
Peer reviewed version

Link to published version (if available): 10.1021/acs.joc.6b02418

Link to publication record in Explore Bristol Research
PDF-document

This is the accepted author manuscript (AAM). The final published version (version of record) is available online via American Chemical Society at http://doi.org/10.1021/acs.joc.6b02418. Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research
General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available: http://www bristol ac uk/pure/about/ebr-terms
Nitro-, Azo- and Amino Derivatives of Ebselen: Synthesis, Structure and 
Cytoprotective Effects

Vijay P. Singh,*† Jia-fei Poon,† Jiajie Yan,† Xi Lu,† Marjam Karlsson Ott,‡ Ray J. Butcher,§ Paul J. Gates§ and Lars Engman*†

†Department of Chemistry – BMC, Uppsala University, Box 576, SE-751 23 Uppsala, Sweden
‡Division of Applied Materials Science, Department of Engineering Sciences, Uppsala University, Sweden
§Department of Chemistry, Howard University, Washington D.C. 20059, United States
¶University of Bristol, School of Chemistry, Bristol, BS8 1TS, United Kingdom

Abstract: Novel azo-bis-ebson and 1,2-benziselenazol-3(2H)-ones 3 and 6 with sodium benzenetellurolate, NaTeC₆H₅, and by reaction of 2-bromo-3-nitrobenzamides with Na₂Se₂. The X-ray structure of 7b showed that the molecule, due to strong intramolecular secondary Se···N interactions, is completely planar. Azo-compounds 7 upon further reaction with NaTeC₆H₅ were reductively cleaved to provide two equivalents of the corresponding aromatic amine. The weak Se—N bond was not stable enough to survive the reaction conditions and diselenides 8 were isolated after work-up. Whereas azo-bis-ebson 7 were poor mimics of the glutathione peroxidase (GPx)-enzymes, nitroebson 3, 6 and 11b and diselenides 8 were 3-6 fold more active than ebson. Based on ⁷⁷Se NMR spectroscopy, a catalytic cycle for diselenide 8b, involving aminoebson 14, was proposed. As assessed by chemiluminescence measurements, the good GPx-mimics could reduce production of reactive oxygen species (ROS) in stimulated human mononuclear cells more efficiently than Trolox. No toxic effects of the compounds were seen in MC3T3-cells at 25 μM.

Introduction

Selenocysteine (Sec, 1) is recognized as the 21st proteinogenic amino acid. It is an essential part of the active site of selenoproteins in humans and animals. So far, 23 selenoprotein families have been annotated.¹ The human selenoproteome consists of 25 selenoproteins.² Whereas some of these have a known structure and function (GPx, iodothyronine deiodinases, thioredoxin reductases),³ others are as yet insufficiently characterized. The substitution of selenium for sulfur in cysteine (Cys) has many
implications. At physiological pH, the more acidic selenol ($\text{pK}_a = 5.43$) is essentially deprotonated. Sec is therefore a more reactive nucleophile than Cys. Also, redox-cycling (oxidation/reduction) occurs more readily in Sec than in Cys. In addition to catalase, the GPx-enzymes are the most important hydroperoxide-decomposing enzymes in humans. Glutathione (GSH) is used as the stoichiometric reducing agent (eq 1). In the proposed catalytic cycle for the action of the GPx-enzymes selenium is present in the form of selenol, selenenic acid and selenosulfide.

\[
\text{HOOH/ROOH} + 2 \text{GSH} \xrightarrow{\text{GPx-enzymes}} \text{H}_2\text{O/ROH} + \text{GSSG} + \text{H}_2\text{O} \quad (1)
\]

The reports on the existence of GPx triggered a search for small-molecule compounds that could mimic the action of the large enzyme. The benzoselenazolone ebselen (2) was the first compound of this kind. Since the mid 1980s, the suitability of ebselen as a pharmaceutical agent has been extensively probed. Due to its GPx-activity, it has been found to reduce oxidative stress. It is/has been used in clinical trials for the prevention or treatment of cardiovascular diseases, arthritis, stroke and cancer. Considered as a safe drug-like compound with a history of use in human clinical trials, ebselen has also been included in the National Institutes of Health Clinical Collection. Ebselen has been subjected to numerous structural modifications in order to improve its GPx-activity. An early study by Parnham and co-workers showed that the nitro-derivative 3 of ebselen was 9-fold better than the parent as a catalyst for the glutathione-induced reduction of t-BuOOH. However, this finding has not been much explored and the reason for the rate enhancement has been attributed both to electronic and steric effects.

We thought it would be interesting to prepare benzoselenazolones carrying a variety of N-substituents (nitro-, azo- and amino groups) in position 7. In the following we describe their synthesis and structure as well as their GPx-like activities and cytoprotective effects.

**Results and Discussion**

**Synthesis:** We envisaged obtaining azo- and amino derivatives of ebselen by reduction of the corresponding nitro compounds. Nitro-substituted ebselens were prepared using a slightly modified version of the procedure developed by Christians and co-workers. The required benzamides 4 a-c (eq 2) for this reaction were obtained in high yields by addition of aniline, para-toluidine and para-anisidine, respectively, to 2-bromo-3-nitrobensoyl chloride. Their conversion to
benzisoselenazolones 3/6b-c involved aromatic nucleophilic substitution with "BuSeNa, generated in situ from Bu₂Se₂ and NaBH₄ in ethanol, followed by bromine induced cyclization.

Reduction of the nitro group in the presence of the weak Se-N bond turned out to be difficult. Sodium benzenetellurolate, NaTePh, generated in ethanol by sodium borohydride reduction of diphenyl ditelluride (Ph₂Te₂), was found to cleanly reduce compound 3 to azo-bis-ebselen derivative 7a in 51% yield. However, in the case of 6b, the corresponding azo-derivative 7b was isolated as the minor product (9%) along with diselenide 8b (16%). Obviously, in 8b, the nitro group has been reduced all the way to an amine and the Se-N bond cleaved reductively. Oxidation of the resulting selenol then provided the diselenide product. Reduction of azo-compound 7b with NaTePh produced diselenide 8b as the only product (87%).

An alternative method for the preparation of azo-derivatives of ebselen was also tried. This is based on the finding that 2-bromo-3-nitrobenzylic alcohols, when reacted with disodium diselenide (Na₂Se₂) produced azo-derivatives 9 of 2-oxaselenaindane. Reaction of in situ-prepared Na₂Se₂ with compounds 4a-d at ambient temperature produced the corresponding azo-bis-ebselens 7a (29%), 7b (39%), 7c (21% when heated at reflux) and 7d (68%) (eq 3). Due to poor solubility, we were unable to characterize the methoxy derivative 7c.
Further reaction of compound 7d with NaTePh afforded diselenide 8d in 52% yield. Under the similar reaction conditions, benzamides 5a-c, carrying a butylseleno group in position 2, were cleanly reduced to the corresponding amines 10a-c in high yields. We recently reported on the cytoprotective effects of the radical-trapping and hydroperoxide-decomposing ebselenol 11a. Curious about the effects of an ortho-coordinating nitro group, compound 6c was O-demethylated with 3 equivalents of BBr3 in CH2Cl2 to afford the nitroebselenol 11b in 88% yield.

