Anti-Thyroid Drugs and Thyroid Hormone Synthesis: Effect of Methimazole Derivatives on Peroxidase-Catalyzed Reactions

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · DECEMBER 2005

Impact Factor: 12.11 · DOI: 10.1021/ja054497u · Source: PubMed

2 AUTHORS:

Gouriprasanna Roy
Shiv Nadar University
21 PUBLICATIONS **451** CITATIONS
SEE PROFILE

Govindasamy Mugesh
Indian Institute of Science
155 PUBLICATIONS **4,332** CITATIONS
SEE PROFILE

J|A|C|S
ARTICLES
Published on Web 10/08/2005

Anti-Thyroid Drugs and Thyroid Hormone Synthesis: Effect of Methimazole Derivatives on Peroxidase-Catalyzed Reactions

Gouriprasanna Roy and G. Mugesh*

Contribution from the Department of Inorganic & Physical Chemistry, Indian Institute of Science, Bangalore 560 012, India

Received July 7, 2005; E-mail: mugesh@ipc.iisc.ernet.in

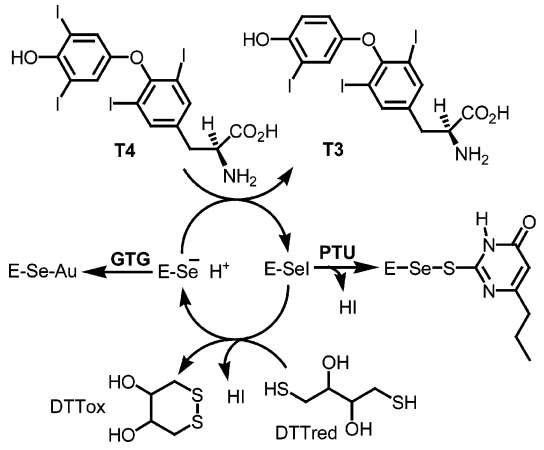
Abstract: Syntheses and characterization of the selenium analogue (**MSeI**) of anti-thyroid drug methimazole and a series of organoselenium compounds bearing *N*-methylimidazole pharmacophore are described. In contrast to the sulfur compound that exists predominantly in its thione form, the selenium analogue exists in a selenol form, which spontaneously oxidizes in air to produce the corresponding diselenide. The reduction of the diselenide by GSH or $NaBH_4$ affords the biologically active selenol, which effectively inhibits the lactoperoxidase (LPO) activity in vitro. The monoselenides having *N*-methylimidazole moiety are found to be much less active than the selenol, suggesting that the presence of a selenol moiety is important for the LPO inhibition. The kinetic and mechanistic studies reveal that **MSeI** inhibits the LPO activity by reducing the H_2O_2, providing a novel method to reversibly inhibit the enzyme. Although **MSeI** strongly inhibits LPO, the enzyme's activity can be completely recovered by increasing the H_2O_2 concentration. On the other hand, the inhibition by methimazole (**MMI**), the sulfur analogue, cannot be reversed by increasing the H_2O_2 concentration, leading to a complete inactivation of the enzyme. The reversible inhibition of LPO by some of the selenium derivatives is correlated with their glutathione peroxidase (GPx) activity, and the high GPx activity of the selenium compounds as compared with their sulfur analogues suggests that the selenium derivatives may protect the thyroid gland from oxidative damage.

Introduction

Thyroxine (**T4**), the main secretory hormone of the thyroid gland, is produced from thyroglobulin by thyroid peroxidase (TPO)/hydrogen peroxide/iodide system. The synthesis of **T4** by TPO involves two independent steps: iodination of tyrosine and phenolic coupling of the resulting iodotyrosine residues.[1] The prohormone **T4** is then converted to its biologically active form **T3** by a selenium-containing iodothyronine deiodinase (ID-I), which is present in highest amounts in liver, kidney, thyroid and pituitary. The 5′-deiodination catalyzed by ID-I is a ping-pong, bisubstrate reaction in which the selenol (or selenolate) group of the enzyme (E-SeH or E-Se$^-$) first reacts with thyroxine (**T4**) to form a selenenyl iodide (E-SeI) intermediate. Subsequent reaction of the selenenyl iodide with an as yet unidentified intracellular cofactor (1,4-dithiothreitol (DTT, *Cleland*'s reagent) in vitro) completes the catalytic cycle and regenerates the selenol (Scheme 1).[2]

