**Antioxidants**

Deutsche Ausgabe: DOI: 10.1002/ange.201510947
Internationale Ausgabe: DOI: 10.1002/anie.201510947

# Multifunctional Antioxidants: Regenerable Radical-Trapping and Hydroperoxide-Decomposing Ebselenols

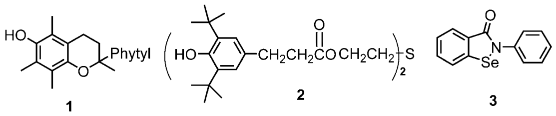
*Shailesh Kumar, Jiajie Yan, Jia-fei Poon, Vijay P. Singh, Xi Lu, Marjam Karlsson Ott, Lars Engman,* and Sangit Kumar**

***Abstract:*** *Regenerable, multifunctional ebselenol antioxidants were prepared that could quench peroxyl radicals more efficiently than α-tocopherol. These compounds act as better mimics of the glutathione peroxidase enzymes than ebselen. Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in human mononuclear cells was considerably decreased upon exposure to the organoselenium compounds. At a concentration of 25 μM, the ebselenol derivatives showed minimal toxicity in pre-osteoblast MC3T3 cells.*

All organic compounds undergo degradation when exposed to air. Decreasing the rate of such processes is of paramount importance to food technologists,[1] polymer producers,[2] the oil industry,[3] and to pharmaceutical companies with an interest in antioxidant pharmacotherapy.[4] Autoxidation is responsible for the oxidative deterioration of organic materials. Both radical-trapping and peroxide decomposing antioxidants are usually needed to delay the process.[5]

Evolution has provided nature with many types of antioxidants. In humans, α-tocopherol (**1**) is the most prominent chain-breaking donating agent in the lipid phase (Figure 1).[6] Ascorbate may have a similar function in hydrophilic environments.[7] Several enzymatic systems in vivo have preventive antioxidant functions. The selenium-containing glutathione peroxidases (GPx)[8] cause reduction of hydrogen peroxide and organic hydroperoxides to water and alcohols, respectively, in the presence of the tripeptide glutathione (GSH) as a stoichiometric reductant [Eq. (1)].

$$\mathrm{ROOH} + 2\,\mathrm{GSH} \xrightarrow{\mathrm{GPx}} \mathrm{ROH} + \mathrm{H_2O} + \mathrm{GSSG} \qquad (1)$$



**1** **2** **3**

***Figure 1.*** Structures of antioxidants **1**–**3**.

Organic materials, such as oils and man-made polymers, are often stabilized with a blend of additives containing both chain-breaking (phenols and aromatic amines)[9] and preventive antioxidants (peroxide decomposing phosphites and sulfur compounds, metal-complexing agents, and UV absorbers). Occasionally, functional groups with different antioxidant functions are built into the same molecule. Irganox 1035 (**2**; Figure 1) is an example of such a multifunctional antioxidant where the sterically hindered phenol contributes chain-breaking activity and the sulfide can serve as a peroxide decomposer.

The organoselenium compound ebselen, specifically 2-phenyl-1,2-benzisoselenazol-3(*2H*)-one (**3**; Figure 1), has been known for more than thirty years to mimic the action of the GPx enzymes.[10,11] A variety of derivatives of the selenacycle have been prepared[12–14] and evaluated for their capacity to mimic the natural enzymes.[15,16] The many interesting biological activities of the ebselen family of compounds, both in vitro and in vivo, have also stimulated the search for other small-molecule organoselenium compounds with a capacity to mimic the GPx enzymes.[17]

Previously, we found that suitably positioned chalcogen substituents (alkylseleno[18] and alkyltelluro[19] groups) in phenols could increase the rate constant for quenching of peroxyl radicals by up to four orders of magnitude. We therefore thought it would be interesting to install hydroxy groups into one or the other of the aromatic rings in ebselen. In fact, some compounds of this sort, denoted herein as ebselenols, are known.[14b]

Methyl-protected isoselenazolones were prepared as shown in Scheme 1 from the corresponding 2-halobenzamides and selenium by means of copper catalysis.[12] Ebselenols **4**–**7** were obtained in 70–75 % yields by O-demethylation induced by boron tribromide. Addition of a stoichiometric quantity of $BBr_3$ was crucial. An excess amount of $BBr_3$ caused cleavage of the Se–N bond and substoichiometric amounts led to incomplete O-demethylation.