Structure: The structures of azo-bis-ebselen derivative 7b and diselenide 8b were determined by X-ray crystallography. Dark black crystals of 7b suitable for X-ray crystallographic analysis were obtained by slow evaporation of a CHCl3-solution at -20 ºC. The structure (Figure 1) shows strong intramolecular secondary Se···N interactions [Se1···N1 2.442(5) Å] notably shorter than the sum of the van der Waals radii of Se and N (3.45 Å). Normally, the bond length of a Se─N covalent bond is 1.87 Å. The strong secondary Se···N interactions causes a slight elongation of this bond (Se1─N2 = 1.940(3) Å). The coordination geometry is strongly distorted from a linear arrangement with the bond angle N2─Se1─N1 = 156.56(13)º, thus, indicating a T-shaped geometry around the Se atom. Because of these strong interactions, compound 7b adopts a completely planar conformation. The crystal structure of 7b showed π-π stacking interactions between the benzene ring with a centroid-centroid distance of 3.711 Å (Figure S3 in the Supporting Information).
Suitable orange crystals of diselenide 8b were obtained for X-ray crystallographic analysis by slow evaporation of an ethyl acetate/pentane solution at room temperature. The structure (Figure 2) indicates a V-shaped geometry around the selenium atom with bond angles C1a─Se1─Se2 and C1b─Se2─Se1 of 104.26(5)° and 103.65(5)°, respectively. The C1a─Se1─Se2─C1b dihedral angle of 98.47(7)° indicates a “transoid” conformation. As indicated in Figure 2, selenium is hydrogen bonded to the –NH₂ group in the ortho position. The Se─H distance of 2.734 Å is significantly shorter than the sum of the van der Waals radii of selenium and hydrogen (3.10 Å).²²
**Figure 2.** ORTEP diagram of 8b. Hydrogen atoms are omitted for clarity. Thermal ellipsoids are set at 50% probability. Significant bond lengths [Å] Se1—C1a 1.9171(15); Se1—Se2 2.3602(2); Se2—C1b 1.9156(15), and angles [°] C1a—Se1—Se2 104.26(5); C1b—Se2—Se1 103.65(5); C1a—Se1—Se2—C1b 98.47(7).

**Bonding:** To find out more about the effect of intramolecular secondary Se···N/O interactions on bonding and $^{77}$Se NMR chemical shifts, density functional theory (DFT) calculations were carried out. The geometries of 6b, 6c, 7a, 7b, 7d, and 11b were fully optimized in the gas phase at the B3LYP/6-311+G(d) level of theory (for optimized geometries and coordinates see the Supporting Information). The optimized geometry of 7b showed good agreement with the X-ray crystal structure. The structures of 7a and 7d were also found to be completely planar with strong Se···N interactions. The second perturbation energies $E_{\text{Se···N}}$ and $E_{\text{Se···O}}$ shown in Table 1 were obtained by NBO analysis. The values reflect the effectiveness of orbital interactions between the low-lying $\sigma^*_{\text{Se—N}}$ and nN/nO. The magnitude of the nN→$\sigma^*_{\text{Se—N}}$ and nO→$\sigma^*_{\text{Se—N}}$ orbital interactions suggests a significant covalent interaction between the nitrogen lone pair and the low-lying antibonding orbital of the Se atom. The NBO results indicate that the Se···N interactions were much stronger than the Se···O interactions. The calculated $^{77}$Se NMR chemical shifts were in good agreement with the experimental values.
Table 1. Data for 3, 6b-c, 7a-b, 7d, and 11b obtained by DFT calculations at the B3LYP/6-311+G(d,p) level in the gas phase. The NBO analysis was calculated at the B3LYP/6-311+G(d,p) level using the B3LYP/6-311+G(d)-level-optimized geometries.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>( r_{\text{Se} \cdots \text{O/N}} ) [Å]</th>
<th>( E_{\text{Se} \cdots \text{O/N}} ) (kcal/mol)</th>
<th>( r_{\text{Se} \cdots \text{N}} ) [Å]</th>
<th>( q_{\text{Se}} )</th>
<th>(^{77}\text{Se} ) NMR (in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3(^{17,23})</td>
<td>2.593 (2.573)</td>
<td>12.63</td>
<td>1.924 (1.896)</td>
<td>+0.753</td>
<td>910 (924)</td>
</tr>
<tr>
<td>6b</td>
<td>2.598</td>
<td>12.35</td>
<td>1.922</td>
<td>+0.753</td>
<td>905 (929)</td>
</tr>
<tr>
<td>6c</td>
<td>2.604</td>
<td>12.00</td>
<td>1.921</td>
<td>+0.750</td>
<td>910 (932)</td>
</tr>
<tr>
<td>7a</td>
<td>2.532</td>
<td>17.32</td>
<td>1.946</td>
<td>+0.734</td>
<td>904 (931)</td>
</tr>
<tr>
<td>7b</td>
<td>2.537 (2.442)</td>
<td>16.89</td>
<td>1.944 (1.941)</td>
<td>+0.733</td>
<td>903 (927)</td>
</tr>
<tr>
<td>7d</td>
<td>2.539</td>
<td>16.57</td>
<td>1.943</td>
<td>+0.732</td>
<td>925 (946)</td>
</tr>
<tr>
<td>11b</td>
<td>2.601</td>
<td>12.22</td>
<td>1.922</td>
<td>+0.751</td>
<td>912 (923)</td>
</tr>
</tbody>
</table>

[a] The \(^{77}\text{Se} \) NMR values are referenced to Me\textsubscript{2}Se (δ = 0). The experimental values are given in parentheses.

The topology of the electron density at the Se···N/O bond critical point (BCP) was evaluated according to Bader’s theory of atoms in molecules (AIM) using the AIM2000 software package (for molecular graphs, see Figure S6 in the Supporting Information). Parameters were calculated in order to support the NBO calculations in the form of electron density (\( \rho_{\text{Se} \cdots \text{N/O}} \)), Laplacian (\( \nabla^2 \rho_{\text{Se} \cdots \text{N/O}} \)) and the total electron energy density (\( H_{\text{Se} \cdots \text{N/O}} \)) for the Se···N/O interactions (Tables S19 and S20 in the Supporting Information). Whereas compounds 7a-b and 7d showed negative values for \( H_{\text{Se} \cdots \text{N}}, H_{\text{Se} \cdots \text{O}} \) for compounds 6b-c and 11b were positive, suggesting weaker electrostatic interactions between Se and O atoms.

