Tertiary amine-based glutathione peroxidase mimics: Some insights into the role of steric and electronic effects on antioxidant activity

ARTICLE *in* TETRAHEDRON · APRIL 2013

Impact Factor: 2.64 · DOI: 10.1016/j.tet.2012.09.020

2 AUTHORS:

Debasish Bhowmick
Indian Institute of Science
15 PUBLICATIONS **39** CITATIONS
SEE PROFILE

Govindasamy Mugesh
Indian Institute of Science
155 PUBLICATIONS **4,326** CITATIONS
SEE PROFILE

Tetrahedron xxx (2012) 1–11

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Tertiary amine-based glutathione peroxidase mimics: some insights into the role of steric and electronic effects on antioxidant activity

Debasish Bhowmick, Govindasamy Mugesh *

Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore 560 012, India

ARTICLE INFO

Article history:
Received 6 July 2012
Received in revised form 29 August 2012
Accepted 4 September 2012
Available online xxx

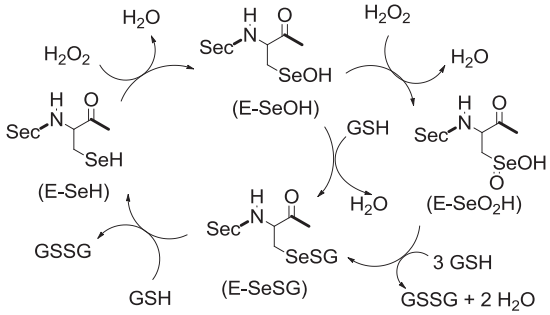
Keywords:
Antioxidant activity
GPx mimics
Selenoenzymes
Selenium
Thiol exchange

ABSTRACT

In this work, several tertiary amine-based diaryl diselenides were synthesized and evaluated for their glutathione peroxidase (GPx)-like antioxidant activities using hydrogen peroxide, *tert*-butyl hydroperoxide and cumene hydroperoxide as substrates and thiophenol (PhSH) and glutathione (GSH) as co-substrates. A comparison of the GPx-like activity of 4-methoxy-substituted *N*,*N*-dialkylbenzylamine-based diselenides with that of the corresponding 6-methoxy-substituted compounds indicates that the activity highly depends on the position of the methoxy substituent. Although the methoxy group at 4- and 6-position alters the electronic properties of selenium, the substitution at the 6-position provides the required steric protection for some of the key intermediates in the catalytic cycle. A detailed experimental and theoretical investigation reveals that the 6-methoxy substituent prevents the undesired thiol exchange reactions at the selenium centers in the selenenyl sulfide intermediates. The 6-methoxy substituent also prevents the formation of seleninic and selenonic acids. When PhSH is used as the thiol co-substrate, the 4-methoxy-substituted diselenides exhibit GPx-like activity similar to that of the parent compounds as the 4-methoxy substituent does not block the selenium center in the selenenyl sulfide intermediates from thiol exchange reactions. In contrast, the 4-methoxy substituent significantly enhances the GPx-like activity of the diselenides when glutathione (GSH) is used as the co-substrate.

1. Introduction

Glutathione peroxidase (GPx) is a selenoenzyme that functions as antioxidant by catalyzing the reduction of harmful peroxides by using glutathione (GSH) as co-substrate.[1] During the catalysis, the selenol group present in the selenocysteine (Sec) residue of the enzyme (E-SeH) reacts with peroxides to produce the corresponding selenenic acid (E-SeOH) with the elimination of a water molecule. The selenenic acid then undergoes reaction with GSH to generate the enzyme-bound selenenyl sulfide (E-Se-SG). The nucleophilic attack of GSH at the selenenyl sulfide linkage regenerates the active site selenol with the release of glutathione disulfide, GSSG (Scheme 1).[1e–g] At high concentrations of peroxide (oxidative stress conditions) and low concentrations of GSH (depletion of GSH), the selenenic acid may undergo further reaction with peroxides to produce the corresponding seleninic acid (E-SeO_2H). The rapid reactions of the selenenic acid with GSH and of the resulting selenenyl sulfide (E-Se-SG) with a second GSH to produce the selenol (E-SeH) are important for the catalytic activity.



Scheme 1. Proposed catalytic cycle for the reduction of H_2O_2 by GPx. At high concentrations of peroxides (oxidative stress conditions) and low concentrations of GSH (GSH depletion), the formation of seleninic acid (E-SeO_2H) may be formed.

The interesting redox chemistry at the active site of GPx[2] led to the design and development of simple organoselenium compounds that can reduce peroxides catalytically in the presence of aliphatic and aromatic thiols.[3] These GPx model compounds include the well-known anti-inflammatory compound ebselen (**1**)[4] and its analogues,[5,6] camphor-based selenenyl amide (**2**),[7] *tert*-amino substituted diselenides (**3**–**6**),[6e,8] diselenides **7**–**8** having Se···O interactions,[9] allyl and alkyl selenides (**9**–**10**),[10] cyclodextrin-based diselenide (**11**),[11] 3,3-diselenopropionic acid (**12**),[12] nicotinoyl

* Corresponding author. Tel.: +91 80 2360 2566; fax: +91 80 2360 1552; e-mail address: mugesh@ipc.iisc.ernet.in (G. Mugesh).

0040-4020/$ – see front matter © 2012 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.tet.2012.09.020

based diselenide (**13**),[13] selenocysteine derivative (**14**),[14] the cyclic selenelate ester (**15**),[15] and the spirodiazaselenuranes (**16**) (Fig. 1).[16] Diaryl diselenides, such as **3**, **4** and **6** having *tert*-amino substituents attracted significant attention as the amino groups play several positive roles in the catalytic cycle. Iwaoka and Tomoda proposed that the *tert*-amino groups in the close proximity of selenium abstract the proton from selenols to produce selenolates, which are more reactive than the undissociated selenols.[8b] These amino substituents may also facilitate the reduction of −Se−Se− bond by thiols to produce the selenenyl sulfide intermediates. However, the strong Se···N non-bonded interactions between the amino groups and selenium atom in the selenenyl sulfide intermediates significantly reduce the GPx-like activity. In selenenyl sulfides having *tert*-amine-substituents, the nucleophilic attack of thiol at selenium is more favored than at sulfur, leading to thiol exchange reactions[8] (Fig. 1).

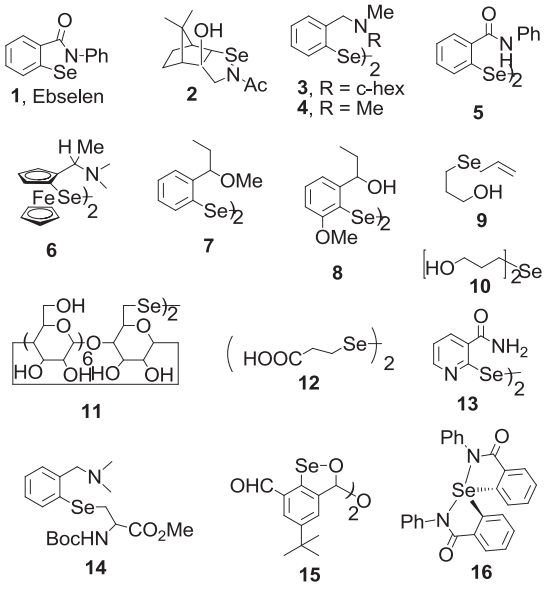


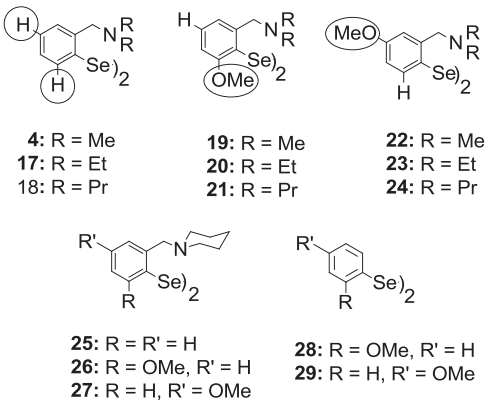
Fig. 1. Some small-molecule GPx mimics reported in the literature.[3–16]

Recently, we have shown that the substitution of hydrogen atom at 6-position in *N*,*N*-dialkylbenzylamine-based diselenides by a methoxy group increases the catalytic activity.[8f] For example, the GPx-like activity of the methoxy-substituted compounds **19**–**21** has been shown to be much higher than that of **4**, **17** and **18**.[8f] It has been suggested that the electronic and steric effects of the 6-methoxy group play key roles in the enhancement of 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. In this paper, we report the synthesis and characterization of a series of *tert*-amine-based diselenides having 4-methoxy group and a comparison of their GPx-like activity with that of the corresponding 6-methoxy-substituted compounds.

2. Results and discussion

2.1. Synthesis of diselenides

Amino-substituted diselenides **4**, **17**–**27** used in this study were synthesized from 2-bromo-*N*,*N*-dialkylbenzylamines, 3-methoxy-*N*,*N*-dialkylbenzylamines or 2-bromo-5-methoxy-*N*,*N*-dilkylbenzylamines via the *ortho*-lithiation method.[17] The treatment of amines with *n*-BuLi afforded the corresponding *ortho*-lithiated compounds, which upon reaction with selenium powder followed by oxidative work up afforded the corresponding diselenides in moderate yield. The GPx-like activities of these diselenides were studied using three different peroxides—hydrogen peroxide (H_2O_2), cumene hydroperoxide (Cum-OOH), and *tert*-butyl hydroperoxide (*t*-BuOOH)—as the substrate. Glutathione (GSH) and thiophenol (PhSH) were used as the thiol co-substrate. The catalytic reduction of peroxides by GSH in the presence of various selenium compounds were monitored by UV−vis method using glutathione reductase (GR) and NADPH. The decrease in concentration of NADPH was monitored at 340 nm. The reduction of peroxides by PhSH in the presence of selenium compounds was studied using a HPLC method. The amount of PhSSPh produced in each reaction was determined and the time required for 50% conversion of PhSH into PhSSPh ($t_{1/2}$ values) was calculated from the peak areas at different time intervals.