Ebselenols substituted with an OH group in the benzisoselenazolone ring were characterized by X-ray crystallography (Figure 2). Attempts to synthesize N-phenyl analogues of ebselenols **4** and **5** were unsuccessful since O-demethylation resulted in the formation of complex reaction mixtures.

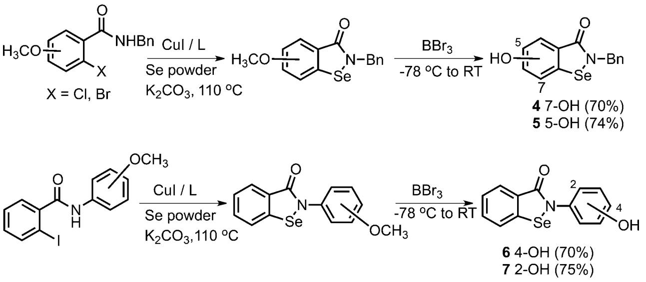
---

[*] S. Kumar, Prof. Dr. S. Kumar
Department of Chemistry
Indian Institute of Science Education and Research (IISER)
Bhopal-462066 (India)
E-mail: sangitkumar@iiserb.ac.in

J. Yan, J. Poon, Dr. V. P. Singh, Prof. Dr. L. Engman
Department of Chemistry—BMC, Uppsala University
Box-576, 75123 Uppsala (Sweden)
E-mail: lars.engman@kemi.uu.se

Dr. X. Lu, Dr. M. Karlsson Ott
Division of Applied Materials Science
Department of Engineering Sciences
Uppsala University (Sweden)

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/anie.201510947.



**Scheme 1.** Synthetic routes to ebselenols **4**–**7**. L = 1,10-phenanthroline.

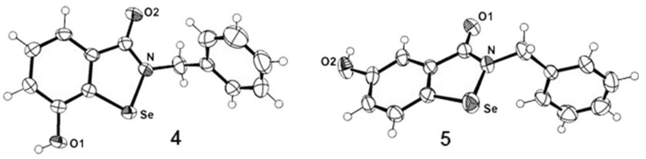


**Figure 2.** ORTEP diagrams of ebselenols **4** and **5**.[27]

Ebselenols **4**–**7** were evaluated for their capacity to inhibit azo-initiated peroxidation of linoleic acid in a two-phase chlorobenzene/water system open to the atmosphere.[20] Autoxidation of linoleic acid will give rise to the formation of conjugated diene hydroperoxides that can be analyzed by HPLC with UV detection. A plot of concentration versus time is linear and the slope of the line has a characteristic value, $R_{\text{uninh}}$, for the uninhibited peroxidation reaction (Figure 3).

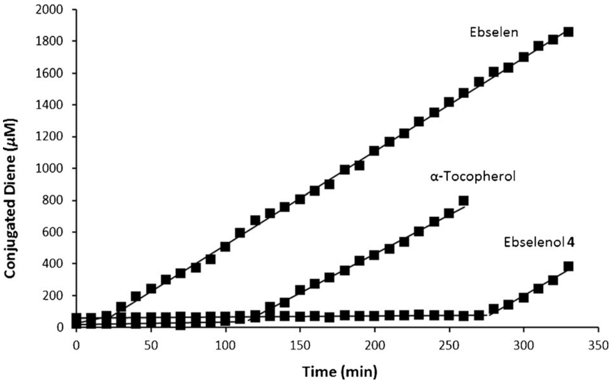


**Figure 3.** Peroxidation traces (conjugated diene concentration versus time) recorded with α-tocopherol (**1**), ebselen (**3**), and ebselenol (**4**) as antioxidants in the chlorobenzene layer and ascorbic acid (0.5 mM) in the aqueous phase.

