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An Ebselen like Catalyst with Enhanced GPx Activity via a Selenol Intermediate

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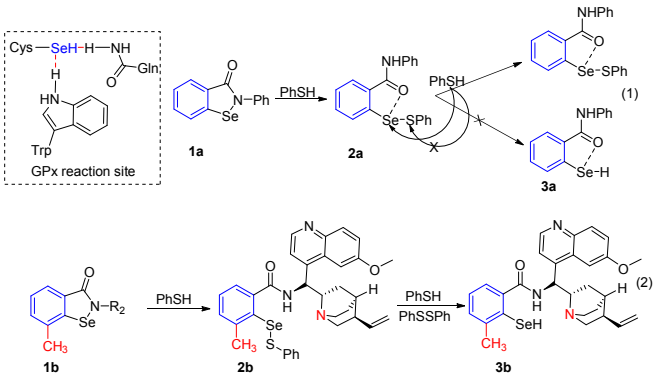
The reaction of KSeO^{t}Bu with 2-iodo-arylbenzamides gave benzamide ring-substituted, quinine-derived isoselenazolones 1b-1d. The reaction of PhSH with *ortho*-methyl-substituted isoselenazolone 1b gave selenol 3b, which is oxidized by H_2O_2 to regenerate 1b. Isoselenazolone 1b shows a high rate (0.33 x 10^3 μM.min^{-1}) of oxidation of PhSH with H_2O_2, which is ~10^3-fold more active than ebselen (1a) and ≥30-fold more active than the other isoselenazolones of this study. Compound 1b shows less inhibition of the growth of yeast cells than 1a.

The selenoenzyme glutathione peroxidase (GPx) is present in the human body and functions as a catalytic antioxidant for the reduction of various hydroperoxides using glutathione as the stoichiometric co-reductant. The reactive site of GPx contains selenocysteine (CySeH), which is responsible for the high catalytic activity for the reduction of hydroperoxides (Scheme 1).[1] The GPx mimic ebselen (**1a**) or 2-phenyl-1,2-benzisoselenazol-3(2H)-one (PZ 51) is a biologically non-toxic (LD_{50} = 6.81 g/kg), well-studied organoselenium compound with anti-inflammatory and antioxidant therapeutic properties as well as the potential to treat indications such as bipolar disorder.[2,3] The diverse remedial properties of **1a** are apparently due to its reduction of hydroperoxides together with its low toxicity. Unfortunately, **1a** is a relatively inefficient catalyst for the reduction of hydroperoxides, which encourages attempts to synthesize effective GPx-mimics with improved catalytic activity for the reduction of peroxides while maintaining low toxicity. The poor catalytic reactivity of **1a** is presumably due to the lack of reactivity of selenenylsulfide **2a** to produce selenol **3a** when it reacts with an additional molecule of thiol (eq. 1 Scheme 1).[4] The generation of a selenol by the reaction of an organothiol with an organoselenium compound is a challenging task in general and from isoselenazolones in particular.[5-7] Several *N*-substituted isoselenazolones have been described; however, none appear to form a stable selenol upon their reaction with organothiols.[8-13] Therefore, these isoselenazolones are limited in their catalytic activity for the reduction of H_2O_2 via an ebselen-like pathway.