Reactions of selenenyl amides, such as **1** or diselenides, such as **4** with thiols produce the corresponding selenenyl sulfides. In general, the reduction of selenenyl sulfides to selenols need to overcome substantial energy barriers.[18] Therefore, the nucleophilic attack of aromatic thiols at the selenium centers of the selenenyl sulfides are energetically more favorable than at sulfur. The thiol attack at the selenium center can be further enhanced by increasing the electrophilic reactivity of selenium. It is known that ebselen (**1**) exhibits a very weak GPx-like activity in the presence of PhSH. This is due to the Se···O non-bonded interactions in the selenenyl sulfide intermediate, which favors the nucleophilic attack of PhSH at the selenium center.[6a] Previous studies have shown that the *N*,*N*-dialkylbenzylamine-based diselenides **4**, **17**, **18** exhibit much better catalytic activity than ebselen in the presence of aromatic thiols.[8b,d,f] The higher activity of these compounds is due to the presence of a basic amino group, which can deprotonate the selenol to generate a more reactive selenolate.

From Table 1 and Fig. 2, it is clear that the GPx-like activity of all the amine-based diselenides in the presence of PhSH is much higher than that of ebselen in all three peroxide systems. Wirth and co-workers had previously shown that the replacement of aryl protons with methoxy substituents in diselenides improves the stereoselectivity in asymmetric selenenylation reactions.[19] We reported that simple replacement of hydrogen atoms by methoxy substituents in *N*,*N*-dialkylbenzylamine-based diselenides enhances the catalytic activity.[8f] Compounds **19**–**21** having 6-methoxy substituents were found to be much better catalysts than the diselenides **4**, **17** and **18**, respectively. The protection of the selenium moieties from overoxidation by peroxides and the prevention of thiol exchange reactions at the selenium centers in the selenenyl sulfide intermediates upon introduction of the methoxy substituents have been shown to be the important factors for the improvement in the catalytic activity.[8f] In the present study, all the 6-methoxy-substituted diselenides (**19**–**21** and **26**) exhibited much better activity than the corresponding parent compounds **4**, **17**, **18** and **25**, respectively. However, the increase in activity upon

Please cite this article in press as: Bhowmick, D.; Mugesh, G., Tetrahedron (2012), http://dx.doi.org/10.1016/j.tet.2012.09.020

Table 1
Reduction of peroxides by PhSH in the absence of and the presence of compounds **1**, **4**, **17**–**31**

Compd	$t_{1/2}$ (min)[a]		
	H_2O_2	*t*-BuOOH	Cum-OOH
Control	512.0±20.2	781.0±41.0	471.0±33.7
1	422.0±10.0	329.0±2.0	375.0±9.2
4	18.3±0.1	23.9±3.4	22.4±1.5
17	19.8±0.7	35.5±4.6	24.3±0.8
18	27.1±0.5	47.3±11.2	23.8±2.2
19[b]	5.8±0.1	12.5±1.2	4.9±0.2
20	13.2±0.3	37.8±5.1	11.9±1.1
21	15.7±0.6	40.3±2.9	14.1±0.9
22	14.5±1.2	12.5±0.8	11.6±0.1
23	16.8±1.7	16.9±0.6	12.9±0.6
24	23.9±2.1	17.3±0.9	15.0±1.3
25	53.7±3.8	60.4±5.1	46.5±2.1
26	39.1±4.3	45.0±1.5	13.0±1.4
27	24.6±3.1	28.1±3.7	16.9±3.1
28	365.0±23.4	592.0±29.4	213.0±11.8
29	413.0±18.5	699.0±46.8	246.5±23.0

[a] The reactions were carried out in MeOH at 22 °C. Catalyst: 10.0 μM [except compound **19**]; PhSH: 1.0 mM; peroxide: 2.0 mM. The control reactions were performed under identical condition in the absence of selenium compounds.
[b] A lower concentration of compound **19** (5 μM) was used as the conversion was too fast to be measured at 10 μM concentration.

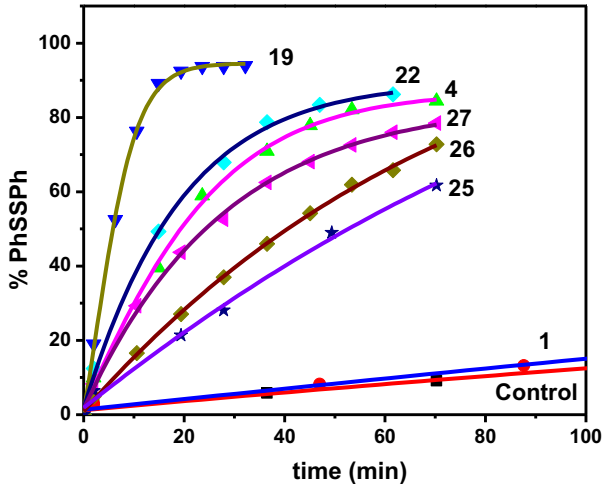


Fig. 2. Catalytic reduction of H_2O_2 by PhSH in the presence of various selenium compounds. The formation of PhSSPh was followed by a reverse-phase HPLC and the % conversion was calculated from calibration plot. Assay condition: catalyst: 10.0 μM (except compound **19**, [catalyst **19**]=5.0 μM); PhSH: 1.0 mM; H_2O_2: 2.0 mM in MeOH at 22 °C.

introduction of methoxy groups appears to be dependent upon the nature of amino groups. While compound **19** having an *N*,*N*-dimethylamino substituent exhibits the highest activity, the introduction of a sterically bulky *N*-cyclohexylamine significantly reduces the catalytic activity. The X-ray crystal structure of compound **25** indicates the existence of non-covalent Se···N interactions (Fig. 3).

To understand whether it is the steric effect or the electronic effect that plays an important role in the enhancement of catalytic activity, we have determined the GPx-like activity of compounds **22**–**24**, and **27** having 4-methoxy substituents. Back and co-workers have reported the effect of substituents on the activity of aromatic cyclic seleninate esters and spirodioxyselenuranes.[20] They have concluded that the introduction of methoxy groups at the 4-position enhances the GPx-like activity. In these compounds, the electronic effects of the substituents play an important role in modulating the redox properties of selenium. From Table 1 and Fig. 2, it is observed that the introduction of a methoxy substituent at the 4-position of

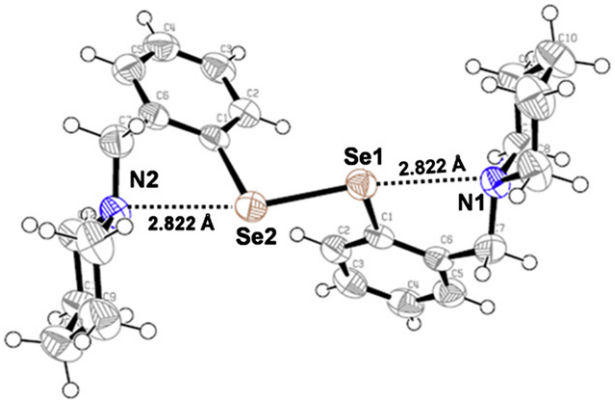


Fig. 3. X-ray crystal structure of compound **25**. ORTEP diagram with the ellipsoids representing 50% probability. Significant bond lengths and bond angles: Se1–Se2: 2.355 Å; Se1–Se2–N2: 168.86°. Se2–Se1–N1: 168.86°.

compounds **4**, **17** and **18** marginally enhances the GPx-like activity. A slight increase in the activity was observed for compound **22** as compared to **4** when H_2O_2 was used as substrate. In contrast, a significant enhancement in the activity was observed upon introduction of a methoxy group at the 4-position of compound **25**. Compound **27** was found to be almost two times more active than **25** in all three peroxide systems. Interestingly, compound **27** was found to be more active than compound **26**, which contains a methoxy group in the 6-position. The lower activity of compound **26** as compared to that of **27** is probably due to the presence of *N*-cyclohexyl and methoxy groups, which increases the steric hindrance around selenium, and hence blocks the –Se–Se– bond cleavage by thiols. However, in both 4- and 6-methoxy-substituted diselenides, the presence of *tert*-amino group is important for the catalytic activity. Compounds **28** and **29**, which lack the amino groups, were found to be poor catalysts in all three peroxide assays. The 2-methoxy-substituted diselenides **28** was found to be marginally better than the 4-methoxy derivative **29**.

The catalytic reduction of peroxides by diselenides was also studied by using GSH as the substrate. The initial rates (v_o) for the reactions were determined by following the oxidation of NADPH at 340 nm using GSH–GSSG coupled assay in phosphate buffer.[3-a,6a,b,8a] The initial rates, summarized in Table 2, indicate that most

Table 2
Initial rates for the reduction of peroxides by GSH in the presence of compounds **1**, **4** and **17**–**29**

Compd	Initial rate (v_o) [μM min^{-1}][a]		
	H_2O_2	*t*-BuOOH	Cum-OOH
1	137.1±5.5	28.8±1.1	42.8±1.5
4	509±12.1	192.6±8.6	480.3±11.5
17	496.8±14.3	179.9±5.9	334.1±12.1
18	255.1±7.7	47.9±2.5	127.3±6.1
19	537.4±14.3	199.8±11.7	472.5±4.6
20	410.2±9.5	84.0±1.5	195.1±8.4
21	372.2±2.7	74.6±1.6	112.3±2.3
22	562.6±14.3	244.3±9.2	595.3±7.5
23	558.0±11.2	207.7±8.1	535.0±5.1
24	537.2±16.4	104.2±3.0	357.7±7.1
25	144.08±6.3	26.9±0.5	96.5±1.7
26	205.3±6.6	61.1±3.2	110.0±6.8
27	561.8±10.7	132.3±1.4	481.8±7.2
28	178.0±6.9	43.0±1.1	75.9±8.6
29	151.9±5.1	27.5±1.7	45.8±3.1

[a] Assay condition: the reactions were carried out in phosphate buffer (100 mM, pH 7.5) at 23 °C. Catalyst: 80.0 μM; GSH: 2.0 mM; NADPH: 0.4 mM; EDTA: 1 mM; glutathione disulfide reductase: 1.7 units/ml; peroxide: 1.6 mM. The control reactions were performed under identical condition in the absence of selenium compounds. For the peroxidase activity, rates were corrected for the background reaction between thiol and peroxide.