Glutathione Peroxidase-like Activity: The GPx-like activity of antioxidants 2-3, 6b-c, 7a-b, 7d, 8b, 8d and 11b was assessed by the coupled reductase assay, using H\textsubscript{2}O\textsubscript{2} as a substrate and GSH as a thiol cofactor in the presence of glutathione reductase (GR). GR serves to reduce oxidized glutathione (GSSG) formed by the action of the GPx-catalyst on H\textsubscript{2}O\textsubscript{2}/GSH, using β-nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. GPx-activities were assessed as the initial rates (\( v_0 \)) for the consumption of NADPH by UV-spectroscopy at 340 nm during the initial 10 seconds of reaction. The values shown in Table 2 were corrected for the spontaneous oxidation of GSH by H\textsubscript{2}O\textsubscript{2} (24.3 ± 1.2 μM-min\(^{-1}\)). Ebselen (46.5 ± 0.8 μM-min\(^{-1}\)) and 3 (111.4 ± 4.6 μM-min\(^{-1}\)) were included as bench-marks and references in the study. Nitroebelsons 6b, 6c and 11b were found
to be nearly three times more active than ebselen (2), and slightly better than 3. This enhanced activity of the nitro compounds can probably be ascribed to intramolecular secondary Se···O interactions. Such or similar effects are well described in the literature. 14,17,20b,24 Azoebselens 7a, 7b and 7d turned out to be considerably poorer GPx-mimics than 2 and 3. Interestingly, diselenide 8b showed an activity (271.6 ± 5.7 µM-min⁻¹) almost six-fold larger than recorded for ebselen. The other diselenide 8d, carrying a bulky naphthyl group, also showed good GPx-activity (133.8 ± 2.7 µM-min⁻¹).

Table 2. GPx-like activities of ebselen, 3, 6b-c, 7a-b, 7d, 8b, 8d and 11b as determined by the initial rate of NADPH consumption (v₀) in the presence of H₂O₂, GSH and glutathione reductase

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>GPx-like activity, v₀ (µM·min⁻¹)</th>
<th>Activity relative to ebselen</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, ebselen</td>
<td>46.5 ± 0.8</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>111.4 ± 4.6</td>
<td>2.4</td>
</tr>
<tr>
<td>6b</td>
<td>135.2 ± 4.1</td>
<td>2.9</td>
</tr>
<tr>
<td>6c</td>
<td>137.4 ± 2.3</td>
<td>3.0</td>
</tr>
<tr>
<td>7a</td>
<td>29.5 ± 0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>7b</td>
<td>2.3 ± 0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>7d</td>
<td>9.7 ± 0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>8b</td>
<td>271.6 ± 5.7</td>
<td>5.8</td>
</tr>
<tr>
<td>8d</td>
<td>133.8 ± 2.7</td>
<td>2.9</td>
</tr>
<tr>
<td>11b</td>
<td>123.9 ± 0.7</td>
<td>2.7</td>
</tr>
</tbody>
</table>

[a] Assay conditions: Phosphate buffer (100 mM), pH 7.5 with ethylenediaminetetraacetic acid (EDTA; 1 mM), GSH (1 mM), NADPH (0.2 mM), GR (1.3 unit·mL⁻¹), H₂O₂ (0.80 mM) and catalysts (20 µM). Stock solutions (2 mM) of catalysts 2, 3 and 6a-b were prepared in MeOH and stock solutions of catalysts 7, 8 and 11b were prepared in DMSO. Initial rates (v₀) were corrected for the spontaneous oxidation of GSH (24.3 ± 1.2 µM-min⁻¹). Errors correspond to ± SD for triplicates.
Consumption of Hydrogen Peroxide: The performance of some of the best GPx-mimics \(8b, 8d\) and \(11b\) (10 mol\%) together with GSH, NADPH, GR and \(H_2O_2\) in phosphate buffer was also followed for much longer time (250 min). As shown in Figure 3, a significant decrease in the absorbance of NADPH was seen during ca. 80 min. Thereafter, the consumption of NADPH corresponds more or less to the background reaction. Ebselen (2), included as a reference in Figure 3, could not match the activity of \(8b, 8d\) and \(11b\).

![Figure 3](image)

**Figure 3.** NADPH-consumption with time in the absence (control) and presence of catalysts 2 (ebselen), \(8b, 8d\) and \(11b\). A stock solution of catalyst 2 was prepared in MeOH and stock solutions of catalysts \(8b, 8d\) and \(11b\) were prepared in DMSO. Assay conditions: reactions were carried out with phosphate buffer (100 mM), pH 7.5, with EDTA (1 mM), GSH (0.10 mM), NADPH (0.20 mM), GR (1.3 unit·mL\(^{-1}\)), \(H_2O_2\) (20 \(\mu\)M) and catalyst (2 \(\mu\)M).

Mechanistic studies: In order to obtain some mechanistic insight, \(^{77}\)Se NMR experiments in DMSO-\(d_6\) were carried out with diselenide \(8b\) (\(\delta = 369\) ppm). Addition of 1 equivalent of \(H_2O_2\) (Scheme 1) did not cause much of a change in the spectrum (see the \(^{77}\)Se NMR spectrum in the Supporting Information). Upon addition of another equivalent of \(H_2O_2\) to the above mixture, one new signal appeared at \(\delta = 1156\) ppm, corresponding to seleninic acid \(12\) (or a dehydrated product thereof - an Se-oxide of aminoebselen). Now, 1 equivalent of GSH was added to the mixture produced
containing unreacted 8b and 12. This caused the disappearance of the peak at $\delta = 1156$ and the appearance of two new peaks at $\delta = 935$ ppm and $\delta = 344$ ppm, corresponding to amino derivative 14 of ebselen and selenol 16, respectively. Presumably, compound 14 is formed by cyclization of an intermediate selenenic acid 13. 17,24,25 Further addition of 2 equivalents of GSH to the mixture caused the disappearance of the peaks corresponding to 12, 14 and 16 and only the peak at 369 ppm, corresponding to diselenide 8b, remained in the $^{77}$Se NMR spectrum. When GSH (1 equivalent) was added to a solution of 8b in DMSO-d$_6$, the peak for 16 at $\delta = 346$ ppm appeared.

**Scheme 1.** Proposed mechanism for the reduction of H$_2$O$_2$ in the presence of GSH and diselenide 8b

It is not surprising that the peak from selenol 16 is shifted only 25 ppm upfield from that of diselenide 8b. This is due to strong intramolecular secondary Se···O interactions with the carbonyl group and additional substituent effects from the aromatic rings. A similar downfield chemical shift of 232 ppm for the corresponding selenol derived from ebselen was also accounted for in terms of strong Se···O interactions.24b In none of the above experiments did we see a peak corresponding to selenosulfide 15. It is probably a highly reactive species. We speculate that hydrogen bonding of Se to the ortho-amino group facilitates attack by thiol.

**Cytoprotective effects:** An overproduction of ROS/reactive nitrogen species (RNS) in biological systems has many deleterious effects. For example, oxidative damage of DNA is directly linked to
cardiovascular and neurodegenerative diseases.\textsuperscript{26,27} Since many of the novel antioxidants showed excellent hydroperoxide-decomposing activities, we decided to test their protective effects in cellular systems. Freshly isolated human mononuclear cells (MNC) were stimulated with phorbol myristate acetate (PMA) to produce ROS/RNS in the presence of antioxidants 3, 6b-c, 7a-b, 7d, 8b, 8d, 11b, ebselen (2) and Trolox at 25 µM. The total chemiluminescence (CL; extra- and intracellular) was then recorded in a luminol amplified assay. As shown in Figure 4, CL was significantly reduced for all compounds tested. Good GPx-mimics such as nitro compounds 6b, 6c and 11b and diselenide 8b all afforded better cytprotection than Trolox. Nitroebseleol 11b was by far the most potent protective agent.