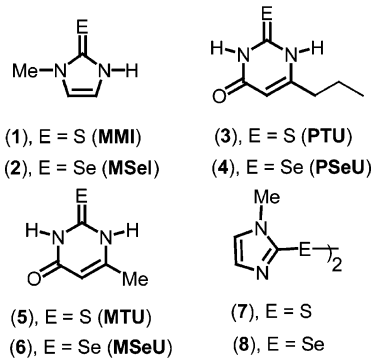
Although the deiodination reactions are essential for the function of the thyroid gland, the activation of thyroid stimulating hormone (TSH) receptor by autoantibodies leads to an overproduction of thyroid hormones. In addition, these antibodies stimulate ID-I and probably other deiodinases[3] to produce relatively more **T3**. As these antibodies are not under the pituitary feedback control system, there is no negative influence on the thyroid activity, and therefore, the uncontrolled production of thyroid hormones leads to a condition called "hyperthyroidism". Under these conditions, the overproduction of **T4** and **T3** can be controlled by specific inhibitors, which either block the thyroid hormone biosynthesis or reduce the conversion of **T4** to **T3**. A unique class of such inhibitors is the thiourea

Scheme 1. Proposed Mechanism for the Deiodination of Thyroxine by ID-I and Inhbition of ID-I by *n*-Propyl-2-thiouracil (PTU) and Gold Thioglucose (GTG)



(1) (a) Doerge, D. R. *Xenobiotica* **1985**, *25*, 761−767. (b) Taurog, A. Thyroid Hormone Synthesis. In *Werner's The Thyroid*; Braverman, L. E.; Utiger, R. D., Eds. 1991, 51−97. (c) Taurog, A.; Doriss, M. L.; Doerge, D. R. *Arch. Biochem. Biophys.* **1994**, *315*, 82−89. (d) Doerge, D. R.; Taurog, A.; Doriss, M. L. *Arch. Biochem. Biophys.* **1994**, *315*, 90−99. (e) Taurog, A.; Doriss, M. L.; Doerge, D. R. *Arch. Biochem. Biophys.* **1996**, *330*, 24−32.

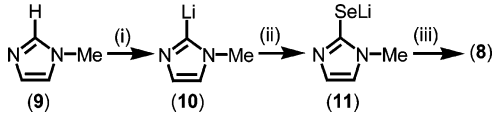
drugs, methimazole (**1**, MMI), 6-*n*-propyl-2-thiouracil (**3**, PTU), and 6-methyl-2-thiouracil (**5**, MTU).



Although these compounds are the most commonly employed drugs in the treatment of hyperthyroidism, the detailed mechanism of their action is still not clear. According to the initially proposed mechanism, these drugs may divert oxidized iodides away from thyroglobulin by forming stable electron donor−acceptor complexes with diiodine, which can effectively reduce the thyroid hormone biosynthesis.[4] It has also been proposed that these drugs may block the thyroid hormone synthesis by coordinating to the metal center of thyroid peroxidase (TPO).[5] After the discovery that the ID-I is responsible for the activation of thyroxine, it has been reported that PTU, but not MMI, reacts with the selenenyl iodide intermediate (E-SeI) of ID-I to form a selenenyl sulfide as a dead end product, thereby blocking the conversion of **T4** to **T3** during the monodeiodination reaction.[2] The mechanism of anti-thyroid activity is further complicated by the fact that the gold-containing drugs such as gold thioglucose (GTG) inhibit the deiodinase activity by reacting with the selenol group of the native enzyme (Scheme 1).[2]

Recently, the selenium analogues **2** (**MSeI**), **4** (**PSeU**) and **6** (**MSeU**) attracted considerable attention because these compounds are expected to be more nucleophilic than their sulfur analogues and the formation of an −Se−Se− bond may occur more readily than the formation of an −Se−S− bond with the ID-I enzyme.[6] However, the data derived from the inhibition of TPO by selenium compounds show that these compounds may inhibit the TPO activity by a different mechanism. Therefore, further studies are required to understand the mechanism by which the selenium compounds exert their inhibitory action. We have shown, in a preliminary communication, that the unexpected behavior of the selenium compounds as compared to that of their sulfur analogues may be due to their facile oxidation to the corresponding diselenides.[7] Our initial attempts to isolate **2** were unsuccessful, and the final stable compound in the synthesis was characterized to be the diselenide (**8**). In view of the current interest in anti-thyroid drugs and their mechanism, we extended our approach to the synthesis and biological activities of a number of sulfur and selenium derivatives bearing the methimazole pharmacophore. In this article, we provide experimental evidence that the replacement the sulfur atom in methimazole by selenium leads to a completely different mechanism. In addition, we describe the effect of a range of sulfur and selenium compounds bearing methimazole moiety on peroxidase-catalyzed oxidation reactions. We also describe the effect of substituents attached to the imidazole moiety on the selenol−selone tautomerism by theoretical calculations.