Good chain-breaking antioxidants such as α-tocopherol will quench most of the peroxyl radicals formed and the slope of the line (the rate of peroxidation $R_{\text{inh}}$) during this inhibited phase of peroxidation will be substantially decreased. However, when the antioxidant is fully consumed, the rate of peroxidation will increase rapidly from $R_{\text{inh}}$ to $R_{\text{uninh}}$. This time is defined as the inhibition time ($T_{\text{inh}}$) of the antioxidant. The aqueous phase, which is in contact with the chlorobenzene layer by constant stirring, contains a water-soluble co-antioxidant that could hopefully regenerate the antioxidant and thus extend the $T_{\text{inh}}$ value.

Ebselenols **4**–**7** were first evaluated in the absence of any reducing agents in the aqueous phase (Table 1). α-Tocopherol (**1**) was used as a reference and benchmark antioxidant. Under our standard conditions (40 μM of antioxidant), both ebselenols **4** ($R_{\text{inh}} = 11$ μM h$^{-1}$) and **6** ($R_{\text{inh}} = 16$ μM h$^{-1}$) could outperform α-tocopherol ($R_{\text{inh}} = 28$ μM h$^{-1}$) in the quenching of peroxyl radicals. Although the $T_{\text{inh}}$ values for **6** (105 min) and α-tocopherol (109 min) were very similar, compound **4** could inhibit peroxidation for a considerably longer time (155 min). α-Tocopherol is known to trap two peroxyl radicals per molecule (the stoichiometric number $n$ is 2). The long inhibition time of ebselenol **4** seems to indicate that it is quenching peroxyl radicals with a stoichiometric number of $n = 3$. Increased $n$ values in the quenching of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals were recently reported as a consequence of phenylseleno substitution in zingerones (vanillylacetones).[21]

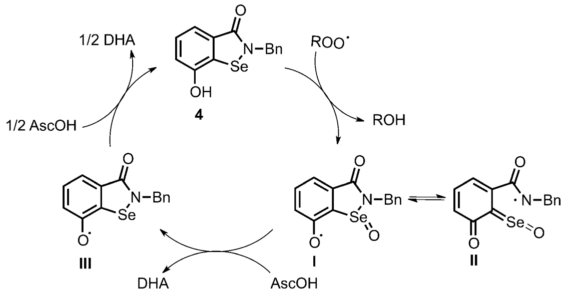
**Table 1:** Inhibited rate of peroxidation ($R_{\text{inh}}$) and time of inhibition ($T_{\text{inh}}$) for antioxidants (AO) tested with and without reducing agents.

| AO | NAC (1 mM) | | Ascorbic acid (0.5 mM) | | Without reducing agent | |
|---|---|---|---|---|---|---|
| | $R_{\text{inh}}$ [μM h$^{-1}$][a] | $T_{\text{inh}}$ [min][b] | $R_{\text{inh}}$ [μM h$^{-1}$][a] | $T_{\text{inh}}$ [min][b] | $R_{\text{inh}}$ [μM h$^{-1}$][a] | $T_{\text{inh}}$ [min][b] |
| **1** | 25 ± 1 | 97 ± 5 | 8 ± 3 | 114 ± 6 | 28 ± 2 | 109 ± 2 |
| **3** | 326 ± 8 | 0 | 127 ± 4 | 25 ± 1 | 312 ± 4 | 0 |
| **4** | 129 ± 3 | 0 | 5 ± 1 | 292 ± 9 | 11 ± 1 | 155 ± 2 |
| **5** | 318 ± 6 | 0 | 80 ± 2 | 230 ± 2 | 67 ± 5 | 75 ± 7 |
| **6** | 83 ± 6 | 26 ± 1 | 10 ± 2 | 222 ± 7 | 16 ± 3 | 105 ± 8 |
| **7** | 18 ± 2 | 43 ± 4 | 50 ± 8 | 93 ± 6 | 58 ± 10 | 70 ± 9 |

[a] Rate of peroxidation during the inhibited phase (uninhibited rate circa 479 μM h$^{-1}$). Uninhibited rate = 430 μM h$^{-1}$ with ascorbic acid in the aqueous phase. Uninhibited rate = 425 μM h$^{-1}$ with NAC in the aqueous phase. Errors correspond to ± SD (standard deviation) for triplicate measurements. [b] Duration of the inhibited phase of peroxidation. Errors correspond to ± SD for triplicate measurements.