Please cite this article in press as: Bhowmick, D.; Mugesh, G., Tetrahedron (2012), http://dx.doi.org/10.1016/j.tet.2012.09.020

of the diselenides exhibit much better catalytic activity than ebselen, which is in agreement with the previous studies on amine-based diaryl diselenides.[8b,d,f] Similar to the effect of 6-methoxy group on the GPx-like activity of compounds **19**–**21** and **26** in the presence of PhSH, a significant enhancement in the catalytic activity in the presence of GSH was observed upon introduction of 6-methoxy substituent except for compound **17**. On the other hand, compounds **22**–**24** and **27** having 4-methoxy substituents were found to be more active than the corresponding 6-methoxy-substituted diselenides. This is in contrast to the PhSH assay in which the 6-methoxy derivatives (**19**–**21**) were found to be more active than the corresponding 4-methoxy-substituted compounds (**22**–**24**). The lower activity of **19**–**21** and **26** as compared to that of **22**–**24** and **27** can be ascribed to the larger size of thiol co-substrate (GSH). The 6-methoxy substituent may inhibit the nucleophilic attack of GSH at the selenium center in compounds **19**–**21** and **26**, although this substituent may play an important role in preventing the thiol exchange reactions at the selenium centers in the selenenyl sulfide intermediates.

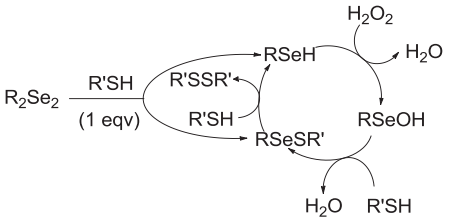
To further understand the catalytic behavior of compounds **4**, **17**–**27**, we have carried out detailed kinetic experiments. The catalytic parameters, such as maximum velocity (V_{max}), Michaelis constant (K_m), catalytic constant (k_{cat}) and catalytic efficiency (η) for the reduction of H_2O_2 by PhSH in the presence of compounds **4**, **17**–**27**, were obtained by plotting the reciprocal of initial rates ($1/v_o$) against the reciprocal of substrate concentration (1/[S]).[6b,e,8d,f] It is clear from Table 3 that the catalytic efficiencies (η) of compound **22**–**24** are around 1.2–1.5 times higher than that of compound **4**, **17**–**18**, but significantly lower than that of **19**–**21**. The higher catalytic efficiencies of compounds **19**–**21** as compared to that of compounds **4**, **17**–**18** and **22**–**24** is mainly due to the difference in their K_m values. The relatively lower K_m values of compound **19**–**21** and **26** indicate that the selenenyl sulfides formed in the reaction from **19**–**21** and **26** are readily converted to the corresponding selenols by reaction with PhSH. The higher K_m values for other diselenides (**4**, **17**–**18**, **22**–**24**, **25** and **27**) indicate the extensive thiol exchange reactions due to strong Se···N interactions. These compounds, therefore, follow the nonsaturation kinetics. When the initial rates were measured with increasing concentration of PhSH at constant catalyst and peroxide concentrations, a rapid increase in the rate was observed at the beginning, but after certain PhSH concentration, the rate became constant. This indicates that compounds **22**–**27** follow saturation kinetics.

Table 3
Catalytic parameters [maximum velocities (V_{max}), Michaelis constants (K_m for PhSH), catalytic constants (k_{cat}) and catalytic efficiencies (η)] for the reduction of H_2O_2 by PhSH in the presence of diselenides **4**, **17**–**27**

Compd	V_{max} (μM min^{-1})	K_m (mM)	k_{cat} (min^{-1})	η (M^{-1} min^{-1})
4	50.8	2.85	5.08	1.78×10^3
17	24.1	1.40	2.41	1.71×10^3
18	11.3	0.79	1.13	1.42×10^3
19	54.9	0.35	5.49	1.58×10^4
20	10.5	0.12	1.05	8.87×10^3
21	11.2	0.17	1.12	6.58×10^3
22	126.6	6.98	12.66	1.81×10^3
23	52.3	2.05	5.23	2.54×10^3
24	31.4	1.46	3.14	2.15×10^3
25	17.8	2.93	1.78	0.60×10^3
26	4.6	0.10	0.46	4.63×10^3
27	63.8	5.34	6.38	1.19×10^3

Assay condition: PhSH (0.0–4.0 mM), H_2O_2 (1.0 mM), and catalyst (10 μM) in MeOH at 22 °C.

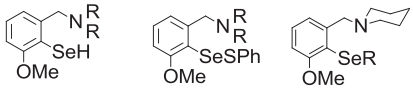
The mechanism for the GPx-like activity of diaryl diselenides generally involves four major steps (Scheme 2). (i) Reductive cleavage of –Se–Se– bond by thiols, leading to the formation of the corresponding selenols. (ii) Reaction of the selenols with peroxides to produce selenenic acids. (iii) Reactions of selenenic acids with thiols to generate the corresponding selenenyl sulfides. (iv) Regeneration of the catalytically active selenols from the selenenyl sulfide intermediates by reaction with thiols. To understand the effect of methoxy substituents on the reactivity of various intermediates in the catalytic mechanism, we have studied the reactivity of diselenides toward thiol (PhSH) and peroxide (H_2O_2). The reactions were followed by ^{77}Se NMR spectroscopy. In some cases, the intermediates were isolated and fully characterized.



Scheme 2. Proposed mechanism for the reduction of H_2O_2 by diaryl diselenides in the presence of a thiol.

2.2. Reactivity of diselenides toward thiol

Reactions of diselenides with thiols generally produce a mixture of selenenyl sulfides and selenols, and the relative amount of selenols produced in these reactions depends on the nature and concentration of the thiols. Although both the 6-methoxy- and 4-methoxy-substituted compounds react readily with PhSH to produce the corresponding selenenyl sulfides and selenols, the conversion of selenenyl sulfides to selenols is more favorable by the 6-methoxy substituent. Treatment of diselenides **19**–**21** and **26** with PhSH (1 equiv in each case) rapidly generated the selenols **30**–**32** and **36**, respectively, with the formation of only trace amount of the corresponding selenenyl sulfides (**33**–**35**, **37**). As 1 equiv of PhSH in each case was not sufficient for quantitative conversion of **19**–**21** and **26** into the selenols, some unreacted diselenides were observed in the reaction mixtures. When 2 equiv of PhSH was added in each case, the diselenides and selenenyl sulfides were converted quantitatively into the corresponding selenols (**30**–**32**, **36**).



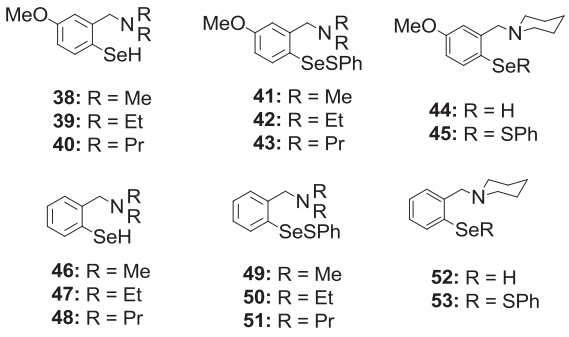
30: R = Me
31: R = Et
32: R = Pr

33: R = Me
34: R = Et
35: R = Pr

36: R = H
37: R = SPh

To understand the effect of 4-methoxy substituent on the reactivity of diselenides toward thiols, compounds **22**–**24** and **27** were treated with PhSH (1 equiv in each case) under identical experimental conditions. While these reactions produced the corresponding selenols (**38**–**40**, **44**) and the selenenyl sulfides (**41**–**43**, **45**) in nearly 1:1 ratios, the addition of 2 equiv of PhSH in each case did not lead to complete conversion of the selenenyl sulfides into the corresponding selenols. This is due to the thiol exchange reactions that take place at the selenium centers in the selenenyl sulfide intermediates.[6a] As previously shown for related compounds, the extent of thiol exchange reactions depend on the nature of substituents in the close proximity of selenium.[8f] The reactions of **41**–**43**, **45** with a different thiol (4-Me–C_6H_4SH) produced new selenenyl sulfide, confirming the thiol exchange reactions (Fig. S65, Supplementary data). In this respect, the 4-methoxy-substituted selenenyl sulfides (**41**–**43**, **45**) behave somewhat similar to the parent selenenyl sulfides (**49**–**51**, **53**). We have shown previously that treatment of compounds **4**, **17** and **18** produces the corresponding selenols **46**–**48**, together with the

selenenyl sulfides **49**–**51**, respectively, even in the presence of 2 equiv of PhSH.[8f] However, the 4-methoxy substituents in compounds **41**–**43** and **45** appear to enhance the formation of selenols, although the introduction of a methoxy group at the 6-position was found to be more effective than at the 4-position in suppressing the thiol exchange reactions.



The ^{77}Se NMR chemical shifts of the three sets of selenenyl sulfides show some interesting features (Table 4). The ^{77}Se NMR chemical shifts for compounds **49**–**51** and **53**, which lack methoxy substituents, are found to be in the range 549–564 ppm. These values are marginally shifted downfield as compared to that of PhSeSPh (526 ppm), indicating the presence of Se···N non-covalent interactions. The strength of these interactions depends on the nature of amino substituent. Sterically bulky amino groups appear to decrease the strength of Se···N interactions. For example, the ^{77}Se NMR chemical shift of compound **53** having a *N*-cyclohexyl amino group is shifted upfield (549 ppm) as compared to that of *N*,*N*-dimethylamino-substituted selenenyl sulfide **49** (564 ppm). Interestingly, the methoxy group at 4- and 6-position shows different effect on the ^{77}Se NMR chemical shifts. The peaks for compounds **33**–**35**, **37** having the methoxy group in the 6-position are shifted 85–100 ppm upfield with respect to compounds **49**–**51**, **53**, respectively. On the other hand, the signals for compounds **41**–**43**, **45** having the methoxy substituents in the 4-position do not show significant shift in the ^{77}Se NMR spectra. While the ^{77}Se NMR chemical shifts for compounds **41**–**43** are shifted ~15 ppm upfield with respect to the parent selenenyl sulfides (**49**–**51**), the *N*-cyclohexylamine-based compounds **45** and **53** exhibit almost identical chemical shift values. These observations indicate that the selenium centers in compounds **33**–**35** and **37** are significantly less electrophilic than that of compounds **41**–**43** and **45**. Therefore, the methoxy group in the 6-position is more effective than in the 4-position in preventing the undesired thiol exchange reactions.