Scheme 2. Synthetic Route to Compound **8**[a]



[a] Reagents and conditions: i) *n*-BuLi, THF, −78 °C, 40 min; ii) Se powder, rt; iii) H_2O, 1 N HCl.

Results and Discussion

The synthesis of selones can be achieved by a variety of methods, which include reactions with electrophilic selenium, nucleophilic selenium and carbon diselenide.[8] The synthesis of **2** was approached by the electrophilic selenium route, which utilizes the high reactivity of elemental selenium toward vinyl anions (Scheme 2). The low-temperature metalation of 1-methylimidazole (**9**) by *n*-BuLi afforded the lithiated species **10**, which upon treatment with elemental selenium produced the corresponding lithium selenolate **11**. The addition of a 1 N HCl solution followed by an aqueous workup afforded a viscous liquid, which slowly solidified to form an orange solid. This method was originally employed by Guziec et al. for the synthesis of selone **2**, and in this particular case, the authors have observed the formation of two types of compounds that differ considerably from each other in their melting points.[6d] However, all our attempts to isolate the selone (**2**) were unsuccessful, and this compound readily oxidized by air to produce the corresponding diselenide (**8**). This is in agreement with the report of Guziec et al. that the recrystallization of **2** affords orange crystals having a melting point of 142 °C,[6d] which is identical with that of **8** in our synthesis. This indicates that the diselenide (**8**) is the final stable compound in the preparation. The structure of this compound was confirmed by a variety of techniques including ^{77}Se NMR studies. The proposed diselenide structure was finally confirmed by single-crystal X-ray studies.[7]

The tautomeric behavior of MMI has been subjected to many investigations,[9] which show that MMI exists almost exclusively as the thione tautomer (**1a**, Figure 1). Recent studies have shown

(2) (a) Behne, D.; Kyriakopoulos, A.; Meinhold, H.; Köhrle, J. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 1143−1149. (b) Berry, M. J.; Banu, L.; Larsen, P. R. *Nature* **1991**, *349*, 438−440. (c) Berry, M. J.; Kieffer, J. D.; Harney, J. W.; Larsen, P. R. *J. Biol. Chem.* **1991**, *266*, 14155−14158. (d) Köhrle, J. *Exp. Clin. Endocrinol.* **1994**, *102*, 63−89. (e) Larsen, P. R.; Berry, M. J. *Annu. Rev. Nutr.* **1995**, *15*, 323−352. (f) St. Germain, D. L.; Galton, V. A. *Thyroid* **1997**, *7*, 655−668. (g) Köhrle, J. *Biochimie* **1999**, *81*, 527−553. (h) du Mont, W.-W.; Mugesh, G.; Wismach, C.; Jones, P. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 2486−2489. (i) Bianco, A. C.; Salvatore, D.; Gereben, B.; Berry, M. J.; Larsen, P. R. *Endocr. Rev.* **2002**, *23*, 38−89. (j) Köhrle, J. *Methods Enzymol.* **2002**, *347*, 125−167.

(3) In addition to ID-I, two other deiodinases (ID-II and ID-III) have been shown to have selenium in their active centers (For more details, see refs 2e−j).

(4) (a) Buxeraud, J.; Absil, A. C.; Claude, J.; Raby, C.; Catanzano, G.; Beck, C. *Eur. J. Med. Chem.* **1985**, *20*, 43−50. (b) Raby, C.; Lagorce, J. F.; Jambut-Absil, A. C.; Buxeraud, J.; Catanzano, G. *Endocrinology* **1990**, *126*, 1683−1691.

(5) Bassosi, R.; Niccolai, N.; Rossi, C. *Biophys. Chem.* **1978**, *8*, 61−69.