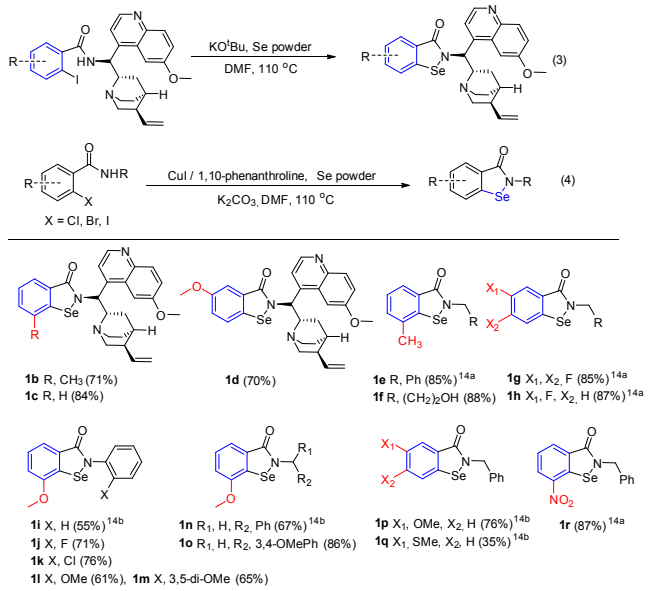


Scheme 1 GPx Reaction Site and Generation of Selenol

Isoselenazolones with additional benzamide ring functionality have not been studied as GPx mimics presumably due to difficulties in their synthesis. Here, in continuation of our work on organochalcogen chemistry,[14] we describe the synthesis of isoselenazolones containing a quinine moiety and the facile generation of selenol **3b** by the reaction of isoselenazolone **1b** with PhSH (eq. 2). The catalytic activity of various isoselenazolones as GPx mimics was also studied with **1b** showing much greater catalytic activity than ebselen (**1a**).

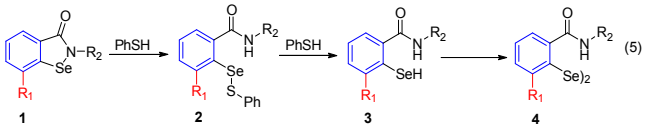
N-Quininamine-substituted [*N*-(1*S*)-(6-methoxyquinolin-4-yl)((2*S*,4*S*,5*R*)-5-vinyl-quinuclidin-2-yl)methyl] isoselenazolones **1b-1d** were prepared using KSeO^{t}Bu as the source of selenium[15] and in higher yields (71% vs 55% for **1b** , 84% vs 72% for **1c** and 70% vs 60% for **1d**) in comparison to copper-catalyzed Se-N bond formation reactions (eq 3, Scheme 2). The use of KSeO^{t}Bu as a selenium source is more efficient for the synthesis of isoselenazolones bearing polar functionality. In the copper catalysed methodology, separation of the copper-1,10-phenanthroline catalyst from the polar quininamine-derived

isoselenazolones **1b-1d** was difficult. Using KSeO^{t}Bu as the source of Se, quininamine-derived isoselenazolones **1b-1d** were obtained in higher yield with fewer side products. However, the use of KSeO^{t}Bu was only applicable to 2-iodo- and selected 2-bromobenzamides. KSeO^{t}Bu was unreactive with 2-chlorobenzamides as substrates. The isoselenazolones ebselen (**1a**) and **1e-1r** were prepared by the Cu-catalysed Se-N bond forming reaction (eq 4, Scheme 2).[14a,b] The isoselenazolone structure was confirmed by ^{77}Se NMR (Table 1) and X-ray structural studies of **1b**, **1c** and **1e**.



Scheme 2 Synthesis of Isoselenazolones

Table 1 Reaction of Isoselenazolones with PhSH[a]



Entry	**1**(δ)	[PhSH]	**2** (δ)	**3** (δ)	**4** (δ)
1	**1a** (912)	1	**2a** (591)	**3a** (-)	**4b** (-)
1	**1b** (851)	1	**2b** (-)	**3b**(-0.2)	**4b** (-)
2	**1b** (851)	2	**2b** (-)	**3b** (4.8)	**4b** (-)
3, 4	**1c** (858)	2	**2c** (589)	**3c** (-)	**4c** (-)
5	**1e** (859)	1	**2e** (496)	**3e** (-)	**4e** (416)
6	**1e** (859)	2	**2e** (496)	**3e** (-)	**4e** (416)
7	**1n** (889)	1	**2n** (454)	**3n** (-)	**4n** (405)
8	**1n** (889)	2	**2n** (-)	**3n** (-)	**4n** (401)

[a] Reaction was carried out in CD_3OD using 1 equiv of PhSH with 1. (-) Not observed.