Table 4
Experimentally obtained ^{77}Se NMR chemical shifts and theoretically calculated $E_{Se\cdots N}$ for the selenenyl sulfides

Compd	^{77}Se NMR chemical shift (ppm)[a]	$E_{Se\cdots N}$ (kcal mol^{-1})[b]	Compd	^{77}Se NMR chemical shift (ppm)[a]	$E_{Se\cdots N}$ (kcal mol^{-1})[b]
33	470	5.78	**43**	540	10.25
34	461	7.02	**45**	553	9.16
35	451	6.59	**49**	564	11.20
37	464	7.04	**50**	558	11.23
41	548	10.96	**51**	555	12.57
42	542	9.00	**53**	549	9.66

[a] The values are cited with respect to Me_2Se.
[b] The optimizations of the geometries were performed at the B3LYP/631+G(d)//B3LYP/6-311+G(d,p) levels.

The density functional theory (DFT) calculations indicate that the strength of Se···N non-covalent interactions depend not only on the nature of amino substituents, but also on the location of the methoxy group. The introduction of methoxy groups in the 6-positions dramatically decreases the strength of Se···N interactions (Fig. 4 and Table 5). The interaction energies between the selenium and nitrogen atoms calculated by natural bond orbital (NBO) analyses, indicate that the incorporation of methoxy group in the 6-position decreases the $E_{Se\cdots N}$ by almost 50% for each amino-

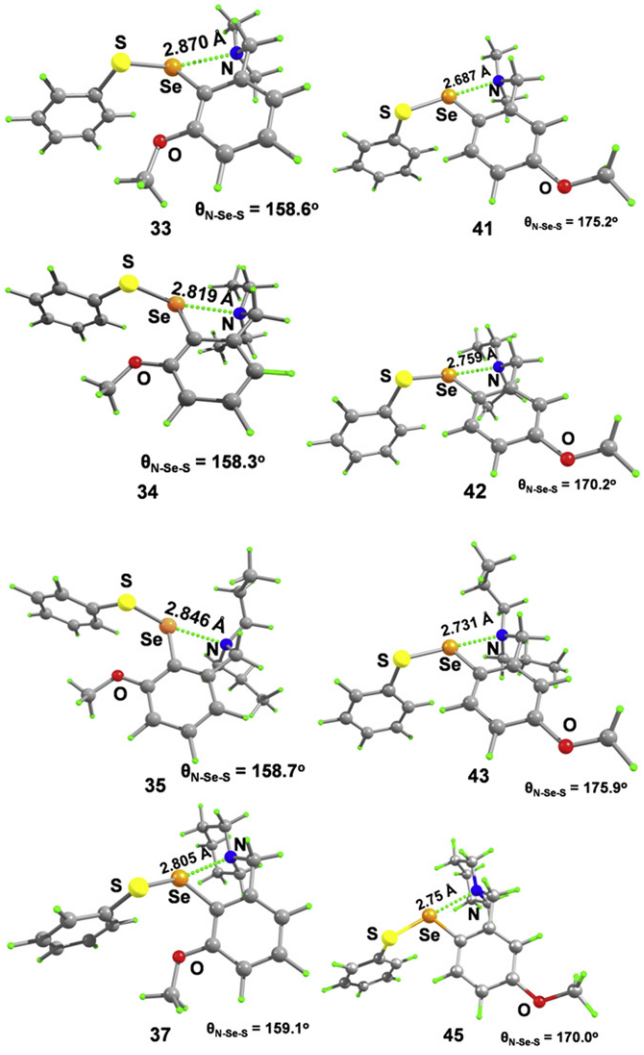


Fig. 4. Energy-optimized geometries of the selenenyl sulfide intermediates **33**–**35**, **37**, **41**–**43** and **45**. The optimizations of the geometries were performed at the B3LYP/6-31+G(d) level of theory.

substituted selenenyl sulfide. In contrast, the Se···N interactions in the 4-methoxy-substituted compounds are much stronger than that of the corresponding 6-methoxy-substituted selenenyl sulfides. For example, the Se···N distance in compound **41** (2.687 Å) is

Table 5
Experimentally determined ^{77}Se NMR chemical shifts and the theoretically calculated charge on selenium atom in the selenols

Compd	^{77}Se NMR chemical shift (ppm)[a]	q_{Se}[b]	Compd	^{77}Se NMR chemical shift (ppm)[a]	q_{Se}[b]
30	−58	−0.246	**40**	17	−0.337
31	−42	−0.279	**44**	13	0.067
32	−37	−0.295	**46**	35	−0.289
36	−44	0.102	**47**	54	−0.312
38	19	−0.294	**48**	56	−0.314
39	16	−0.322	**52**	67	0.069

[a] The values are cited with respect to Me_2Se.
[b] The charge on selenium calculated at the B3LYP/631+G(d)//B3LYP/6-311+G(d,p) levels.

much shorter than that in compound **33** (2.870 Å). Similarly, the $E_{Se\cdots N}$ values for compounds **41**–**43** and **45** (9.00–10.96 kcal mol^{-1}) are significantly higher than that of compounds **33**–**35** and **37** (5.78–7.02 kcal mol^{-1}). However, the strength of Se···N interactions in the 4-methoxy-substituted compounds are slightly weaker than that of the parent selenenyl sulfides **49**–**51** and **53** (Table 4). This is in agreement with the minor shifts in the ^{77}Se NMR chemical shift values.

According to Scheme 2, the reactions of diselenides with 1 equiv of PhSH should produce a 1:1 mixture of the corresponding selenenyl sulfides and selenols. However, the conversion of the selenenyl sulfides into the selenols in the presence of two or more equiv of PhSH depends on the nature of the substituents and the rate of thiol exchange reactions. In addition to the amount of selenol produced in the reactions, the high reactivity of selenol toward H_2O_2 is crucial for the GPx-like activity. Therefore, we have carried out experimental and theoretical investigations to understand the effect of methoxy substituents on the reactivity of selenols. In agreement with our previous study,[8f] treatment of the diselenides **19**–**21** and **26** with PhSH (2 equiv in each case) quantitatively generated the corresponding selenols (**30**–**32** and **36**). A comparison of the ^{77}Se NMR chemical shifts of the 6-methoxy-substituted compounds **30** (−58 ppm), **31** (−42 ppm) **32** (−37 ppm) and **36** (−44 ppm) with that of parent selenols **46** (35 ppm), **47** (54 ppm), **48** (56 ppm) and **52** (67 ppm) indicates that the introduction of a methoxy substituent at the 6-position leads to a remarkable upfield shift in the ^{77}Se NMR signals. Particularly, the chemical shifts for the 6-methoxy-substituted selenol having a *N*-cyclohexyl amino group is shifted almost 100 ppm upfield relative to that for the unsubstituted selenol. These observations indicate that the introduction of a methoxy group in the 6-position dramatically increases the negative charge on selenium (Fig. 5). The selenium moiety in compounds **30**–**32** and **36** are, therefore, significantly more nucleophilic than that in compounds **46**–**48** and **52**. However, the prevention of thiol exchange reactions in the selenenyl sulfide intermediates appears to be more important than the increase in the nucleophilic character of the selenol for the enhancement in the GPx-like activity. This is in agreement with the theoretical investigations of Haverly-Coulson and Boyd, which indicate that the introduction of substituents in the *ortho*, *meta* or *para* positions has little effect on the energy barrier for the peroxide reduction reaction.[21]

In contrast to the effect of 6-methoxy substituent, the introduction of a methoxy group at the 4-position does not lead to a drastic change in the ^{77}Se NMR chemical shifts. The chemical shifts for compounds **38** (19 ppm), **39** (16 ppm), **40** (17 ppm) and **44** (13 ppm) are shifted 16–54 ppm upfield relative to those for compounds **46**–**48** and **52**, which lack the methoxy substituent. However, the moderate change in the chemical shift values indicates that the incorporation of a methoxy substituents at the 4-position does increase the negative charge on selenium. Therefore, the more amount of selenols produced in the reactions of compounds **19**–**21** and **26** with PhSH relative to those of **22**–**24** and **27** may account for the higher activity of the 6-methoxy-substituted diselenides as compared to that of the 4-methoxy-substituted compounds. It should be noted that a quantitative conversion of compounds **22**–**24** and **27** into the corresponding selenols were observed only in the presence of a large excess of PhSH (60 equiv in each case). The quantitative conversion at this concentration of PhSH was confirmed by treating the selenols with iodoacetic acid to get the corresponding alkylated products **54**–**56** (Scheme 3). Although the DFT calculations indicate that the introduction of a 4-methoxy substituent can increase the negative charge on selenium in most of the selenols (Table 5), the solvents may have a significant effect on the zwitterionic character of the selenols in solution.

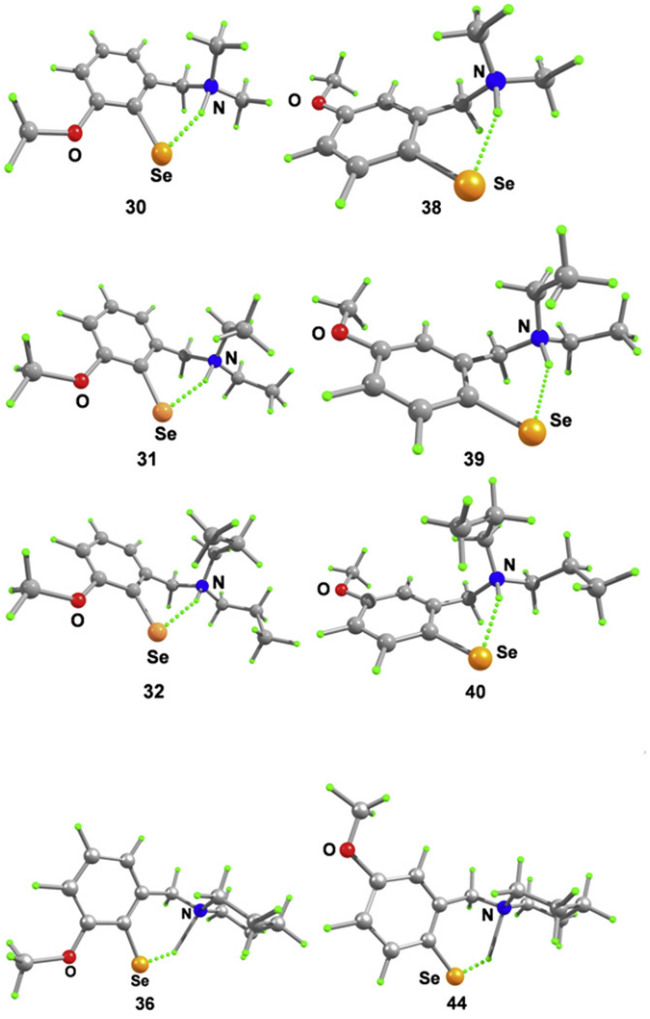
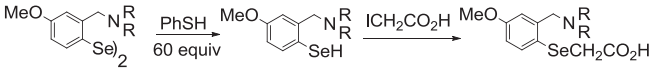


Fig. 5. Energy-optimized geometries of the selenol species **30**–**32**, **36**, **38**–**40** and **44**. The optimizations of the geometries were performed at the B3LYP/6-31+G(d) level of theory.