(6) (a) Visser, T. J.; Kaptein, E.; Aboul-Enein, H. Y. *Biochem. Biophys. Res. Commun.* **1992**, *189*, 1362−1367. (b) Aboul-Enein, H. Y.; Awad, A. A.; Al-Andis, N. M. *J. Enzymol. Inhib.* **1993**, *7*, 147−150. (c) Taurog, A.; Dorris, M. L.; Guziec, L. J.; Guziec, F. S., Jr. *Biochem. Pharmacol.* **1994**, *48*, 1447−1453. (d) Guziec, L. J.; Guziec, F. S., Jr. *J. Org. Chem.* **1994**, *59*, 4691−4692. (e) Taurog, A.; Dorris, M. L.; Hu, W.-X.; Guziec, F. S., Jr. *Biochem. Pharmacol.* **1995**, *49*, 701−709.

(7) Roy, G.; Nethaji, M.; Mugesh, G. *J. Am. Chem. Soc.* **2004**, *126*, 2712−2713.

(8) Murai, T.; Kato, S.; Selenocarbonyls. In *Topics in Current Chemistry*; Wirth, T.; Ed.; Springer-Verlag, Berlin, 2000; Vol. 208, pp 177−199.

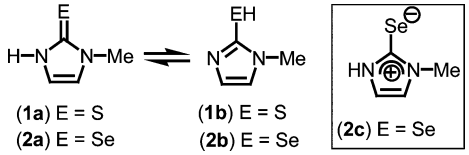


Figure 1. Proposed tautomeric structures of **MMI** and **MSeI**.

that the thiourea-based drugs PTU and MTU also exist as the thione tautomers.[10] The stability of the thione tautomers may prevent these compounds from being oxidized to their corresponding disulfides, which may account for their high antithyroidal activity. Laurence et al. have shown that the thione tautomer of MMI is responsible for its complexation with diiodine and the iodine complex of the thione tautomer **1a** is favored by 13.2 kJ·mol^{-1} compared to that of the thiol tautomer **1b**.[11a] Therefore, the facile oxidation of **2** to the corresponding diselenide (**8**) requires the compound to be in its selenol form (**2b**) and not in the selone form (**2a**). Although compound **2** can exist in both selenol and selone forms in solution, the ^{77}Se NMR spectrum recorded immediately after the workup of the reaction showed a signal at 4 ppm, which can be ascribed to the selenol (**2b**) tautomer. In the presence of air, the selenol slowly oxidizes to the corresponding diselenide, which shifts the equilibrium to the right (Figure 1), and this process continues until all the selone−selenol mixture is converted to diselenide **8**. In contrast, the sulfur analogue, which exists predominantly in its thione tautomer form (**1a**), was found to be very stable and could not be converted to the corresponding disulfide (**7**) even by using oxidizing agents such as O_2, H_2O_2 etc. It should be mentioned that MMI is readily oxidized by the TPO system to form the disulfide. Although the oxidation of this compound by iodine has been postulated to be a possible mechanism,[12] the chemical way through which MMI is transformed into disulfide **7** in vivo is unknown.

The theoretical investigations on selones are highly limited to the compounds having simple substituents, mainly due to the requirement of large basis sets for the calculations.[13] The relatively larger size and more polarizability of selenium as compared with those of sulfur have led to the assumption that the compounds with selone moiety are less stable than their sulfur analogues. Because the inhibition of TPO by anti-thyroid drugs depends on the redox state of sulfur or selenium, we performed detailed quantum chemical calculations on **1** and **2**

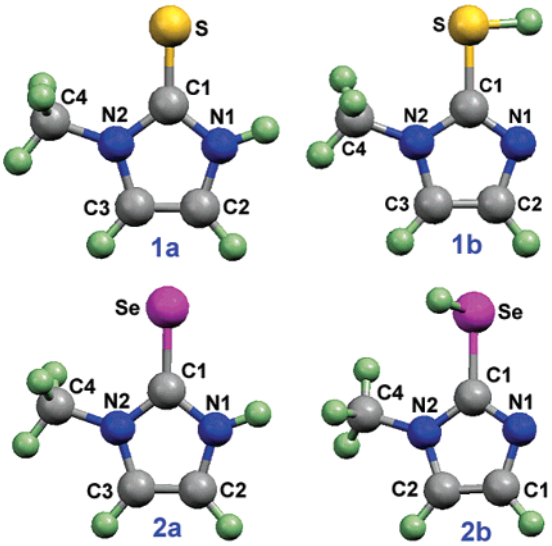


Figure 2. Optimized geometries of **1** and **2**. The conversion of thiol to thione is more favored than the conversion of selenol to selone. The structures were optimized at the B3LYP level of theory using 6-311++G-(d,p) basis set.