Several chalcogen-containing antioxidants were found to be regenerable in the presence of *N*-acetylcysteine (NAC).[18,19] NAC was therefore added to the aqueous phase (1 mM initial concentration) and ebselenols **4**–**7** were tested again. Disappointingly, the compounds could no longer match the $T_{\text{inh}}$ values recorded for α-tocopherol (**1**) and the $R_{\text{inh}}$ value was often much higher than recorded in the absence of NAC., suggesting that they acted to retard rather than inhibit autoxidation. Ebselen is known to be readily ring-opened by thiols to form the corresponding selenenyl sulfides.[22] We speculate that compounds of this sort are formed under the conditions of the two-phase assay. Among other co-antioxidants tried, ascorbic acid (AscOH; 0.5 mM) showed an interesting synergistic effect. In the absence of any lipid-soluble antioxidant it did not show any inhibiting effect on peroxidation ($T_{\text{inh}} = 0$ and $R_{\text{inh}} \approx R_{\text{uninh}}$). However, in combination with the ebselenol antioxidants, the $T_{\text{inh}}$ value was often significantly extended (almost three-fold longer for compound **4** than for α-tocopherol). This suggests that ascorbic acid can cause a regeneration of the ebselenol antioxidants. Ebselen itself acted as a poor retarder of peroxidation. Shown in Figure 3 are peroxidation traces recorded with ebselen, α-tocopherol, and ebselenol **4**. There is a trend that the $R_{\mathrm{inh}}$ values are smaller in the presence of the co-antioxidant. It may be that the ebselenols during peroxidation are converted into the corresponding selenoxides (for example by O atom transfer from peroxyl radicals) which are likely to be poorer quenchers of peroxyl radicals than the parents. Ascorbic acid is known to act as a two-electron reducing agent towards selenoxides and could thus lower the $R_{\mathrm{inh}}$ value. α-Tocopherol also showed a lower $R_{\mathrm{inh}}$ value and a slightly longer $T_{\mathrm{inh}}$ in the presence of ascorbic acid.

Ebselenol **4** is unlikely to quench peroxyl radicals more rapidly than α-tocopherol if the reaction occurs by formal H atom transfer. We therefore propose a mechanism analogous to the one recently suggested[23] for alkyltelluro phenols. We propose that peroxyl radicals transfer an oxygen to selenium and the resulting alkoxyl radical abstracts a hydrogen atom from the nearby phenol, leading to phenoxide radical selenoxide **I** which could equilibrate with the ring-opened aminyl radical **II** (Scheme 2). Reduction of selenoxide **I** to selenide **III** occurs by interaction with aqueous-phase AscOH and is accompanied by dehydroascorbate (DHA) formation. Finally, regeneration of the antioxidant from the corresponding phenoxyl radical is brought about by AscOH. Some evidence for the proposed mechanism was obtained from EPR experiments (see page S54 in the Supporting Information for details). Photolysis of ebselenol **4** together with di-*tert*-butyl peroxide (DTBP) produced a single peak in the spectrum (attributable to the phenoxyl radical). After addition of $H_2O_2$ to the mixture of **4** and DTBP, a triplet signal was detected in the decoupled spectrum, indicative of an N-centered radical.



*Scheme 2.* Proposed mechanism for the chain-breaking antioxidant activity of ebselenol **4**. Bn = benzyl.