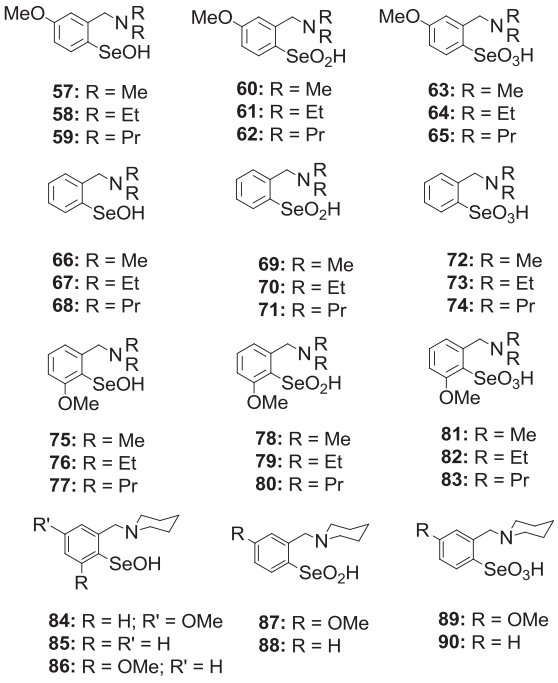
Scheme 3. Reactions of diselenides **22**–**24** with an excess amount of PhSH (60 equiv). The selenols produced in these reactions could be trapped by treating with iodoacetic acid.

2.3. Reactions of diselenides with peroxides

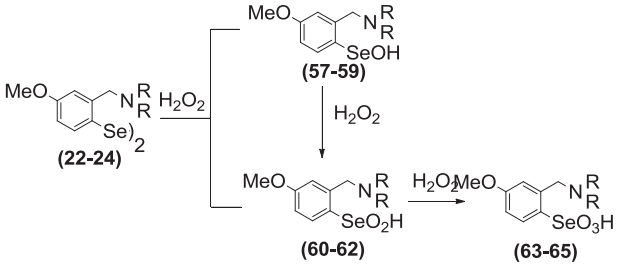
In addition to the experiments to understand the role of methoxy substituents in the selenols and selenenyl sulfides, we have also studied the effect of Se···N interactions in the selenenic acids that are considered to be a crucial type of intermediates in the GPx catalytic cycle. It is known that the amine-based diselenides readily react with peroxides to produce selenenic/seleninic acids. Treatment of compounds **22**–**24** with H_2O_2 produced a mixture of the corresponding selenenic acids (**57**–**59**) and seleninic acids (**60**–**62**). When compound **22** was treated with H_2O_2, two ^{77}Se peaks were observed at 1163 ppm and 1189 ppm, which could be assigned to the selenenic acid **57** and seleninic acid **60**, respectively. In the presence of an excess amount (10 equiv) of H_2O_2, these two compounds were converted to the selenonic acid **63**, which exhibited a signal in ^{77}Se NMR at 1018 ppm. The signal is

Please cite this article in press as: Bhowmick, D.; Mugesh, G., Tetrahedron (2012), http://dx.doi.org/10.1016/j.tet.2012.09.020

significantly shifted upfield as compared to that of the seleninic acid **60** (1189 ppm). This is in agreement with the report of Iwaoka and Tomoda that the selenonic acid derived from compound **4** exhibits an upfield shift in ^{77}Se NMR with respect to the corresponding selenenic and seleninic acids,[8b] which is probably due to a decrease in the strength of Se···N interactions upon overoxidation.



Similarly to compounds **4** and **22**, the other 4-methoxy-substituted compounds **23** and **24** invariably produced the selenonic acids **64** (1017 ppm) and **65** (1015 ppm), respectively, upon treatment with an excess amount (10 equiv) of H_2O_2 (Scheme 4). The introduction of a sterically bulky cyclohexyl group on the nitrogen atom does not appear to block the overoxidation as the reaction of compound **27** with H_2O_2 produced the selenonic acid **89** (1018 ppm) as the major oxidized product. Therefore, the electronic effects introduced by the 4-methoxy substitution in compounds **22**–**24** and **27** are not sufficient to stabilize the selenenic and seleninic acids against overoxidation. In this regard, the reactivity of compounds **22**–**24** and **27** toward H_2O_2 very similar to that of their parent compounds **4**, **17**, **18** and **25**, which readily produce the selenonic acids **72** (1019 ppm), **73** (1019 ppm), **74** (1017 ppm) and **90** (1018 ppm), respectively. Interestingly, the introduction of methoxy substituents at their 6-positions prevents the overoxidation of the selenenic acids to the corresponding seleninic acids. For example, the energy difference between **75** and **81** has been shown to be almost 4.0 kcal mol^{-1} higher than that between **66** and **72**, indicating that the conversion of **75** into **81** is less favored than the conversion of **66** into **72**.[8f] The higher stability of the 6-methoxy-substituted selenenic acids **75**–**77** as compared to that of **57**–**59** or **66**–**68** may contribute to the enhanced GPx-like activity of compounds **19**–**21**.



Scheme 4. Reaction of diselenides **22**–**24** and **27** with H_2O_2 to produce corresponding selenenic acids (**57**–**59**); seleninic acids (**60**–**62**); and selenonic acids (**63**–**65**).

The optimized geometries (Fig. 6) and NBO analysis (Table 6) indicate that both the 4-methoxy- and 6-methoxy-substituted selenenic acids exhibit strong Se···N interactions, which help nucleophilic attack by incoming thiols at the selenium centers. Although the Se···N distances in the 6-methoxy-substituted compounds **75**–**77** and **86** are slightly longer than that observed in the corresponding 4-methoxy-substituted compounds (**57**–**59** and **84**) (Fig. 6), they exhibit similar reactivity toward thiols. Therefore, the oxidation of selenenic acids to the corresponding seleninic and selenonic acids may partly be responsible for the lower activity of compounds **22**–**24** as compared to that of **19**–**21**. Haverly-Coulson and Boyd have shown that *ortho*-substituents generally alter the environment around the selenium atom such a way that it becomes less favorable for the H_2O_2 to form a close association with the selenium center.[21] In agreement with this, the 6-methoxy substituents in compounds **75**–**77** may disfavor the formation of seleninic and selenonic acids by preventing the interactions between the selenium atom and peroxide molecule.

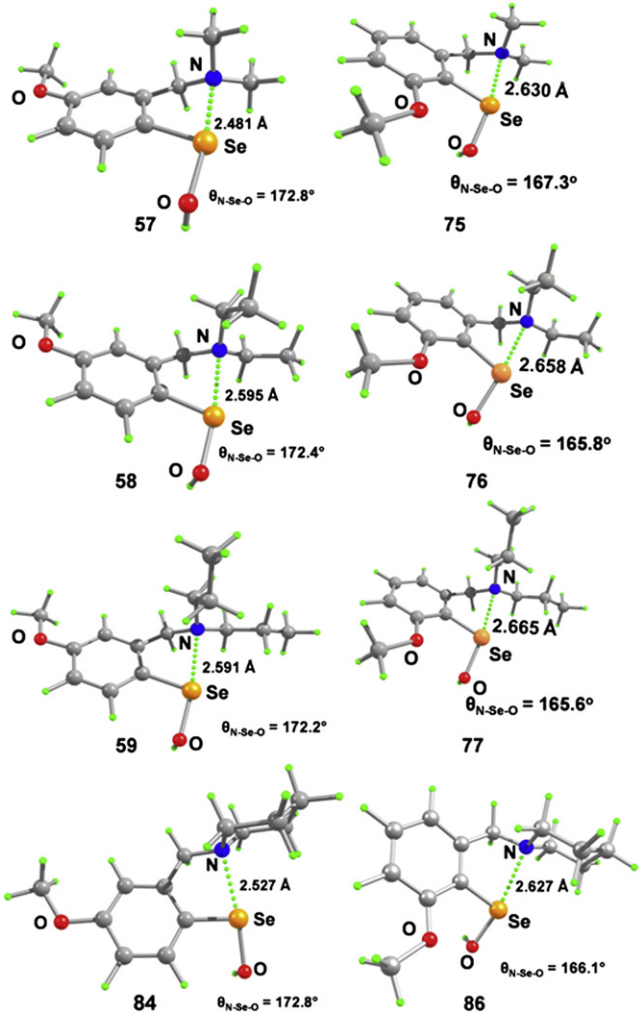


Fig. 6. Energy-optimized geometries of the selenenic acid intermediates **57**–**59**, **75**–**77**, **84** and **86**. The optimizations of the geometries were performed at the B3LYP/6-31+G(d) level of theory.