![Figure 4](image)

**Figure 4.** Chemiluminescence (normalized to positive control) monitored at $\lambda = 425$ nm in PMA-stimulated MNC-cells exposed to 25 µM of antioxidants Ebselen (2), 3, 6b-c, 7a-b, 7d, 8b, 8d, 11b and Trolox. N = 3 to 6 for each group from 4 independent experiments.

**Cell Viability:** In order to reveal any toxicity of antioxidants 3, 6b-c, 7a-b, 7d, 8b, 8d and 11b, MC3T3-cells (a preosteoblast cell line) were exposed to 25 µM of compound and cell viability checked after 1 and 3 days by using the Alamar Blue assay (Figure 5). No toxic effects of the compounds were seen after 3 days.
Figure 5. Relative cell viability of MC3T3 cells in the presence of 25 µM of antioxidants 3, 6b-c, 7a-b, 7d, 8b, 8d and 11b as determined by Alamar Blue measurements after 1 and 3 days at $\lambda_{em} = 590$ nm.

Conclusion

In search for novel ebselen derivatives with improved antioxidative properties we have described straightforward syntheses of azo-bis-ebselens from nitroebselens and 2-bromo-3-nitrobenzamides. Reduction of the azo-group to the corresponding amine was effected by NaTePh, but this transformation was accompanied by cleavage of the Se-N bond, resulting in formation of diaryl diselenides carrying both amino- and benzamide groups in the ortho positions. As revealed by X-ray crystallography and DFT-calculations, the intramolecular Se···N-coordination in azo-bis-ebselens was much stronger than the corresponding Se···O-interaction in nitroebselens. This may be the reason for the poor GPx-activity of the azo-bis-ebselen. Both nitroebselens 6 and diselenides 8 outperformed ebselen when it comes to catalysis of GSH-induced reduction of hydrogen peroxide. Considering their low toxicity and ability to inhibit ROS/RNS in stimulated human MNC-cells, we feel that compounds of this sort would be useful as models and tools in the development of novel treatments for diseases with a component of oxidative stress (cardiovascular diseases, stroke, Alzheimer’s and Parkinson’s diseases).
Experimental Section

2-Bromo-3-nitrobenzoic acid (98% purity) was purchased and used as such. $^1$H and $^{13}$C NMR spectra for all compounds prepared were recorded on 300 MHz ($^1$H: 300 MHz; $^{13}$C: 75 MHz) and 400 MHz ($^1$H: 399.97 MHz; $^{13}$C: 100 MHz) spectrometers, using the residual solvent peaks of CDCl$_3$ ($^1$H: δ 7.26; $^{13}$C: δ 77.2), CD$_3$OD ($^1$H: δ 3.31; $^{13}$C: δ 49.0) and DMSO-d$_6$ ($^1$H: δ 2.50; $^{13}$C: δ 39.5), as indirect references to TMS (δ = 0 ppm). $^{77}$Se NMR spectra were recorded on 300 MHz ($^{77}$Se: 57 MHz) and 400 MHz ($^{77}$Se: 76 MHz) spectrometers with Ph$_2$Se$_2$ (δ = 460 ppm) as an indirect reference to Me$_2$Se (δ = 0 ppm). Flash column chromatography was performed using silica gel (0.04-0.06 mm). Melting points are uncorrected. The high resolution mass spectra (HRMS) were obtained using a time of flight (TOF) instrument equipped with electrospray ionization (ESI) and electron impact (EI$^+$) operating in the positive ion mode. Tetrahydrofuran (THF) was dried in a solvent purification system by passing it through an activated alumina column before use.

Typical procedure for benzamide formation: 2-Bromo-3-nitro-N-phenylbenzamide (4a). A mixture of 2-bromo-3-nitrobenzoic acid (1.0 g, 4.06 mmol) and thionyl chloride (5 mL) was refluxed for 3 h in the presence of a catalytic amount of N,N-dimethyl formamide (DMF). Excess thionyl chloride was then removed under reduced pressure. The residue was dissolved in dichloromethane (10 mL). Aniline (0.74 g, 8.13 mmol) in CH$_2$Cl$_2$ (15 mL) was added dropwise at room temperature. The resulting solution was then stirred for overnight. The reaction mixture was poured in water (10 mL) and extracted with dichloromethane. The separated organic layers were combined, dried over anhydrous Na$_2$SO$_4$ and the solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel using ethyl acetate/pentane (1:1) as an eluent to give the title product (1.13 g, 87%) as a solid. Physical and spectroscopic data were in good agreement with the literature. The $^1$H and $^{13}$C NMR spectra of 4a have been included in the Supporting Information.

2-Bromo-3-nitro-N-p-tolylbenzamide (4b). White solid. Yield: 1.30 g (96%); Mp 166-168 °C; $^1$H NMR (CDCl$_3$): δ 2.36 (s, 3H), 7.18 (d, $J$ = 8.2 Hz, 2H), 7.48 (d, $J$ = 8.5 Hz, 2H), 7.53 (t, $J$ = 7.8 Hz, 1H), 7.62 (br s, 1H), 7.71 (dd, $J$ = 1.7, 7.7 Hz, 1H), 7.76 (dd, $J$ = 1.6, 7.9 Hz, 1H); $^{13}$C NMR (CDCl$_3$): δ 21.1, 111.6, 120.5, 126.2, 128.9, 129.9, 131.9, 134.6, 135.4, 141.5, 151.3, 164.2; HRMS (TOF MS ES$^+$) m/z calcd for C$_{14}$H$_{11}$N$_2$O$_3$Br [M+H]$^+$: 335.0031; found: 335.0035.

2-Bromo-N-(4-methoxyphenyl)-3-nitrobenzamide (4c). The crude residue was purified by chromatography on silica gel with 60% ethyl acetate/pentane as an eluent to afford the title
compound as a white solid. Yield: 1.34 g (94%); Mp 180-182 °C; ¹H NMR (CD₂OD): δ 3.80 (s, 3H), 6.93 (dd, J = 2.2, 6.9 Hz, 2H), 7.57 (dd, J = 2.2, 6.9 Hz, 2H), 7.66 (t, J = 7.8 Hz, 1H), 7.74 (dd, J = 1.8, 7.8 Hz, 1H), 7.89 (dd, J = 1.7, 7.9 Hz, 1H); ¹³C NMR (CD₂OD): δ 55.9, 112.0, 115.1, 123.2, 126.6, 130.2, 132.3, 143.2, 152.8, 158.5, 166.9; HRMS (TOF MS ES⁺) m/z calcd for C₁₄H₁₁N₂O₄Br [M+H]⁺: 350.9980; found: 350.9975.

2-Bromo-N-(1-naphthyl)-3-nitrobenzamide (4d). The crude residue was washed with pentane to obtain the title compound of sufficient purity. Yield: 0.89 g (59%); Mp 243-246 °C; ¹H NMR (DMSO-d₆): δ 7.56-7.59 (several peaks, 3H), 7.80 (m, 3H), 7.89 (d, J = 8.0 Hz, 1H), 7.97-8.00 (several peaks, 2H), 8.10 (dd, J = 1.2, 6.8 Hz, 1H), 8.20 (t, J = 7.2 Hz, 1H), 8.25 (br s, 1H); ¹³C NMR (DMSO-d₆): δ 110.5, 122.6, 122.9, 125.3, 125.6, 126.2, 126.3, 126.4, 128.2 (2C), 129.5, 131.8, 132.7, 133.8, 141.9, 150.9, 165.6; HRMS (TOF MS EI⁺) m/z calcd for C₁₇H₁₁N₂O₃Br [M]⁺: 369.9953; found: 369.9959.

