deprotonate the thiol will be less. Therefore, introduction of an additional amino group into the diselenide may increase the catalytic activity further, as the additional amine can effectively deprotonates the thiol to generate more nucleophilic thiolate. A series of compounds **25-30** with additional amine were synthesized which is discussed in the **fourth chapter** (Figure 4).

A comparison of the catalytic activities of compounds **25-30** with that of the corresponding diselenides lacking the additional amino group showed almost 1.5-2.0 times higher activity in the presence of additional amine. It is observed from the ^{77}Se NMR experiments and DFT calculations that the catalytic intermediates such as selenenyl sulfides, selenols and selenenic acids were unaffected by the additional amino group, which strongly suggests that the higher activity is due to the deprotonation of the thiol moiety by the additional amino group increasing the thiolate concentration in the reaction medium. Replacement of the amino groups in compounds **25-26** with the alcohol moieties (**31-32**) lead to a considerable decrease in the catalytic activities indicating that additional amino group really plays some role to increase the GPx-like activity.

In the **fifth chapter**, synthesis and antioxidant activities of some intramolecular diselenides are discussed. The *tert*-amine-based diselenides, reported till now, are intermolecular, which after cleavage of the –Se-Se-bonds by the reducing agent generate two separate selenol species where each selenium atom interacts with one nitrogen atom. It is discussed in chapter one that strong Se···N non-covalent interaction leads to an extensive thiol exchange reaction, therefore, reduction of this interaction may increase the GPx-like activity. Thus, intramolecular diselenides **33-36** were synthesized containing one amino group near to the selenium atoms. In contrast to the intermolecular diselenides, two selenium atoms interact non-covalently with only one nitrogen atom in the case of intramolecular diselenides. For the comparison, compounds **37-40**, which contain two nitrogen atoms, were synthesized and their catalytic activities were studied (Figure 5) using PhSH and GSH as the cofactors and different peroxides as the substrates.

33. R = H
34. R = Me
35. R = Et
36. R = Pr



37. R = H
38. R = Me
39. R = Et
40. R = Pr

Figure 5. Chemical structures of the intramolecular diselenides **33-40**.

A comparison of their activities show that these compounds are much higher active than ebselen, however, significantly less active than the respective intermolecular diselenides **13-15**. Lower catalytic activities of these compounds are due to the longer Se···N distances in the molecules, which is confirmed from some of the crystal structures. Very large Se···N distance resulted very week or negligible Se···N noncovalent interaction in the molecules. However, Se···N noncovalent interaction is also very important for the catalytic activity. Therefore, these compound **33-40** exhibit very poor GPx-like activity. Significant steric interaction between the diselenide and the nucleophilic thiol is also responsible for the poor activity. Compounds **34** and **37** showed the highest activities in the presence of GSH and PhSH, respectively, as the thiol cofactor among all the intramolecular diselenides. Due to less Se···N interaction, most of the diselenides do not react with PhSH to cleave the –Se-Se-bonds. Thus, they react with peroxides first to produce the oxidized products. However, compounds **34** and **37** react readily with PhSH generating the corresponding selenenyl sulfides and selenols. Based on the experimental studies, catalytic mechanisms of compounds **34** and **37** show that catalytic cycles are almost like that of the intermolecular diselenides involving selenol, selenenic acid and selenenyl sulfides as the intermediates.

The progress so far towards the design and synthesis of small molecule organoselenium compounds as the GPx mimics shows a trend given in Figure 6. The first reported GPx mimic ebselen **1** (1984) shows very poor catalytic activity in the presence of aromatic thiols due to the extensive thiol exchange reaction. In 1989, Wilson and coworkers reported tert-amine-based diselenide **10**, which exhibit very high activity compared to that of the ebselen. However, the thiol exchange reaction could not be reduced in the selenenyl sulfide intermediate due to the strong Se···N non-covalent interaction. In 2008, Bhabak and Mugesh reported that compounds **13-15** exhibit very high catalytic activities. Particularly, compound **13** exhibits activity of almost one order of magnitude higher than that of the parent diselenide **10**. The methoxy substituent in the *ortho*-position reduces the thiol exchange reaction significantly in the selenenyl sulfide intermediate and increases the selenolate character of the selenol species. Based on these observations, we have replaced the *tert*-amino moiety with *sec*-amino substituent to synthesized compound **22-24**, which show enhanced activity than the compounds **13-15**. The higher basicity of the *sec*-amino group helps to deprotonate the selenol species more efficiently than the *tert*-amine. The activity can be further increased by attaching an additional amino group on the nitrogen center. The additional amino substituent can deprotonate the thiol to generate a more reactive thiolate anion in the reaction medium, which facilitates the nucleophilic attack of the thiol. Therefore, compound **28** was found to be the most active GPx mimic for the reduction of peroxides in the presence of aromatic thiols (PhSH).

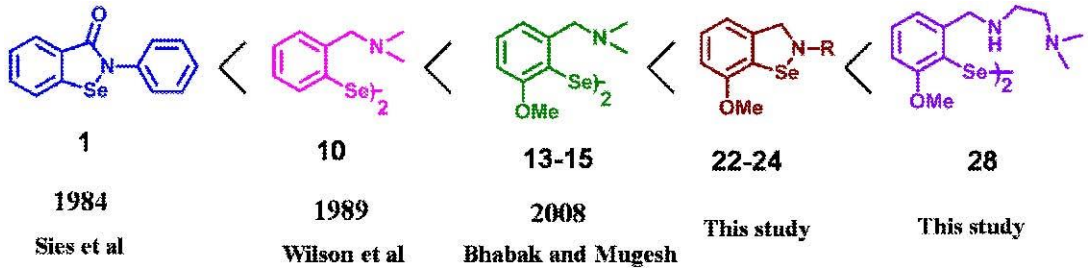


Figure 6. Enhancement of the GPx-like catalytic activity by introduction of methoxy and amino groups in diaryl diselenides.