The GPx-like activity [Eq. (1)] of ebselenols **4–7** was assessed by a coupled reductase assay, using hydrogen peroxide ($H_2O_2$) and *tert*-butyl hydroperoxide (TBHP) as substrates in the presence of glutathione reductase.[24] All ebselenols were better GPx mimics than the parent ebselen (**3**; Table 2).

For every compound **3–7**, the catalytic activity was higher with $H_2O_2$ than with TBHP, in good agreement with previous studies.[14b] Additionally, when the catalysts were arranged according to performance in the two series of experiments, the ordering was the same: **4** > **5** > **6** ≈ **7**. The thiol peroxidase activity was studied in methanol containing hydrogen peroxide and PhSH using the UV method described by Tomoda and Iwaoka (Table 2).[25] Whereas ebselenols **5**, **6**, and **7** showed an activity similar to ebselen (**3**), compound **4** was a significantly better catalyst (15-fold more active than ebselen; Figure 4). The high activity of **4** may be the result of a proximity effect (the OH group and the Se center are close) as suggested recently.[14c,26]

*Table 2:* Initial rates ($\nu_o$) for the reduction of peroxides by thiols in the presence of ebselenol compounds.

| AO | $\nu_o$ [µM min$^{-1}$] with PhSH[a] $H_2O_2$ | $\nu_o$ [µM min$^{-1}$] with GSH[b] $H_2O_2$ | TBHP |
|---|---|---|---|
| **3** | 7.5 ± 0.3 | 142 ± 4 | 71 ± 3 |
| **4** | 129.9 ± 3.4 | 332 ± 4 | 229 ± 5 |
| **5** | 3.5 ± 0.4 | 240 ± 3 | 180 ± 1 |
| **6** | 9.9 ± 0.6 | 214 ± 2 | 136 ± 5 |
| **7** | 9.5 ± 1.0 | 210 ± 2 | 134 ± 3 |

[a] $\nu_o$ = Initial rates for the reduction of $H_2O_2$. Assay conditions: test compound (0.1 mM), benzenethiol (1 mM), $H_2O_2$ (3.75 mM) in MeOH. [b] Assay conditions: phosphate buffer (100 mM, pH 7.5), GSH (2 mM), NADPH (0.4 mM), EDTA (1 mM), glutathione reductase (0.67 unit mL$^{-1}$), peroxide (1.6 mM), and test compound (80 µM). EDTA = ethylenediaminetetraacetic acid.

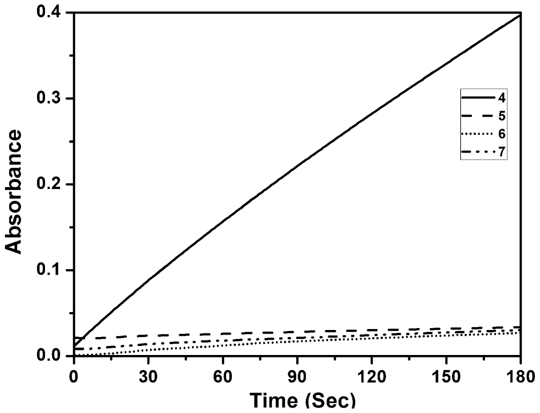


*Figure 4.* Thiol peroxidase activity of ebselenols **4–7**, measured by plotting the absorbance intensity of PhSSPh (monitoring at λ = 305 nm) versus time.

Stimulation of human mononuclear cells (MNC) can induce an "oxidative burst", a process in which a cocktail of reactive oxygen (ROS) and nitrogen (RNS) species, including hydrogen peroxide, superoxide, hydroxyl radicals, and peroxynitrite, is produced. Since our ebselenols could act both as chain-breaking and peroxide decomposing agents, we thought it would be interesting to see if they could relieve the effects of an oxidative burst. Freshly isolated human MNC were therefore stimulated with phorbol myristate acetate (PMA) and the amount of reactive intermediates formed were quantified by luminol-enhanced chemiluminescence (CL).