Typical procedure for the introduction of a butylseleno group by nucleophilic aromatic substitution: 2-Butylseleno-3-nitro-N-phenylbenzamide (5a). Benzamide 4a (0.75 g, 2.33 mmol) was added to an in situ-prepared solution of BuSeNa (4 equivalents) in EtOH (30 mL) at 0 °C under inert atmosphere. The mixture was stirred at room temperature for 2 h and then warmed at 50 °C for 20 h. The solvent was removed under reduced pressure and the residue dissolved in CHCl₃ and washed with water. After drying over anhydrous Na₂SO₄, evaporation of the solvent and column chromatography using 20% ethyl acetate/pentane as eluent, the pure title compound (0.51 g, 55%) was isolated. Physical and spectroscopic data were in good agreement with the literature.¹⁷ The ¹H, ¹³C and ⁷⁷Se NMR spectra of 5a have been included in the Supporting Information.

2-Butylseleno-3-nitro-N-p-tolylbenzamide (5b). Orange liquid (semi-solid). Solidified in the freezer. Yield: 0.59 g (67%); Mp 69-73 °C; ¹H NMR (CDCl₃): δ 0.78 (t, J = 7.3 Hz, 3H), 1.22-1.29 (several peaks, 2H), 1.45-1.55 (several peaks, 2H), 2.36 (s, 3H), 2.87 (t, J = 7.5 Hz, 2H), 7.20 (d, J = 8.2 Hz, 2H), 7.49-7.55 (several peaks, 3H), 7.83 (dd, J = 1.3, 7.9 Hz, 1H), 7.91 (dd, J = 1.4, 7.6 Hz, 1H), 8.24 (br s, 1H); ¹³C NMR (CDCl₃): δ 13.5, 21.1, 22.7, 31.1, 31.9, 120.0, 122.4, 125.5, 128.9, 129.9, 133.4, 135.0, 135.1, 142.9, 155.1, 165.2; ⁷⁷Se NMR (CDCl₃): δ 283; HRMS (TOF MS ES⁺) m/z calcd for C₁₈H₂₀N₂O₃Se [M+H]⁺: 393.0717; found: 393.0721.
2-Butylseleno-N-(4-methoxyphenyl)-3-nitrobenzamide (5c). Yellow solid. Yield: 0.50 g (56%); Mp 94-97 °C; 1H NMR (CDCl3): δ 0.78 (t, J = 7.3 Hz, 3H), 1.26 (m, 2H), 1.51 (m, 2H), 2.88 (t, J = 7.5 Hz, 2H), 3.82 (s, 3H), 6.93 (dd, J = 2.2, 7.7 Hz, 2H), 7.51 (t, J = 7.8 Hz, 1H), 7.57 (dd, J = 2.2, 5.4 Hz, 2H), 7.82 (dd, J = 1.5, 8.0 Hz, 1H), 7.90 (dd, J = 1.5, 7.6 Hz, 1H), 8.22 (br s, 1H); 13C NMR (CDCl3): δ 13.5, 22.7, 31.0, 31.9, 55.6, 114.5, 121.7, 122.3, 125.3, 128.8, 130.7, 133.1, 143.0, 155.0, 157.0, 165.2; 77Se NMR (CDCl3): δ 283; HRMS (TOF MS ES+) m/z calcd for C18H20N2O4Se [M+H]+: 409.0667; found: 409.0663.

Typical procedure for the synthesis of nitroebselein analogues: 7-Nitro-2-phenyl-1,2-benzisoselenazol-3(2H)-one (3). To a stirred solution of selenide 5a (0.72 g, 1.84 mmol,) in dry CHCl3 (10 mL) was added Br2 (0.10 ml, 1.84 mmol) in dry CHCl3 (5 mL) containing Et3N (0.257 ml, 1.84 mmol) at 0 °C under an inert atmosphere. The reaction mixture was then stirred for 3 h at room temperature and water (10 mL) was added. The separated organic layer was dried over anhydrous Na2SO4. Removal of the solvent and purification of the residue by silica gel column chromatography (elution with 25% ethyl acetate/n-pentane) afforded the title compound (0.58 g, 95%) as a red solid. Physical and spectroscopic data were in good agreement with the literature.17 The 1H, 13C and 77Se NMR spectra of 3 have been included in the Supporting Information.

7-Nitro-2-p-tolyl-1,2-benzisoselenazol-3(2H)-one (6b). Yield: 0.50 g (82%); Mp 184 °C; 1H NMR (CDCl3): δ 2.39 (s, 3H), 7.27 (d, J = 7.9 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.72 (t, J = 7.8 Hz, 1H), 8.46 (dd, J = 0.9, 7.5 Hz, 1H), 8.57 (dd, J = 0.9, 8.1 Hz, 1H); 13C NMR (CDCl3): δ 21.3, 125.2, 127.6, 127.9, 130.3, 131.6, 135.3, 135.8, 136.6, 137.4, 142.2, 164.0; 77Se NMR (CDCl3): δ 929; HRMS (TOF MS ES+) m/z calcd for C14H10N2O4Se [M+H]+: 334.9935; found: 334.9943.

2-(p-Methoxyphenyl)-7-nitro-1,2-benzisoselenazol-3(2H)-one (6c). Purification with 40% ethyl acetate/n-pentane afforded the pure title compound as a red solid. Yield: 67%; Mp 206-209 °C; 1H NMR (CDCl3): δ 3.86 (s, 3H), 6.99 (d, J = 9.0 Hz, 2H), 7.51 (d, J = 8.8 Hz, 2H), 7.73 (t, J = 7.6 Hz, 1H), 8.47 (d, J = 7.6 Hz, 1H), 8.58 (d, J = 8.1 Hz, 1H); 13C NMR (CDCl3): δ 55.8, 114.9, 127.1, 127.6, 127.9, 131.0, 131.4, 135.3, 136.6, 142.2, 158.9, 164.2; 77Se NMR (CDCl3): δ 932; HRMS (TOF MS ES+) m/z calcd for C14H10N2O4Se [M+H]+: 350.9884; found: 350.9876.