The reaction between PhSH and isoselenazolones was monitored by ^{77}Se NMR spectroscopy and mass spectrometry.[16] An equimolar mixture of isoselenazolone **1b** and PhSH in CD_3OD gave a small peak at -0.2 ppm due to selenol **3b** (Table 1). Surprisingly, formation of the expected selenenylsulfide **2b** was not observed by ^{77}Se NMR. Addition of a second equiv of PhSH to the stoichiometric reaction mixture of **1b** and PhSH gave a sharp peak at 4.8 ppm. A freshly prepared solution of **1b** and PhSH was monitored by mass spectrometry and showed formation of selenenylsulfide **2b** (*m/z* 629.1615+H$^+$) which suggests that the reaction proceeds *via* the formation of selenenylsulfide **2b**. However, this is a transient intermediate in the formation of selenol **3b**. Selenol **3b** is stable for at least two weeks in solution as ^{77}Se NMR shows a constant signal at -0.9 ppm. Signals due to the formation of diselenide **4b** or isoselenazolone **1b** were not observed.

The ^{77}Se NMR chemical shift of **3b** is similar to reported ^{77}Se NMR chemical shifts of *N,N*-dimethylbenzylamine selenol (9.9 ppm),[6b] the aryl-selenol BmtSeH (6 ppm),[7a] and a camphor-derived selenol (-49 ppm).[9a] However, the ^{77}Se NMR chemical shift of **3b** is upfield relative to PhSeH (145 ppm).

Several other isoselenazolones (**1a-1c**, **1e**, and **1n**) were reacted with PhSH and results are summarized in Table 1. Isoselenazolone **1c** having a quininamine moiety and lacking the *ortho*-CH_3 substituent forms only selenenylsulfide **2c** in the presence of 1-3 equivalents of PhSH (entries 3-4, Table 1). However, *ortho* CH_3- substituted isoselenazolones **1e** gave selenenylsulfides **2e** and diselenide **4e** when reacted with one and two equivalents of PhSH. Similarly, **1n** gave **2n** and **4n** with one equivalent of PhSH (entry 7, Table 1). In contrast, addition of a second equivalent of PhSH led to complete conversion of **2n** into diselenide **4n** (entry 8, Table 1).

The formation of diselenides **4e** and **4n** could occur *via* either of two processes: oxidation of the respective selenols **3e** and **3n** or disproportionation of selenenylsulfides **2e** and **2n**.[17] Mixtures of isoselenazolones **1b**, **1e**, or **1n** and PhSH (1:2 molar ratios) were reacted with CH_3I. Indeed, complete conversion of *in-situ* generated selenol **3b** into the corresponding methylselenide was observed. In contrast, isoselenazolones **1e** and **1n** failed to produce the corresponding methylselenides under similar conditions. This implies that the formation of diselenides **4e** and **4n** occurred by the disproportionation of selenenylsulfides **2e** and **2n** rather than *via* the formation of the corresponding selenols.

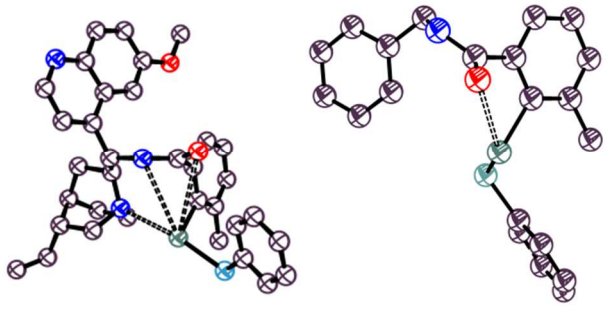
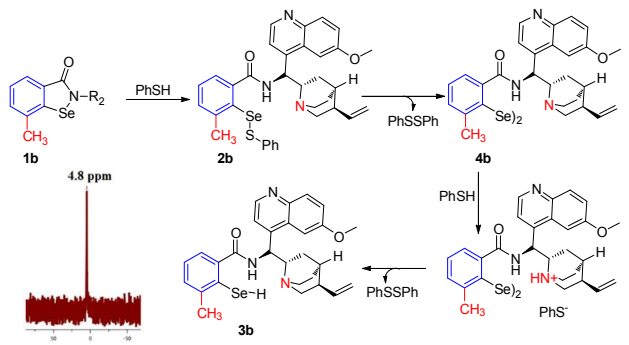


Figure 1 Optimized Structures of **2b** and **2e**. Se…O 3.811 Å (C=O), Se…N 2.825 Å (tert-N quininamine), Se…N (NH) in **2b**. Se…O 2.42 Å (C=O) in 2e. Se…O 4.083 Å (C=O), Se…N 2.79 Å (tert-N), Se…N (NH) in **2c**.