As shown in Figure 5, CL was significantly decreased in the presence of all four compounds **4–7** at 25 µM. Both ebselen (**3**) and the water-soluble phenolic antioxidant Trolox

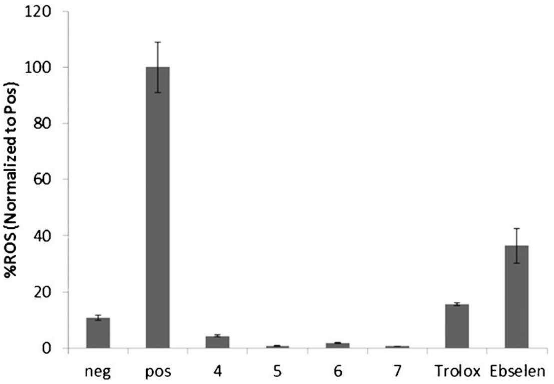


***Figure 5.*** Percentage ROS calculated on the basis of chemiluminescence intensity (monitoring at $\lambda = 425$ nm; normalized to the positive control)from PMA-stimulated MNC cells exposed to 25 μM concentration of antioxidants **4–7**, ebselen, and Trolox. $N = 6$ for each group from three independent experiments.

were tested at the same concentration but none of them could match the ROS/RNS-scavenging activity of the ebselenols.

To probe the toxicity of ebselenols **4–7**, MC3T3 cells (a pre-osteoblast cell line) were exposed to 1, 25, and 50 μM of ebselenols **4–7** and cell viability was checked after one and three days by using the Alamar Blue viability assay. No toxic effects of the compounds were seen at the two lower concentrations but a slight decrease was detected at 50 μM (Figure 6).

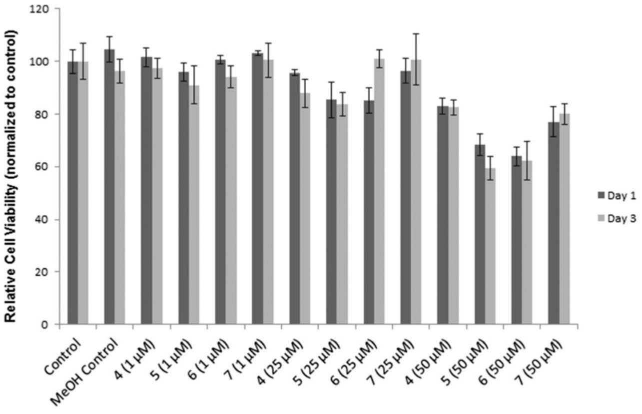


***Figure 6.*** Relative cell viability (normalized to control) of MC3T3 cells in the presence of 1, 25, and 50 μM concentrations of ebselenols **4–7** as determined by an Alamar Blue assay after 1 and 3 days.

In conclusion, we have described for the first time the ability of ebselenols to act as regenerable chain-breaking antioxidants in the presence of stoichiometric amounts of ascorbic acid. In the absence of the reducing agent, ebselenol **4** could trap peroxyl radicals with a stoichiometric number as high as $n = 3$. The substitution of a hydroxy group in ebselen also serves to improve its glutathione-peroxidase-like, hydroperoxide-decomposing activity. In cell systems, the ebselenols had a scavenging effect on reactive oxygen and nitrogen species produced by human MNCs.

## Acknowledgements

This study was supported financially by the DRDO and DST New Delhi (EMR/2015/000061), the IISER Bhopal, the Swedish Research Council, the Carl Tryggers Stiftelse (CTS 13:346 and CTS 13:120) and the Ollie and Elof Ericssons Stifelse för Vetenskaplig Forskning. Part of this work was facilitated by the BioMat facility/Science for Life Laboratory at Uppsala University.

**Keywords:** antioxidants · ascorbic acid · ebselen · selenium · cytotoxicity

**How to cite:** *Angew. Chem. Int. Ed.* **2016**, *55*, 3729–3733
*Angew. Chem.* **2016**, *128*, 3793–3797

[1] J. Pokorny, N. Yanishlieva, M. Gordon, *Antioxidants in Foods*, Woodhead Publishing, Cambridge, **2001**.