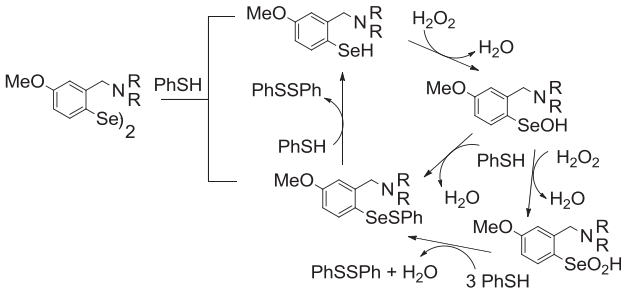
Table 6
Experimentally obtained ^{77}Se NMR chemical shifts and theoretically calculated $E_{Se\cdots N}$ for the selenenic acids

Compd	^{77}Se NMR chemical shift (ppm)[a]	$E_{Se\cdots N}$ (kcal mol^{-1})[b]	Compd	^{77}Se NMR chemical shift (ppm)[a]	$E_{Se\cdots N}$ (kcal mol^{-1})[b]
57	1163	19.92	**75**	1170	12.84
58	1165	15.29	**76**	1174	12.41
59	1168	15.42	**77**	1172	12.04
66	1168	20.47	**84**	1160	18.43
67	1171	16.02	**85**	1163	18.94
68	n.d[c]	16.24	**86**	1172	13.40

[a] The values are cited with respect to Me_2Se.
[b] The optimizations of the geometries were performed at the B3LYP/631+G(d)//B3LYP/6-311+G(d,p) levels.
[c] Not detected.

2.4. Catalytic mechanism

From the experimental and theoretical observations, we propose a catalytic mechanism for the reduction of peroxides by PhSH in the presence of diselenides **22**–**24** and **27** as shown in Scheme 3. The initial reductive cleavage of –Se–Se– bond in the diselenides by PhSH produces the corresponding selenenyl sulfides (**41**–**43** and **45**) and selenols (**38**–**40** and **44**). The selenols thus produced undergo reaction with H_2O_2 to produce the corresponding selenenic acids (**57**–**59** and **84**), which upon reaction with 1 equiv of PhSH generates the selenenyl sulfide intermediates. Addition of an excess amount of thiol regenerates the selenols with the formation of PhSSPh. When the concentration of H_2O_2 is higher than that of thiol, the selenenic acids generated from the selenols undergo further oxidation to produce the corresponding seleninic acids (**60**–**62** and **87**) and selenonic acid (**63**–**65** and **89**). However, in the presence of an excess amount of PhSH, the selenenic, seleninic, and selenonic acids are converted to the corresponding selenenyl sulfides. This catalytic cycle is very similar to that of diselenide **4**, **17**, **18** and **25**, but slightly different from that of compounds **19**–**21** and **26** in which no overoxidized product (**78**–**83**) is generated in the presence of an excess amount of peroxides (Scheme 5).



Scheme 5. The proposed catalytic mechanism for the reduction of peroxides by the diselenides **22**–**24**, and **27** in the presence of thiol.

3. Conclusion

In this study we have compared the glutathione peroxidase-like activity of a series of 4-methoxy-substituted *N*,*N*-dialkylbenzylamine-based diselenides with that of the corresponding 6-methoxy-substituted compounds. While both 4- and 6-methoxy substituents can alter the electronic properties of the selenium center, the substitution at the 6-position provides the required steric protection for some of the catalytically active species. Particularly, the 6-methoxy group prevents the thiol exchange reactions at the selenium atoms in the selenenyl sulfide intermediates. Furthermore, the introduction of a substituent at the 6-position prevent the formation of seleninic and selenonic acids by disfavoring the interactions between selenium center and peroxides. When PhSH is used as the thiol co-substrate, the 4-methoxy substituent neither blocks the selenium center in the selenenyl sulfide intermediates from thiol exchange reactions nor prevents the formation of seleninic and selenonic acids. On the other hand, when larger thiols, such as glutathione (GSH) are used in assay, the introduction of steric hindrance decreases the catalytic activity by blocking the nucleophilic attack of GSH at the selenium center in the selenenic acids. In such cases, the substitution at the 4-position significantly enhances GPx-like activity of the diselenides.

4. Experimental

4.1. General procedure

n-Butyllithium (*n*-BuLi) was purchased from Acros Chemical Co. (Belgium). Methanol was obtained from Merck and dried before use. All other chemicals were of the highest purity available. All the reactions were carried out under nitrogen with use of standard vacuum-line techniques. Because of the unpleasant odors and toxic nature of several of the reaction mixtures involved, most manipulations were carried out in a well-ventilated fume hood. Et_2O was dried over sodium metal with benzophenone. Thin-layer chromatography analyses were carried out on pre-coated silica gel plates (Merck), and spots were visualized with UV radiation. Column chromatography was performed on glass columns loaded with silica gel or on automated flash chromatography systems (Biotage) with use of preloaded silica cartridges. ^{1}H (400 MHz), ^{13}C (100.56 MHz), and ^{77}Se (76.29 MHz) NMR spectra were obtained on a Bruker 400 MHz NMR spectrometer. Chemical shifts are cited with respect to $SiMe_4$ as internal (^{1}H and ^{13}C) and Me_2Se as external (^{77}Se) standard. A Perkin–Elmer Lambda 5 UV/Vis spectrophotometer was used to measure the GPx-like activities. Mass spectral studies were carried out on a Bruker Daltonics Esquire 6000 plus mass spectrometer with ESI-MS mode analysis. Compound **1**, **4**, **17**–**21** were synthesized by the literature method.[4,8a,f]

4.2. General procedure for the synthesis of compound 22–25

n-BuLi (2.5 equiv of a 1.6 M solution in hexane) was added with stirring at −78 °C to a solution of 2-bromo-5-methoxy-*N*,*N*-dialkylbenzylamine in dry THF and the mixture was allowed to stir at the same temperature. After 1.5 h finely ground selenium powder (1.1 equiv) was added to the mixture at 0 °C. After the addition of the selenium powder the solution turned to brownish color and the reaction mixture was stirred for 12 h at room temperature. The solution was poured to the saturated solution of ammonium chloride (NH_4Cl) and stirred for 10 min. Then it was extracted with diethyl ether three times. It was dried with anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to obtain yellow-colored liquid. Diselenides were purified in flash chromatography using petroleum ether and ethyl acetate as the solvent. The compounds were characterized by NMR spectroscopy.

4.2.1. Compound ***22***. Yield: 0.388 g (41%), ^{1}H NMR ($CDCl_3$), δ (ppm): 2.25 (s, 12H, NCH_3), 3.50 (s, 4H, ArCH_2N), 3.61 (s, 6H, OCH_3), 6.71–6.73 (d, 2H, ArH), 6.77 (s, 2H, ArH), 7.69–7.71 (d, 2H, ArH); ^{13}C NMR ($CDCl_3$), δ (ppm): 44.9, 55.8, 65.0, 113.8, 115.0, 124.8, 133.6, 141.0, 158.9; ^{77}Se NMR ($CDCl_3$), δ (ppm): 420. ESI-MS: *m*/*z*: calcd for $C_{20}H_{28}N_2O_2Se_2$ $[M+H]^+$: 489.0559; found: 489.0557.

4.2.2. Compound ***23***. Yield: 0.41 g (38%), ^{1}H NMR ($CDCl_3$), δ (ppm): 1.00–1.04 (t, 12H, NCH_2CH_3), 2.54–2.58 (m, 8H, NCH_2CH_3), 3.64 (s,

4H, ArCH_2N), 3.77 (s, 6H, OCH_3), 6.68–6.70 (d, 2H, ArH), 6.80 (s, 2H, ArH), 7.65–7.68 (d, 2H, ArH); ^{13}C NMR ($CDCl_3$), δ (ppm): 11.2, 45.5, 55.7, 59.5, 113.4, 114.7, 124.2, 133.3, 134.9, 141.9, 158.8; ^{77}Se NMR ($CDCl_3$), δ (ppm): 417. ESI-MS: *m*/*z*: calcd for $C_{24}H_{38}N_2O_2Se_2$ $[M+H]^+$: 546.1264; found: 545.1079.

4.2.3. Compound ***24***. Yield: 0.47 g (39%), ^{1}H NMR ($CDCl_3$), δ (ppm): 0.82–0.85 (t, 12H, $NCH_2CH_2CH_3$), 1.47–1.57 (m, 8H, $NCH_2CH_2CH_3$), 2.39–2.43 (t, 8H, $NCH_2CH_2CH_3$), 3.63 (s, 4H, ArCH_2N), 3.77 (s, 6H, OCH_3), 6.67–6.70 (d, 2H, ArH), 6.83 (s, 2H, ArH), 7.64–7.66 (d, 2H, ArH); ^{13}C NMR ($CDCl_3$), δ (ppm): 12.5, 19.8, 55.3, 55.7, 60.7, 113.4, 114.9, 124.1, 133.5, 142.2, 158.9; ^{77}Se NMR ($CDCl_3$), δ (ppm): 414. ESI-MS: *m*/*z*: calcd for $C_{28}H_{46}N_2O_2Se_2$ $[M+H]^+$: 602.1890; found: 601.2214.

4.2.4. Compound ***25***. It was crystallized in $CHCl_3$/ether mixture. Yield: 0.75 g (47%), ^{1}H NMR ($CDCl_3$), δ (ppm): 1.43–1.46 (br, 4H, CylH_a), 1.56–1.61 (m, 8H, CylH_b), 2.42 (br, 8H, CylH_c), 3.55 (s, 4H, NCH_2N), 7.08–7.13 (br, 4H, ArH), 7.79–7.81 (d, 2H, ArH), 7.20–7.22 (t, 2H, ArH); ^{13}C NMR ($CDCl_3$), δ (ppm): 25.0, 26.5, 54.1, 64.5, 126.0, 128.5, 128.9, 131.8, 134.8, 139.8; ^{77}Se NMR ($CDCl_3$), δ (ppm): 425. ESI-MS: *m*/*z*: calcd for $C_{24}H_{32}N_2Se_2$ $[M+H]^+$: 508.0896; found: 508.9519.