Typical procedure A for the preparation of azo-bis-ebselein compounds: Bis-[2-phenyl-1,2-benzisoselenazol-3-(2H)-one-7-yl]diazene (7a). Nitroebselein 3 (275 mg, 0.86 mmol) was added to
an in situ prepared colourless solution of PhTeNa (1.72 mmol) from the reaction of Ph$_2$Te$_2$ (352 mg, 0.86 mmol) and NaBH$_4$ (65 mg, 1.73 mmol) in EtOH (10 mL) at room temperature under an inert atmosphere. The mixture was then heated at reflux for 20 h, allowed to cool, poured into water and extracted with CHCl$_3$. The separated CHCl$_3$-extract was dried over anhydrous Na$_2$SO$_4$, the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel. The column was first eluted with pentane/ethyl acetate (50:50) then with CHCl$_3$ and finally with CHCl$_3$/MeOH (98:2) to give the title compound as a dark brown solid. Yield: 0.125 g (51%); Mp >320 °C; $^1$H NMR (CDCl$_3$): $\delta$ 7.32 (t, $J$ = 7.2 Hz, 2H), 7.49 (t, $J$ = 7.2 Hz, 4H), 7.66 (dd, $J$ = 8.0 Hz, 4H), 7.80 (t, $J$ = 7.6 Hz, 2H), 8.40 (d, $J$ = 7.2 Hz, 2H), 8.61 (d, $J$ = 7.6 Hz, 2H); $^{13}$C NMR (CDCl$_3$): $\delta$ 125.3, 126.7, 128.3, 129.6, 130.2, 131.7, 131.8, 132.1, 139.4, 144.5, 164.0; $^{77}$Se NMR (CDCl$_3$): $\delta$ 931; HRMS (MALDI) m/z calcd for C$_{28}$H$_{16}$N$_4$O$_2$Se$_2$ [M+H]$^+$: 576.9682; found: 576.9689.

**Bis-[2-p-tolyl-1,2-benziselenzol-3-(2$H$)-one-7-yl]diazene (7b).** Heating at reflux for 40 h. Purification by column chromatography using 4% MeOH/CHCl$_3$. Yield: 30 mg (9%); Mp >320 °C; $^1$H NMR (CDCl$_3$): $\delta$ 2.41 (s, 6H), 7.29 (d, $J$ = 7.6 Hz, 4H), 7.52 (d, $J$ = 8.0 Hz, 4H), 7.78 (t, $J$ = 7.2 Hz, 2H), 8.38 (d, $J$ = 7.6 Hz, 2H), 8.57 (d, $J$ = 7.6 Hz, 2H); $^{13}$C NMR (CDCl$_3$): $\delta$ 21.3, 125.4, 128.2, 130.2, 131.2, 131.8, 132.1, 136.7 (2C), 144.6, 164.1; $^{77}$Se NMR (CDCl$_3$): $\delta$ 927; HRMS (TOF MS ES$^+$) m/z calcd for C$_{28}$H$_{20}$N$_4$O$_2$Se$_2$ [M+H]$^+$: 604.9995; found: 604.9970.

**Typical procedure B for the preparation of azo-bis-ebselen compounds: Bis-[2-phenyl-1,2-benziselenzol-3-(2$H$)-one-7-yl]diazene (7a).** To a brown suspension of in situ prepared Na$_2$Se$_2$ (1.24 mmol) in dry THF (20 mL) under an inert atmosphere was slowly added a solution of benzamide 4a (0.20 g, 0.62 mmol) in THF (10 mL) at room temperature. After heating at reflux for 5h and cooling to room temperature, water (20 mL) was added and stirring was continued for 10 min. Following extraction with CHCl$_3$, the combined organic layers were dried over anhydrous Na$_2$SO$_4$. Removal of the solvent and purification of the residue by silica gel column chromatography, eluting first with pentane/ethyl acetate (50:50) then with CHCl$_3$ and finally with CHCl$_3$/MeOH (98:2), afforded compound 7a (62 mg, 35%). Similarly, compound 7b (340 mg, 36%) was prepared from 4b (1.00 g, 2.98 mmol).

**Bis-[1-naphthyl-1,2-benziselenzol-3-(2$H$)-one-7-yl]diazene (7d).** The solution of 4d and Na$_2$Se$_2$ was stirred for 8 h at room temperature before workup. Purification by column chromatography using 5% MeOH/CHCl$_3$ as an eluent afforded the title compound (68%) as a dark brown solid. Mp
>320 °C; ¹H NMR (CDCl₃): δ 7.49-7.61 (several peaks, 8H), 7.77 (t, J = 8.0 Hz, 2H), 7.85 (d, J = 8.0 Hz, 2H), 7.95 (t, J = 6.0 Hz, 4H), 8.42 (d, J = 7.6 Hz, 2H), 8.53 (d, J = 8.0 Hz, 2H); ¹³C NMR (CDCl₃): δ 123.5, 125.8, 126.8 (2C), 127.1, 128.3, 128.7, 129.1, 130.8, 130.9, 131.4, 131.7, 132.4, 134.8, 135.0, 144.5, 165.3; ⁷⁷Se NMR (CDCl₃): δ 946; HRMS (TOF MS EI⁺) m/z calcd for C₃₄H₂₆N₄O₂Se₂ [M⁺]: 675.9917; found: 675.9913.

**Typical procedure for the synthesis of diselenides: Bis[3-amino-N-(1-naphthyl)benzamide-2-yl] diselenide (8d).** The azo-bis-ebseilen 7d (50 mg, 0.074 mmol) was added to an in situ-prepared colorless solution of NaTePh (0.592 mmol) prepared from Ph₂Te (61 mg, 0.148 mmol) and NaBH₄ (11 mg, 0.296 mmol) in EtOH (10 mL) at room temperature under an inert atmosphere. The mixture was then heated at 80 °C for 1 h. and allowed to cool to room temperature. After addition of water, the mixture was extracted with ethyl acetate and dried over anhydrous MgSO₄. Evaporation of the solvent and purification by column chromatography, using ethyl acetate as an eluent afforded the title compound (25 mg, 52%) as a yellow solid. Mp 236-240 °C; ¹H NMR (DMSO-d₆): δ 5.63 (s, 4H), 6.45 (d, J = 7.1 Hz, 2H), 6.80 (d, J = 7.8 Hz, 2H), 6.90 (t, J = 7.5 Hz, 2H), 7.25 (d, J = 7.0 Hz, 2H), 7.25-7.52 (several peaks, 6H), 7.87 (d, J = 8.2 Hz, 2H), 7.91 (d, J = 8.2 Hz, 2H), 7.97 (d, J = 7.9 Hz, 2H), 10.27 (s, 2H); ¹³C NMR (DMSO-d₆): δ 110.1, 114.3, 114.8, 122.6, 123.0, 125.3, 125.3, 125.5, 125.7, 125.8, 127.8, 128.4, 130.3, 133.1, 133.5, 144.5, 150.9, 169.2; ⁷⁷Se NMR (DMSO-d₆): δ 364; HRMS (TOF MS ES⁺) m/z calcd for C₃₄H₂₆N₄O₂Se₂ [M+Na⁺]: 705.0286; found: 705.0304.

**Bis[3-Amino-N-(p-tolyl)benzamide-2-yl] diselenide (8b).** Orange solid. Yield: 44 mg (87%); Mp 219-221 °C; ¹H NMR (DMSO-d₆): δ 2.25 (s, 6H), 5.46 (s, 4H), 6.60 (d, J = 7.0 Hz, 2H), 6.79 (d, J = 8.2 Hz, 2H), 7.05 (d, J = 8.2 Hz, 4H), 7.10 (t, J = 7.8 Hz, 2H), 7.33 (d, J = 8.2 Hz, 4H), 10.08 (s, 2H); ¹³C NMR (DMSO-d₆): δ 20.5, 110.2, 114.6, 115.0, 120.0, 128.8, 130.4, 132.4, 136.3, 144.7, 150.8, 167.3; ⁷⁷Se NMR (DMSO-d₆): δ 368; HRMS (TOF MS ES⁺) m/z calcd for C₂₈H₂₆N₄O₂Se₂ [M+H⁺]: 611.0464; found: 611.0475.