[2] H. Zweifel, *Stabilization of Polymeric Materials*, Springer, Berlin, **1997**.

[3] *Chemistry and Technology of Lubricants*, (Eds.: R. M. Mortier, S. T. Orszulik), Blackie Academic & Professional, London, **1997**.

[4] a) C. A. Rice-Evans, A. T. Diplock, *Free Radical Biol. Med.* **1993**, *15*, 77; b) A. Bast, *Drug News Perspect.* **1994**, *7*, 465; c) C. M. Andersson, A. Hallberg, T. Högberg, *Adv. Drug Res.* **1996**, *28*, 65; d) C. Santi, C. Tidei, C. Scalera, M. Piroddi, F. Galli, *Curr. Chem. Biol.* **2013**, *7*, 25.

[5] G. Scott in *Free Radicals: Chemistry, Pathology and Medicine* (Eds.: C. Rice-Evans, T. Dormandy), Richelieu Press, London, **1988**, p. 103.

[6] a) G. W. Burton, K. U. Ingold, *Acc. Chem. Res.* **1986**, *19*, 194; b) E. Niki, N. Noguchi, *Acc. Chem. Res.* **2004**, *37*, 45.

[7] B. Halliwell, J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, 4th ed., Oxford University Press, Oxford, **2007**.

[8] J. Lu, A. Holmgren, *J. Biol. Chem.* **2009**, *284*, 723.

[9] a) L. Valgimigli, R. Amorati, S. Petrucci, G. F. Pedulli, D. Hu, J. J. Hanthorn, D. A. Pratt, *Angew. Chem. Int. Ed.* **2009**, *48*, 8348; *Angew. Chem.* **2009**, *121*, 8498; b) J. J. Hanthorn, L. Valgimigli, D. A. Pratt, *J. Am. Chem. Soc.* **2012**, *134*, 8306; c) L. Valgimigli, D. A. Pratt, *Acc. Chem. Res.* **2015**, *48*, 966; d) R. Shah, E. A. Haidasz, L. Valgimigli, D. A. Pratt, *J. Am. Chem. Soc.* **2015**, *137*, 2440.

[10] H. Sies, *Free Radical Biol. Med.* **1993**, *14*, 313, and references therein.

[11] B. K. Sarma, G. Mugesh, *Chem. Eur. J.* **2008**, *14*, 10603.

[12] a) S. J. Balkrishna, B. S. Bhakuni, D. Chopra, S. Kumar, *Org. Lett.* **2010**, *12*, 5394; b) S. J. Balkrishna, B. S. Bhakuni, S. Kumar, *Tetrahedron* **2011**, *67*, 9565.

[13] a) G. Mugesh, H. B. Singh, *Chem. Soc. Rev.* **2000**, *29*, 347; b) S. S. Zade, H. B. Singh, R. J. Butcher, *Angew. Chem. Int. Ed.* **2004**, *43*, 4513; *Angew. Chem.* **2004**, *116*, 4613; c) S. S. Zade, S. Panda, S. K. Tripathi, H. B. Singh, G. Wolmershäuser, *Eur. J. Org. Chem.* **2004**, 3857; d) M. Pietka-Ottlik, P. Potaczek, E. Piasecki, J. Młochowski, *Molecules* **2010**, *15*, 8214; e) K. Selvakumar, P. Shah, H. B. Singh, R. J. Butcher, *Chem. Eur. J.* **2011**, *17*, 12741; f) K. Selvakumar, H. B. Singh, N. Goel, U. P. Singh, R. J. Butcher, *Chem. Eur. J.* **2012**, *18*, 1444; g) A. J. Pacuła, J. Ścianowski, K. B. Aleksandrzak, *RSC Adv.* **2014**, *4*, 48959.

[14] For oxygen-substituted GPx mimics, see: a) T. Wirth, *Molecules* **1998**, *3*, 164; b) K. P. Bhabak, G. Mugesh, *Chem. Eur. J.* **2007**, *13*, 4594; c) D. Bhowmick, S. Srivastava, P. D′Silva, G. Mugesh, *Angew. Chem. Int. Ed.* **2015**, *54*, 8449; *Angew. Chem.* **2015**, *127*, 8569; d) T. Wirth, *Angew. Chem. Int. Ed.* **2015**, *54*, 10074; *Angew. Chem.* **2015**, *127*, 10212.