4.2.5. Synthesis of ***26***. *n*-BuLi (2.1 ml of a 1.6 M solution in hexane) was added with stirring at 0 °C to a solution of 1-(3-methoxybenzyl)piperidine (0.60 g, 2.92 mmol) in dry THF (15 ml) and the mixture was allowed to stir at the same temperature. After 2 h finely ground selenium powder (0.125 g, 3.21 mmol) was added to the mixture at 0 °C. After the addition of the selenium powder the solution turned to brownish color and the reaction mixture was stirred for 12 h. The solution was poured to the saturated solution of ammonium chloride (NH_4Cl) and stirred for 10 min. Then it was extracted with diethyl ether (20 ml) three times. It was dried with anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to obtain yellow-colored liquid, which was purified in flash chromatography using petroleum ether and ethyl acetate as the solvent. The compound was characterized by NMR spectroscopy. Yield: 0.68 g (43%), ^{1}H NMR ($CDCl_3$), δ (ppm): 1.43–1.46 (br, 4H, CylH_a), 1.56–1.61 (m, 8H, CylH_b), 2.38 (br, 8H, CylH_c), 3.46 (s, 4H, ArCH_2N), 3.81 (s, 6H, OCH_3), 6.78–6.81 (d, 2H, ArH), 6.90–6.92 (m, 2H, ArH), 7.20–7.22 (t, 2H, ArH); ^{13}C NMR ($CDCl_3$), δ (ppm): 24.9, 26.5, 54.9, 55.6, 64.4, 112.7, 115.0, 121.9, 129.4, 140.8, 160.0; ^{77}Se NMR ($CDCl_3$), δ (ppm): 379. ESI-MS: *m*/*z*: calcd for $C_{26}H_{36}N_2O_2Se_2$ $[M+H]^+$: 569.1185; found: 569.0675.

4.2.6. Synthesis of ***27***. *n*-BuLi (3.5 ml of a 1.6 M solution in hexane) was added with stirring at −78 °C to a solution of 1-(2-bromo-5-methoxybenzyl)piperidine (0.64 g, 2.24 mmol) in dry THF (20 ml) and the mixture was allowed to stir at the same temperature. After 2 h finely ground selenium powder (0.195 g, 2.47 mmol) was added to the mixture at −20 °C. After the addition of the selenium powder the solution turned to brownish color and the reaction mixture was stirred for 12 h. The solution was poured to the saturated solution of ammonium chloride (NH_4Cl) and stirred for 10 min. Then it was extracted with diethyl ether (20 ml) three times. It was dried with anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to obtain yellow-colored liquid, which was purified in flash chromatography using petroleum ether and ethyl acetate as the solvent. The compound was characterized by NMR spectroscopy. Yield: 0.40 g (32%), ^{1}H NMR ($CDCl_3$), δ (ppm): 1.42–1.43 (br, 4H, CylH_a), 1.57–1.60 (m, 8H, CylH_b), 2.42 (br, 8H, CylH_c), 3.52 (s, 4H, ArCH_2N), 3.78 (s, 6H, OCH_3), 6.69–6.72 (d, 2H, ArH), 6.77 (s, 2H, ArH), 7.68–7.70 (d, 2H, ArH); ^{13}C NMR ($CDCl_3$), δ (ppm): 24.8, 26.3, 30.2, 54.1, 55.8, 64.3, 113.7, 115.1, 124.9, 133.4, 141.1, 158.7; ^{77}Se NMR ($CDCl_3$), δ (ppm): 418. ESI-MS: *m*/*z*: calcd for $C_{26}H_{36}N_2O_2Se_2$ $[M+H]^+$: 569.1185; found: 569.1508.

4.2.7. Synthesis of ***28***. *n*-BuLi (3.5 ml of a 1.6 M solution in hexane) was added with stirring at 0 °C to a solution of 2-bromoanisole (1.0 g, 5.34 mmol) in dry hexane (10 ml) and the mixture was allowed to stir at room temperature. After 2 h stirring the *n*-butyl bromide formed in the reaction mixture was evaporated under reduced pressure without introduction of air. Dry THF (10 ml) was added followed by the addition of the finely ground selenium powder (0.42 g, 5.34 mmol) at 0 °C. It was stirred for 4 h. It was poured to the ice-cooled saturated $NaHCO_3$ solution and O_2 gas was passed to the mixture in a moderate rate for 15 min. The compound was extracted with diethyl ether and dried with anhydrous Na_2SO_4. The solvent was evaporated to obtain yellow-colored solid. The compound was purified in flash chromatography using petroleum ether and ethyl acetate as solvent. It was characterized by NMR spectroscopy. Yield: 0.58 g (31%), ^{1}H NMR ($CDCl_3$), δ (ppm): 3.91 (s, 6H, OCH_3), 6.80–6.83 (d, 2H, ArH), 6.85–6.89 (m, 2H, ArH), 7.19–7.23 (m, 2H, ArH), 7.53–7.55 (d, 2H, ArH); ^{13}C NMR ($CDCl_3$), δ (ppm): 56.4, 110.6, 119.1, 122.4, 128.6, 131.0, 157.3; ^{77}Se NMR ($CDCl_3$), δ (ppm): 328. ESI-MS: *m*/*z*: calcd for $C_{14}H_{13}O_2Se_2$ $[M+Na]^+$: 396.9222; found: 397.0495.

4.2.8. Synthesis of ***41***. Thiophenol (2 μL, 0.04 mmol) was added at room temperature to the stirred solution of **22** (10 mg, 0.02 mmol) in dichloromethane (1 ml). The reaction mixture was stirred for 30 min, and the solvent was then evaporated. The expected compound was purified by flash chromatography on a silica gel column with ethyl acetate and petroleum ether as eluents to provide pale yellow-colored oil. ^{1}H NMR ($CDCl_3$), δ (ppm): 2.29 (s, 6H, NCH_3), 3.54 (s, 2H, ArCH_2N), 3.77 (s, 3H, OCH_3) 6.70 (s, 1H, ArH), 6.75–6.78 (d, 1H, ArH), 7.11–7.13 (m, 1H, ArH), 7.19–7.23 (t, 2H, ArH), 7.48–7.50 (d, 2H, ArH), 7.78–7.80 (d, 1H, ArH); ^{13}C NMR ($CDCl_3$), δ (ppm): 44.3, 55.8, 64.6, 113.7, 114.5, 126.2, 129.1, 129.3, 130.4, 138.9, 140.1, 158.3; ^{77}Se NMR ($CDCl_3$), δ (ppm): 548 ppm; ESI-MS: *m*/*z*: calcd for $C_{16}H_{18}NOSSe$ $[M+H]^+$: 353.0353; found: 353.9982.

4.2.9. Synthesis of ***63***. Hydrogen peroxide (200 ml, 1.845 mmol, 30% aqueous solution) was added dropwise to a solution of diselenide **22** (50 mg, 0.092 mmol) in methanol/chloroform (4:1). The reaction mixture was stirred for 1 h at room temperature. After 1 h, the yellow color of the diselenide disappeared completely and the solution became colorless. The solvent was removed under reduced pressure to obtain a white solid. ^{1}H NMR (MeOH-d_4), δ (ppm): 3.46 (s, 6H, NCH_3), 3.81 (s, 3H, OCH_3), 5.25 (s, 2H, ArCH_2N), 7.22–7.25 (d, 1H, ArH), 7.27–7.28 (s, 1H, ArH), 8.05–8.07 (d, 1H, ArH); ^{13}C NMR (MeOH-d_4), δ (ppm): 55.7, 56.3, 68.1, 115.9, 121.8, 127.7, 130.5, 138.8, 163.5; ^{77}Se NMR (MeOH-d_4), δ (ppm): 1018; ESI-MS: *m*/*z* calcd for $C_{10}H_{14}NO_4Se$ $[M+Na]^+$: 316.0064; found: 315.9739.

4.3. GPx-like activity-HPLC assay

GPx-like activity was carried out by high-performance liquid chromatography (HPLC) with use of a 2695 separation module and a 2996 photodiode-array detector and a fraction collector. The assays were performed in 1.8 ml sample vials and a built-in autosampler was used for sample injection. In this assay, we used mixtures containing a 1:2 molar ratio of PhSH and peroxide in methanol at room temperature (22 °C) as our model system. Runs with and without catalyst were carried out under the same conditions. Periodically, aliquots were injected onto the reversed-phase column (Princeton C18 column, 4.6×150 mm, 5 μm) and eluted with methanol and water (85:15), and the concentrations of the diphenyl disulfide (PhSSPh) produced in the reaction were determined at 254 nm with the aid of pure PhSSPh as an external

Please cite this article in press as: Bhowmick, D.; Mugesh, G., Tetrahedron (2012), http://dx.doi.org/10.1016/j.tet.2012.09.020

standard. The amount of disulfide formed during the course of the reaction was calculated from the calibration plot for the standard (PhSSPh). The plots for kinetic parameters were obtained by use either of linear or of sigmoidal curve fitting.

4.4. GPx-like activity: GSH–GSSG coupled assay

The GPx-like activity was followed spectrophotometrically. The test mixture contained GSH (2.0 mM), EDTA (1 mM), glutathione disulfide reductase (1.7 units/ml), and NADPH (0.4 mM) in 0.1 M potassium phosphate buffer of pH 7.5. GPx samples (80 μM) were added to the test mixture at 23 °C temperature and the reaction was started by the addition of peroxide (1.6 mM). The initial reduction rates were calculated from the rate of NADPH oxidation at 340 nm in GSH assay. Each initial rate was measured at least three times and calculated from the first 5–10% of the reaction by using 6.22 mM^{-1} cm^{-1} as the molar extinction coefficient for NADPH. For the peroxidase activity, the rates were corrected for background reaction between peroxide and thiol.