Diselenide 8b was also isolated in 16% yield when 6b was allowed to react with NaTePh.

**Typical procedure for reduction of 2-butyllseleno-3-nitrobenzamides: 3-Amino-2-butyllseleno-N-phenyl benzamide (10a).** To in situ-prepared NaTePh (0.96 mmol) from the reaction of Ph₂Te (192 mg, 0.48 mmol) and NaBH₄ (35 mg, 0.94 mmol) in EtOH (10 mL) was added 5a (96 mg 0.24 mmol) at room temperature under nitrogen. The mixture was then heated at reflux for overnight. After cooling to room temperature the solvent was evaporated and the residue dissolved in
dichloromethane. After treatment with water the organic layer was dried over anhydrous MgSO₄ and the solvent removed under reduced pressure. Chromatographic purification, eluting with 40% ethyl acetate/pentane, afforded the title compound (70 mg, 96%) as a white solid; Mp 76-78 °C; ¹H NMR (CDCl₃): δ 0.83 (t, J = 7.5 Hz, 3H), 1.33 (m, 2H), 1.58 (m, 2H), 2.81 (t, J = 7.5 Hz, 2H), 4.62 (s, 2H), 6.83 (d, J = 7.8 Hz, 1H), 6.96 (d, J = 6.9 Hz, 1H), 7.12-7.22 (several peaks, 2H), 7.37 (t, J = 7.8 Hz, 2H), 7.63 (d, J = 7.8 Hz, 2H), 7.79 (s, 1H); ¹³C NMR (CDCl₃): δ 13.6, 23.0, 29.3, 32.6, 110.4, 115.8, 117.6, 120.1, 124.5, 129.2, 130.1, 138.2, 144.4, 149.6, 168.0; ⁷⁷Se NMR (CDCl₃): δ 145; HRMS (TOF MS El⁺) m/z calcd for C₁₇H₂₀N₂OSe [M⁺]: 348.0741; found: 348.0742.

3-Amino-2-butylseleno-N-(p-tolyl)benzamide (10b). Yield: 82%; Mp 88-90 °C; ¹H NMR (CD₂OD): δ 0.84 (t, J = 7.0 Hz, 3H), 1.35 (m 2H), 1.58 (m, 2H), 2.32 (s, 3H), 2.77 (t, J = 7.4 Hz, 2H), 6.74 (dd, J = 1.2, 7.5 Hz, 1H), 6.86 (dd, J = 1.2, 8.2 Hz, 1H), 7.32-7.18 (several peaks, 3H), 7.52 (d, J = 8.3 Hz, 2H); ¹³C NMR (CD₂OD): δ 13.9, 21.0, 23.9, 28.7, 33.6, 111.6, 116.2, 116.7, 121.7, 130.2, 130.9, 135.1, 137.3, 146.7, 151.6, 171.5; ⁷⁷Se NMR (CD₂OD): δ 137; HRMS (TOF MS El⁺) m/z calcd for C₁₈H₂₂N₂OSe [M⁺]: 362.0897; found: 362.0901.

3-Amino-2-(butylseleno)-N-(4-methoxyphenyl)benzamide (10c). Yield: 79%; Mp 100-104 °C; ¹H NMR (DMSO-d₆): δ 0.79 (t, J = 7.4 Hz, 3H), 1.25-1.31 (several peaks, 2H), 1.45-1.51 (several peaks, 2H), 2.71 (t, J = 7.4 Hz, 2H), 3.73 (s, 3H), 5.52 (br s, 2H), 6.58 (d, J = 7.0 Hz, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.89 (d, J = 8.7 Hz, 2H), 7.12 (t, J = 7.8 Hz, 1H), 7.61 (d, J = 8.6 Hz, 2H), 9.99 (s, 1H); ¹³C NMR (DMSO-d₆): δ 13.4, 22.3, 26.9, 31.9, 55.2, 108.6, 113.7, 114.1, 114.4, 120.9, 129.4, 132.7, 145.9, 150.3, 155.2, 167.7; ⁷⁷Se NMR (DMSO-d₆): δ 144; HRMS (TOF MS El⁺) m/z calcd for C₁₈H₂₂N₂O₂Se [M⁺]: 378.0846; found: 378.0844.

2-(4-Hydroxyphenyl)-7-nitrobenzisolelenazol-3(2H)-one (11b). Solution of BBr₃ (0.86 mmol, 0.86 mL) was added into a stirred solution of 6c (0.27 mmol, 0.10 g) in dry CH₂Cl₂ (10 mL) at -78 °C under an inert atmosphere. Stirring was then continued for overnight at room temperature. The reaction mixture was diluted with dichloromethane and poured into a solution of brine. The separated organic layer was dried over anhydrous MgSO₄. The solvent was removed under reduced pressure to afford a red solid which was washed with diethyl ether and dried under vacuum to give the pure title compound. (85 mg, 88%); Mp 263-265 °C; ¹H NMR (DMSO-d₆): δ 6.86 (d, J = 9.0 Hz, 2H), 7.41 (d, J = 9.0 Hz, 2H), 7.86 (t, J = 7.9 Hz, 1H), 8.38 (d, J = 7.4 Hz, 1H), 8.71 (d, J = 8.2 Hz, 1H), 9.77 (s, 1H); ¹³C NMR (DMSO-d₆): δ 115.9, 127.1, 128.3, 128.8, 129.3, 130.3, 134.8, 135.8, 142.0, 156.6,
163.6; $^{77}$Se NMR (DMSO-$d_6$): $\delta$ 923; HRMS (TOF MS EI$^+$) $m/z$ calc'd for C$_{13}$H$_8$N$_2$O$_4$Se [M]$^+$: 335.9649; found: 335.9642.

**X-ray Crystallographic Analysis.** X-ray crystallographic studies for obtaining the structures of compounds 7b and 8b were carried out using graphite-monochromatized Mo Kα radiation ($\lambda = 0.7107$ Å). The structures were solved by direct methods (SHELXS-2013) and refined by a full-matrix least-squares procedure on $F^2$ for all reflections using SHELXL-2013 software. Hydrogen atoms were localized by geometrical means. A riding model was chosen for refinement. The isotropic thermal parameters of hydrogen atoms were fixed at 1.5 times and 1.2 times U(eq) of the corresponding carbon atoms for sp$^3$ C-H and sp$^2$ C-H bonds, respectively. Crystallographic data for the structures reported in this paper, containing supplementary crystallographic data for this paper, have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publications. CCDC 1047473 (for compound 7b), and CCDC 1047474 (for compound 8b). These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

**Crystal data for 7b.** C$_{30}$H$_{22}$Cl$_6$N$_4$O$_2$Se$_2$, $M_r = 841.14$, Monoclinic, space group C2/c, $a = 22.883(7)$ Å, $b = 12.0629(12)$ Å, $c = 15.901(5)$ Å, $\alpha = \gamma = 90^o$, $\beta = 132.43(5)^o$, $V = 3239.5(15)$ Å$^3$, $\lambda = 0.71073$ Å, $Z = 4$, $T = 123(2)$ K, $\rho_{calcd} = 1.725$ Mg/m$^3$, GOF = 1.049, R1 = 0.0707, wR2 = 0.1422 [I>2σ (I)]; R1 = 0.1291, wR2 = 0.1754 (all data). Of the 18727 reflections that were collected, 6174 were unique (R(int) = 0.1199).