[15] K. Satheeshkumar, G. Mugesh, *Chem. Eur. J.* **2011**, *17*, 4849.

[16] Z. Luo, L. Liang, J. Sheng, Y. Pang, J. Li, L. Huang, X. Li, *Bioorg. Med. Chem.* **2014**, *22*, 1355.

[17] K. P. Bhabak, G. Mugesh, *Acc. Chem. Res.* **2010**, *43*, 1408.

[18] S. Kumar, H. Johansson, L. Engman, L. Valgimigli, R. Amorati, M. G. Fumo, G. F. Pedulli, *J. Org. Chem.* **2007**, *72*, 2583.

[19] a) J. Malmström, M. Jonsson, I. A. Cotgreave, L. Hammarström, M. Sjödin, L. Engman, *J. Am. Chem. Soc.* **2001**, *123*, 3434; b) S. Kumar, H. Johansson, T. Kanda, L. Engman, T. Müller, M. Jonsson, G. F. Pedulli, S. Petrucci, L. Valgimigli, *Org. Lett.* **2008**, *10*, 4895; c) S. Kumar, H. Johansson, T. Kanda, L. Engman, T. Müller, H. Bergenudd, M. Jonsson, G. F. Pedulli, R. Amorati, L. Valgimigli, *J. Org. Chem.* **2010**, *75*, 716; d) H. Johansson, D. Shanks, L. Engman, R. Amorati, G. F. Pedulli, L. Valgimigli, *J. Org. Chem.* **2010**, *75*, 7535; e) V. P. Singh, J. Poon, L. Engman, *J. Org. Chem.* **2013**, *78*, 1478; f) J. Poon, V. P. Singh, L. Engman, *J. Org. Chem.* **2013**, *78*, 6008; g) V. P. Singh, J. Poon, L. Engman, *Org. Lett.* **2013**, *15*, 6274; h) V. P. Singh, J. Poon, R. J. Butcher, L. Engman, *Chem. Eur. J.* **2014**, *20*, 12563; i) J. Poon, V. P. Singh, J. Yan, L. Engman, *Chem. Eur. J.* **2015**, *21*, 2447.

[20] a) K. Vessman, M. Ekström, M. Berglund, C.-M. Andersson, L. Engman, *J. Org. Chem.* **1995**, *60*, 4461; b) D. Shanks, R. Amorati, M. G. Fumo, G. F. Pedulli, L. Valgimigli, L. Engman, *J. Org. Chem.* **2006**, *71*, 1033. See Ref. [18] for a figure showing the experimental setup.

[21] D. M. Martinez, A. M. Barcellos, A. M. Casaril, L. Savegnago, G. Perin, C. H. Schiesser, K. L. Callaghan, E. J. Lenardão, *Tetrahedron Lett.* **2015**, *56*, 2243.

[22] B. K. Sarma, G. Mugesh, *J. Am. Chem. Soc.* **2005**, *127*, 11477.

[23] R. Amorati, L. Valgimigli, P. Dinér, K. Bakhtiari, M. Saedi, L. Engman, *Chem. Eur. J.* **2013**, *19*, 7510.

[24] S. R. Wilson, P. A. Zucker, R.-R. C. Huang, A. Spector, *J. Am. Chem. Soc.* **1989**, *111*, 5936.

[25] M. Iwaoka, S. Tomoda, *J. Am. Chem. Soc.* **1994**, *116*, 2557.

[26] S. P. Thomas, K. Satheeshkumar, G. Mugesh, T. N. Guru Row, *Chem. Eur. J.* **2015**, *21*, 6793.

[27] CCDC 1427923 (**4**) and 1427922 (**5**) contain the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre.

Received: November 27, 2015
Revised: January 11, 2016