Scheme 3 Proposed Generation of Selenol **3b** from **1b**

DFT calculations on the related selenenylsulfides **2b**, **2c**, **2e**, **2f** and **2n** were performed to study the nature of Se…O/N interactions (Figure 1) as these interactions appear to be important, not only in the stabilization of the selenol functionality (Scheme 1), but also in its formation from the corresponding selenenylsulfide. Short Se…O/N interactions increase electron density on selenium, which should then favor the nucleophilic attack of PhS^- at sulfur rather than at selenium in the selenenylsulfide intermediate leading to the selenol and disulfide.[6a,c,9a,18] However, other factors must also be considered. In selenenylsulfide **2c**, intramolecular Se…O and Se…N distances are favorable for the generation of selenol **3c**,[19] but neither **3c** or diselenide **4c** is observed.

We next examined the antioxidant properties of isoselenazolones **1a-1r** for the reduction of H_2O_2 in the presence of PhSH as co-reductant in CH_3OH according to eq 6. From the data in Table 2 and Figure 2, isoselenazolone **1b**, which forms selenol **3b**, shows a much higher initial rate of oxidation of PhSH (v_o = 331 μM.min^{-1}) when compared to the remainder of the isoselenazolones of this study.[20] Isoselenazolone **1b** is 10^3-fold more active than ebselen **1a** and 30-fold more active than quininamine-based isoselenazolones **1c** and **1e**. Also, **1b** is 475 times more active than the diphenyl diselenide. We have also evaluated the influence of an external base triethyl amine (0.05 mM) on the GPx activity of **1b** and **1c**. However, the external base gave no significant change in the catalytic activity of **1b** and **1c** (entries 5 and 7, Table 2). In other systems, the introduction of a methoxy group in an aromatic ring enhances the antioxidant property of catalysts.[21] The methoxy-substituted isoselenazolones **1d** and **1i-1p**. However, these catalysts showed ≤5% of the antioxidant activity of **1b**.

Isoselenazolones **1a**, **1c-1d**, **1g**, **1h**, **1p** and **1q**, which form only selenenylsulfides with PhSH via ^{77}Se NMR studies, are poor catalysts for this reaction based on v_o, whereas isoselenazolones **1e**, **1f**, **1i-1o** which form diselenides with PhSH are intermediate in catalytic activity relative to **1b**.

To further understand the mechanism of Gpx activity of the isoselenazolone **1b,** the effects of catalyst and H_2O_2 concentration on v_o were examined. Initial values of v_o increased linearly with respect to catalyst concentration indicating a first-order dependence of GPx activity on catalyst concentration. As the concentration of H_2O_2 was increased, values of v_o increased, but became constant with no further increase in v_o with increasing H_2O_2 concentration. The effect of changing peroxide concentration is consistent with the formation of an oxidized intermediate whose subsequent reduction limits the rate of turnover in the system.

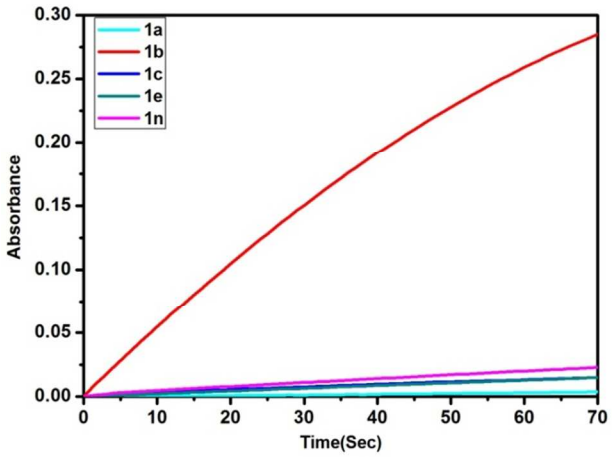


Figure 2 Initial Rates of PhSSPh Formation in the Presence of Catalysts **1a**, **1b**, **1c**, **1e** and **1n**