**Crystal data for 8b.** C$_{28}$H$_{26}$N$_4$O$_2$Se$_2$, $M_r = 608.45$, Monoclinic, space group P 1 2 / c 1, $a = 8.9772(2)$ Å, $b = 26.5501(5)$ Å, $c = 10.9101(3)$ Å, $\alpha = \gamma = 90^o$, $\beta = 96.200(2)^o$, $V = 2585.16(10)$ Å$^3$, $\lambda = 0.71073$ Å, $Z = 4$, $T = 123(2)$ K, $\rho_{calcd} = 1.563$ Mg/m$^3$, GOF = 1.069, R1 = 0.0543, wR2 = 0.0813 [I>2σ (I)]; R1 = 0.0981, wR2 = 0.0921 (all data). Of the 59472 reflections that were collected, 16750 were unique (R(int) = 0.0599).

**Computational details.** Computational calculations for compounds 6b-c, 7a-b, 7d, 11b were executed by using the Gaussian 09 suite of quantum chemical programs. The hybrid B3LYP exchange correlation functional was implemented for density functional theory (DFT) calculations. The geometry optimizations were carried out at the B3LYP level of DFT by using the 6-311+G(d) basis sets. The quantifications of orbital interaction were done by natural bond orbital (NBO) analysis at B3LYP/6–311+G(d,p) level. The $^{77}$Se NMR calculations were performed at B3LYP/6–311+G (d,p) level on B3LYP/6–311+G(d)-level-optimized geometries by using the gauge-including atomic orbital (GIAO) method (referenced with respect to the peak of Me$_2$Se). Atoms in molecules
(AIM)\textsuperscript{33} calculations have also been used to confirm distinct bond critical point between the two interacting atoms.

**Coupled Reductase Assay.** The glutathione peroxidase-like activity of compounds prepared was determined by UV-spectroscopy following the protocol by Wilson\textsuperscript{34} with slight modifications. The test mixture contained GSH (1 mM), ethylene diamine tetra acetate (EDTA, 1 mM), glutathione reductase (GR) (1.3 unit-mL\textsuperscript{-1}), and β-nicotinamide adenine dinucleotide phosphate (NADPH, 0.2 mM) in potassium phosphate buffer (100 mM), pH 7.5. Catalysts (20 µM) were added to the test mixture at 21 °C and the reaction was initiated by addition of H\textsubscript{2}O\textsubscript{2} (0.8 mM). Initial reaction rates were based on the consumption of NADPH as assessed by UV-spectroscopy at 340 nm. The initial reduction rates were triplicated and calculated from the first 10 seconds of reaction by using 6.22 mM\textsuperscript{-1}cm\textsuperscript{-1} as the extinction coefficient for NADPH. GPx-data reported in Table 2 are means ± SD.

**Biological Studies.** Blood buffy coats were diluted in a 1:1 ratio with 1 x phosphate buffered saline (PBS) and mononuclear cells were segregated using Ficoll-Paque plus density gradient centrifugation. Briefly, the diluted blood was gently placed on top of Ficoll-Paque and then centrifuged at 400 g for 30 minutes. After removing the plasma layer, the mononuclear (MNC) layer was collected, washed with PBS, and then spun at 100 g for 15 min. This washing procedure was repeated for a total of 3 times. Total cell number was counted using a hemocytometer and trypan blue exclusion method. The blood pellet was then suspended in 3% dextran/0.9% saline for 20 min. The supernatant was then collected and the cell pellet collected after centrifugation at 250 g for 10 min. To remove erythrocyte, the pellet was subjected to 0.2% saline solution for 20 s and then an equal volume of 1.6% saline was added. Then the cells were spun at 250 g for 10 min and the pellet was suspended in PBS.

**Chemiluminescence Assay.** To measure release of ROS from monocytes, a luminol amplified chemiluminescence assay was conducted. All measurements were performed in white 96 well plates at 37°C in 1 x PBS with 50 mM of luminol, 0.1M NaOH, 2 µg/mL of horse radish peroxidase, different concentrations of ebselen, 3, 6b-c, 7a-b, 7d, 8b, 8d and 11b and Trolox initially diluted in DMSO. Approximately 2 x 10\textsuperscript{5} cells were plated in each well and then stimulated to generate ROS using 500 nM of 1 µM phorbol myristate acetate (PMA). Luminescence readings were taken using a multi-plate reader every 2 min for 2 h. Total ROS was quantified by determining the area under the chemiluminescence kinetic curve. Experiments were repeated 4-5 times with n = 3 per group per
experiment. For statistical analysis, ANOVA was performed with Scheffe post-hoc tests. P values below 0.05 were considered significant.

**Cell proliferation and toxicity assay.** MC3T3 (plating density: 5 x 10^3 and 20 x 10^3 per well respectively in a 96 well plate) was let adhered after plating for 24 h in α-MEM supplemented with 10% FBS and 1% pen/strep. Then the cells were exposed to antioxidants 3, 6b-c, 7a-b, 7d, 8b, 8d and 11b (25 μM) for 24 h, washed and then fed with fresh α-MEM every other day. To assess cell viability, the Alamar Blue assay was conducted on day 1 and 3 after treatment. Briefly, cells were washed with 1 x PBS and then incubated with Alamar Blue (1:20 dilution in phenol red free α-MEM for 1.5 h and then fluorescence was read with a plate reader at 560 nm excitation and 590 nm emissions. Experiments were repeated twice with n = 6 for each group.

**Mechanistic studies.** A solution of H₂O₂ (3.7 μL, 0.033 mmol) was added to an NMR tube containing diselenide 8b (20 mg, 0.033 mmol) in 400 μL of DMSO-d₆ and the ⁷⁷Se NMR spectrum was recorded after 30-60 min. Then another equivalent of H₂O₂ was added and the ⁷⁷Se NMR spectrum recorded again. Several portions (1+2 equivalents) of GSH (10 mg, 0.033 mmol) in 100 μL of H₂O were then syringed into the NMR tube. After every new addition of thiol the ⁷⁷Se NMR spectrum was recorded.

**ASSOCIATED CONTENT**

**Supporting Information**

¹H, ¹³C and ⁷⁷Se NMR data for all new compounds prepared. Results from kinetic studies of compounds prepared and coordinates of optimized geometries. X-ray crystallographic data (CIF files). This material is available free of charge via the Internet at http://pubs.acs.org

**AUTHOR INFORMATION**

**Corresponding Authors**

*E-mail: vijaypalchem@gmail.com

*E-mail: lars.engman@kemi.uu.se

**Notes**

The authors declare no competing financial interest
ACKNOWLEDGEMENTS
Stiftelsen Å-Forsk (16-364) and Stiftelsen Olle Engkvist Byggmästare (2016/159) and Carl Tryggers Stiftelse för Vetenskaplig Forskning (CTS 13:346) are gratefully acknowledged for financial support.

REFERENCES


-Table of Contents-

Nitroebasolans

PhTe, C6H5OH

reflux

Naph/Sey/THF

r / reflux

Bis-azo-ebasolans

24