4.5. Single-crystal X-ray structure determination

X-ray crystallographic studies were carried out using a Bruker CCD diffractometer with graphite-monochromatized Mo Kα radiation (λ=0.71073 Å) controlled by a Pentium-based PC running the SMART software package.[22] Single crystals were mounted at room temperature on the ends of glass fibers, and data were collected at room temperature. The structures were solved by direct methods and refined using the SHELXTL software package.[23] All non-hydrogen atoms were refined anisotropically and hydrogen atoms were assigned idealized locations. Empirical absorption corrections were applied to all structures using SADABS.[24] The structures were solved by direct method (SIR-92) and refined by full-matrix least-squares procedure on F^2 for all reflections (SHELXL-97).[25]

4.6. Computational methods

All calculations were performed by use of the Gaussian98 suite[26] of quantum chemical programs. The hybrid Becke 3-Lee–Yang–Parr (B3LYP) exchange correlation functional was applied for DFT calculations.[27] Geometries were fully optimized at the B3LYP level of theory with use of the 6-31+G(d) basis sets. Orbital interactions were analyzed by the natural bond orbital (NBO) method at the B3LYP/6-311+G(d,p) level, and charges were calculated by natural population analysis (NPA).[28]

4.7. Crystal data for 25

$C_{24}H_{32}N_2Se_2$; M_r=506.4; monoclinic; space group C 2/c; a=16.2254(9), b=11.3658(6), c=12.6999(8) Å; β=100.318(3), V=2304.2(2) $Å^3$; Z=4; ρ_{calcd}=1.46 g cm^{-3}; Mo Kα radiation (λ=0.71073 Å); T=293(2) K; $R1$=0.0282, $wR2$=0.0678 ($I>2\sigma(I)$); $R1$=0.0479, $wR2$=0.0616 (all data). CCDC-847710 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Acknowledgements

This study was supported by the Department of Science and Technology (DST), New Delhi, India. We are grateful to the Alexander von Humboldt Foundation, Bonn, Germany for the donation of an automated flash chromatography system. G.M. acknowledges the DST for the award of Swarnajayanti fellowship and D.B. thanks the CSIR for a research fellowship.

Supplementary data

This data includes NMR and mass spectra, plots, mechanistic study by NMR, coordinates for optimized structures. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2012.09.020.

References and notes

1. (a) Flohé, L.; Günzler, E. A.; Schock, H. H. *FEBS Lett.* **1973**, *32*, 132–134; (b) Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G.; Hoekstra, W. G. *Science* **1973**, *179*, 588–590; (c) Epp, O.; Ladenstein, R.; Wendel, A. *Eur. J. Biochem.* **1983**, *133*, 51–69; (d) *Selenium in Biology and Human Health*; Burk, R. F., Ed.; Springer: New York, NY, 1994; (e) Mugesh, G.; du Mont, W.-W. *Chem.—Eur. J.* **2001**, *7*, 1365–1370; (f) Jacob, C.; Giles, G. I.; Giles, N. M.; Sies, H. *Angew. Chem.* **2003**, *115*, 4890–4907; *Angew. Chem., Int. Ed.* **2003**, *42*, 4742–4758; (g) Roy, G.; Sarma, B. K.; Phadnis, P. P.; Mugesh, G. *J. Chem. Sci.* **2005**, *117*, 287–303.
2. (a) Sies, H. Oxidative Stress: Introductory Remarks In *Oxidative Stress*; Sies, H., Ed.; Academic: London, 1985; pp 1–8; (b) Sies, H. *Angew. Chem.* **1986**, *98*, 1061–1075; *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1058–1071.
3. (a) Mugesh, G.; Singh, H. B. *Chem. Soc. Rev.* **2000**, *29*, 347–357; (b) Mugesh, G.; du Mont, W.-W.; Sies, H. *Chem. Rev.* **2001**, *101*, 2125–2179.
4. (a) Müller, A.; Cadenas, E.; Graf, P.; Sies, H. *Biochem. Pharmacol.* **1984**, *33*, 3235–3240; (b) Wendel, A.; Fausel, M.; Safayhi, H.; Tiegs, G.; Otter, R. *Biochem. Pharmacol.* **1984**, *33*, 3241–3246.
5. (a) Reich, H. J.; Jasperse, C. P. *J. Am. Chem. Soc.* **1987**, *109*, 5549–5551; (b) Jacquemin, P. V.; Christiaens, L. E.; Renson, M. J.; Evers, M. J.; Dereu, N. *Tetrahedron Lett.* **1992**, *33*, 3863–3866.
6. (a) Sarma, B. K.; Mugesh, G. *J. Am. Chem. Soc.* **2005**, *127*, 11477–11485; (b) Bhabak, K. P.; Mugesh, G. *Chem.—Eur. J.* **2007**, *13*, 4594–4601; (c) Sarma, B. K.; Mugesh, G. *Org. Biomol. Chem.* **2008**, *6*, 965–974; (d) Sarma, B. K.; Mugesh, G. *Chem.—Eur. J.* **2008**, *14*, 10603–10614; (e) Bhabak, K. P.; Mugesh, G. *Chem.—Asian. J.* **2009**, *4*, 974–983.
7. Back, T. G.; Dyck, B. P. *J. Am. Chem. Soc.* **1997**, *119*, 2079–2083.
8. (a) Wilson, S. R.; Zucker, P. A.; Huang, R. R. C.; Spector, A. *J. Am. Chem. Soc.* **1989**, *111*, 5936–5939; (b) Iwaoka, M.; Tomoda, S. *J. Am. Chem. Soc.* **1994**, *116*, 2557–2561; (c) Mugesh, G.; Panda, A.; Singh, H. B.; Punekar, N. S.; Butcher, R. J. *Chem. Commun.* **1998**, 2227–2228; (d) Mugesh, G.; Panda, A.; Singh, H. B.; Punekar, N. S.; Butcher, R. J. *J. Am. Chem. Soc.* **2001**, *123*, 839–850; (e) Sarma, B. K.; Mugesh, G. *Inorg. Chem.* **2006**, *45*, 5307–5314; (f) Bhabak, K. P.; Mugesh, G. *Chem.—Eur. J.* **2008**, *14*, 8640–8651; (g) Bhabak, K. P.; Mugesh, G. *Inorg. Chem.* **2009**, *48*, 2449–2455.
9. (a) Wirth, T. *Molecules* **1998**, *3*, 164–166; (b) Jauslin, M. L.; Wirth, T.; Meier, T.; Schoumacher, F. *Hum. Mol. Genet.* **2002**, *11*, 3055–3063.
10. (a) Back, T. G.; Moussa, Z. *J. Am. Chem. Soc.* **2002**, *124*, 12104–12105; (b) Back, T. G.; Moussa, Z.; Parvez, M. *Angew. Chem.* **2004**, *116*, 1288–1290; *Angew. Chem., Int. Ed.* **2004**, *43*, 1268–1270.
11. Sun, Y.; Mu, Y.; Ma, S.; Gong, P.; Yan, G.; Liu, J.; Shen, J.; Luo, G. *Biochim. Biophys. Acta* **2005**, *1743*, 199–204.
12. (a) Kunwar, A.; Bansal, P.; Kumar, S. J.; Bag, P. P.; Paul, P.; Reddy, N. D.; Kumbhare, L. B.; Jain, V. K.; Chaubey, R. C.; Unnikrishnan, M. K.; Priyadarsini, K. I. *Free Radical Biol. Med.* **2010**, *48*, 399–410; (b) Kunwar, A.; Mishra, B.; Barik, A.; Kumbhare, L. B.; Pandey, R.; Jain, V. K.; Priyadarsini, K. I. *Chem. Res. Toxicol.* **2007**, *20*, 1482–1487.
13. Prabhu, C. P.; Phadnis, P. P.; Wadawale, A. P.; Priyadarsini, K. I.; Jain, V. K. *J. Organomet. Chem.* **2012**, *713*, 42–50.
14. Phadnis, P. P.; Mugesh, G. *Org. Biomol. Chem.* **2005**, *3*, 2476–2481.
15. Zade, S. S.; Singh, H. B.; Butcher, R. J. *Angew. Chem.* **2004**, *116*, 4613–4615; *Angew. Chem., Int. Ed.* **2004**, *43*, 4513–4515.
16. Sarma, B. K.; Manna, D.; Minoura, M.; Mugesh, G. *J. Am. Chem. Soc.* **2010**, *132*, 5364–5374.
17. Mugesh, G.; Singh, H. B. *Acc. Chem. Res.* **2002**, *35*, 226–236.
18. (a) Benkova, S.; Kóňa, J.; Gann, G.; Fabian, W. M. F. *Int. J. Quantum Chem.* **2002**, *90*, 555–565; (b) Bachrach, S.; Demoin, D. W.; Luk, M.; Miller, J. V., Jr. *J. Phys. Chem.* **2004**, *108*, 4040–4046 and references therein; (c) Bayse, C. A. *J. Phys. Chem. A* **2007**, *111*, 9070–9075.
19. (a) Wirth, T. *Angew. Chem.* **1995**, *107*, 1872–1873; *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1726–1728; (b) Wirth, T.; Fragale, G. *Chem.—Eur. J.* **1997**, *3*, 1894–1902; (c) Wirth, T. *Liebigs Ann.* **1997**, 2189–2196; (d) Fragale, G.; Neuburger, M.; Wirth, T. *Chem. Commun.* **1998**, 1867–1868; (e) Fragale, G.; Wirth, T. *Eur. J. Org. Chem.* **1998**, 1361–1369; (f) Uehlin, L.; Fragale, G.; Wirth, T. *Chem.—Eur. J.* **2002**, *8*, 1125–1133.
20. Press, D. J.; Mercier, E. A.; Kuzma, D.; Back, T. G. *J. Org. Chem.* **2008**, *73*, 4252–4255.
21. Heverly-Coulson, G. S.; Boyd, R. J. *J. Phys. Chem. A* **2010**, *114*, 10706–10711.
22. SMART, Version 5.05; Bruker AXS: Madison, WI, 1998.
23. (a) Sheldric, G. M. *Acta Crystallogr., Sect. A* **1990**, *46*, 467–473; (b) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Gualardi, A. *J. Appl. Crystallogr.* **1993**, *26*, 343–350.
24. Sheldrick, G. M. *SADABS, a Program for Adsorption Correction with the Siemens SMART Area Detaction System*; University of Göttingen: Göttingen, (Germany), 1996.
25. Sheldrick, G. M. *SHELX-97, Program for Refinement of Crystal Structures*; University of Göttingen: Göttingen, (Germany), 1997.

26. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. J.; Pople, A. J. *Gaussian 98*; Gaussian: Pittsburgh, PA, 1998.
27. (a) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B: Condens. Matter* **1988**, *37*, 785–789; (b) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.
28. (a) Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88*, 899–926; (b) Glendening, E. D.; Reed, J. E.; Carpenter, J. E.; Weinhold, F. Natural Bond Orbital (NBO), Version 3.1.

Please cite this article in press as: Bhowmick, D.; Mugesh, G., Tetrahedron (2012), http://dx.doi.org/10.1016/j.tet.2012.09.020