Table 2 GPx Like Activity of Isoselenazolones

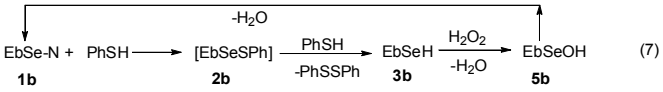
$$2\ PhSH + H_2O_2 \xrightarrow[CH_3OH]{\text{Catalyst (0.01 mM)}} 2\ H_2O + Ph{-}S{-}S{-}Ph \qquad (6)$$

Entry	Cat.	v_o μM.min^{-1}	Entry	Cat.	v_o μM.min^{-1}
1	**$(PhSe)_2$**	0.7±0.1	12	**1h**	13±1
2	**1a**	0.4±0.02	13	**1i**	17±2
3	**1b**	331±2 [b]	14	**1j**	13±1
4	**Et_3N**[c]	1.6±0.1	15	**1k**	17±1
5	**1b+Et_3N**[c]	332±2 [b]	16	**1l**	13±1
6	**1c**	11±1	17	**1m**	14±2
7	**1c+Et_3N**[c]	12.2±0.7	18	**1n**	11.7±0.3
8	**1d**	4.0±0.2	19	**1o**	15±1
9	**1e**	11±2	20	**1p**	1.2±0.3
10	**1f**	21±1	21	**1q**	0.20±0.02
11	**1g**	0.40±0.03	22	**1r**	10±3

The initial rates (v_o) for the oxidation of PhSH (1 mM) with H_2O_2(3.75mM) in the presence of catalyst (0.01 mM) were determined in CH_3OH by monitoring the UV absorption at 305 nm due to the formation of phenyl disulfide.[b] v_o obtained by Lineweaver Burk plot. [c] concentration of Et_3N was 0.05mM

To establish the catalytic cycle, selenol **3b** was treated with one equivalent of H_2O_2 in CD_3OD and the resulting mixture was examined by ^{77}Se NMR and mass spectrometry. A signal was observed at 1093 ppm in the ^{77}Se NMR and is attributed to selenenic acid (R-SeOH) **5b** (based on the observed *m/z* 536.1489) and a second signal at 851 ppm corresponding to

isoselenazolone **1b**. The addition of a second equivalent of PhSH to selenenic acid **5b** gave formation of **1b** and **2b**. The catalytic cycle for **3b** reacting with PhSH and H_2O_2 is summarized in Scheme 4.



Scheme 4 Catalytic cycle for **1b**

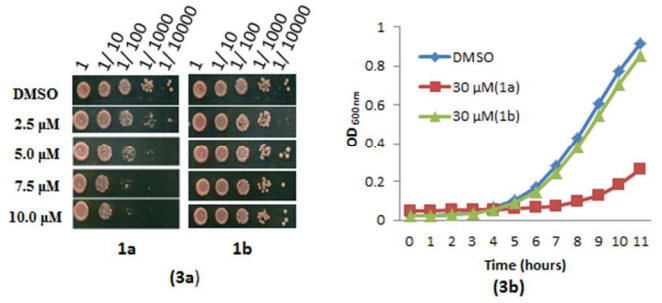


Figure 3 Yeast cells growth in the presence of catalysts

Ebselen (**1a**) is the most studied GPx-mimic in organoselenium chemistry and has demonstrated low toxicity in biological studies. We compared the effect of **1a** and **1b** on the growth of yeast cells to compare relative toxicities.[22] The dose-dependent effect of various concentrations of isoselenazolones on the growth of yeast cells was over a 72 h time period (for more details, please see SI, S174-S175).[23] Figure 3a compares ebselen (**1a**) with isoselenazolone **1b** and shows a higher growth of cells in the presence of **1b** compared to **1a**.

These results were validated by growth curve analysis in liquid culture as shown in Figure 3b, (please see SI, S175-S179 for experimental data).[24] Yeast cells were treated with an increasing dose of either **1a** or **1b** (10, 20 and 30µM) and growth was monitored at OD_{600nm} for 11 h at regular interval. It is apparent that the OD_{600nm} value for **1b** (0.85±0.01) which is significantly higher than **1a** (0.26±0.01). From growth curve analysis, doubling time for the growth of yeast cells was also calculated in presence of **1a** and **1b**. It was significantly higher for **1a** (278±12 min) compared to **1b** (124±3 min) further suggesting that **1b** inhibits cell growth to a lesser extent than ebselen (**1a**).

Conclusions

In summary, the reaction of isoselenazolones with additional substituents on the benzamide ring with PhSH has been investigated. Isoselenazolone **1b** bearing an *ortho*-methyl group on the benzamide ring and an *N*-quininamine group gave selenol **3b** in the presence of PhSH. The remainder of the isoselenazolones of this study formed either selenenylsulfides or diselenides upon reaction with PhSH. We have also shown that short intramolecular Se…N/O interactions are not sufficient for the generation of selenol from selenenylsulfide. The high GPx-like activity of isoselenazolone **1b**, which forms selenol intermediate **3b**, suggests that the presence of the bulky *N*-quininamine substituent and the *ortho*-CH_3 benzamide substituent stabilizes **3b**, which regenerates **1b** following reaction with H_2O_2. In a comparison of ebselen (**1a**) and **1b**, growth of yeast cells in the presence of **1b** was comparable to the DMSO control and was significantly higher than in the presence of **1a**. Currently, we are investigating catalytic role of selenol for various biological activities involving thioredoxin reductase and deiodinase enzymes in which selenol functionality is critical for activity.

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Notes and references

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Electronic Supplementary Information (ESI) available: [Characterization data, NMR and mass spectra on **1a-1r** and CIF files for **1b**, **1c**, and **1e** (CCDC no. 930741, 930742 and 953729), DFT calculations and geometry optimization on **2b**, **2c** and **2e**, GPx-activities, kinetic studies, toxicity tests.]. See DOI: 10.1039/c000000x/

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15. Potassium tert-butoxide base reacted smoothly with selenium powder and produce brown-greenish mixture of potassium *tert*-butoxyselenolate (KSeOtBu) which was characterized by ^{1}H, ^{13}C and ^{77}Se NMR (please see Supporting Information pages S180-S183).
16. The ^{77}Se NMR is very important technique for the characterization of organoselenol in solution. Mass spectrometry provides distinct isotopic pattern due to presence of six selenium isotopes.
17. ^{77}Se NMR experiments were conducted for 10 h which is substantial time for the oxidaton of selenol into diselenide. To preclude this, immediate capture of selenol by electrophile was carried out.
18. Optimized geometries and DFT calculations of **1b**, **1c**, **1e**, **1f**, **1n**, and **2b**, **2c**, **2e**, **2f**, **2n** correlate well with experimentally obtained ^{77}Se NMR chemical shifts, Se…X distances (please see SI S148-S160).
19. Intramolecular Se…N/O (if heteroatom is in conjugation with selenium) interaction decreases electron density around selenium as the case with Se…O (C=O), heteroatom is not in conjugation with Se, interaction (Se…N (quinine N) enhances overall electron density around selenium. See references: (a) M. Iwaoka, S. Tomoda, *J. Am. Chem. Soc.,* 1996, **118**, 8077; (b) M. Iwaoka, H. Komatsu, T. Katsuda, S. Tomoda, *J. Am. Chem. Soc.,* 2004, **126**, 5309; (c) A. J. Mukherjee, S. S. Zade, H. B. Singh, R. B. Sunoj, *Chem. Rev.,* 2010, **110**, 4357.
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