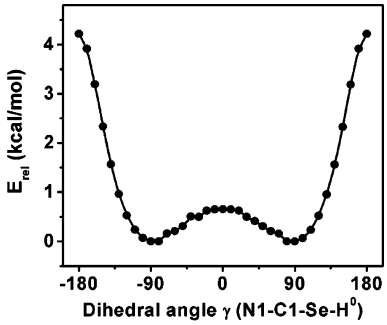


Figure 3. Variation of relative stabilization energy of **2b** for the change in the N1−C1−Se−H dihedral angle. The single-point energies were calculated using B3LYP/6-311++G(d,p) level of theory.

in gas phase. These studies show that the formation of the diselenide (**8**) from **2b** is energetically more favored than the formation of the disulfide (**7**) from the corresponding thiol (**1b**) (see Supporting Information, Scheme S1). Interestingly, the conversion of thiol to thione is more favored than the conversion of selenol to the corresponding selone. This can be rationalized by comparing the relative position of hydrogen on sulfur or selenium with respect to N1 in their most stable conformations. In the selenol (**2b**, Figure 2), the H atom is located away from N1, leading to an increase in the energy barrier for the selenol−selone conversion. In contrast, the H atom is located in the close proximity of N1 in the thiol (**1b**, Figure 2), which may favor the thiol−thione conversion. The calculations performed at the B3LYP level of theory using 6-311++G(d,p) show that the stucture with a N1−C1−Se−H dihedral angle of 107° is the most stable conformation. The variation in the relative stabilization energy of **2b** for changing the position of H atom with respect to the N1−C1−Se plane is shown in Figure 3. The position of this hydrogen with respect to the two nitrogen atoms in the imidazole ring also confirms the delocalized charge as shown in **2c**. As the thione form is calculated to be much more stable than the corresponding thiol, it is quite unlikely that the thiol form contributes to the anti-thyroid activity of MMI. Although the selone (**2a**) was also calculated to be more stable than the selenol (**2b**), the facile oxidation of the selenol to the

(9) (a) Kjellin, G.; Sandström, J. *Acta Chem. Scand.* **1969**, *23*, 2888−2899. (b) Fauré, R.; Vincent, E. J.; Assef, G.; Kister, J.; Metzger, J. *Org. Magn. Reson.* **1977**, *9*, 688−694. (c) Bojarska-Olejnik, E.; Stefaniak, L.; Witanowski, M.; Hamdi, B. T.; Webb, G. A. *Magn. Reson. Chem.*, **1985**, *23*, 166−169. (d) Balestrero, R. S.; Forkey, D. M.; Russell, J. G. *Magn. Reson. Chem.* **1986**, *24*, 651−655.
(10) Antoniadis, C. D.; Corban, G. J.; Hadjikakou, S. K.; Hadjiliadis, N.; Kubicki, M.; Warner, S. Butler, I. S. *Eur. J. Inorg. Chem.* **2003**, *8*, 1635−1640.
(11) (a) Laurence, C.; El Ghomari, M. J.; Le Questel, J.-Y.; Berthelot, M.; Mokhlisse, R. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1545−1551. (b) Although there is no definite evidence for the coordination of the thione moiety of the anti-thyroid drugs to the iron center, this model is given on the basis of the fact that iron−sulfur coordination is very common in metalloenzymes and hydrogen bonding of heme ligands with a distal histidine appears to be common in peroxidases. (For details, see ref 11a.)
(12) Aragoni, M. C.; Arca, M.; Demartin, F.; Devillanova, F. A.; Garau, A.; Isaia, F.; Lippolis, V.; Verani, G. *J. Am. Chem. Soc.* **2002**, *124*, 4538−4539.
(13) (a) Bock, H.; Aygen, S.; Rosmus, P.; Solouki, B.; Weissflog, E. *Chem. Ber.* **1984**, *117*, 187−202. (b) Collin, S.; Back, T. G.; Rauk, A. *J. Am. Chem. Soc.* **1985**, *107*, 6589−6592. (c) Dapprich, S.; Frenking, G. *Chem. Phys. Lett.* **1993**, *205*, 337−342. (d) Kwiatkowski, J. S.; Leszczynski, J. *Mol. Phys.* **1994**, *81*, 119−131. (e) Ha, T.-K.; Puebla, C. *Chem. Phys.* **1994**, *181*, 47−55. (f) Jemmis, E. D.; Giju, K. T.; Leszczynski, J. *J. Phys. Chem. A* **1997**, *101*, 7389−7395.

corresponding diselenide disfavors the selenol−selone conversion by 13.42 kcal/mol.

According to the previous studies on tautomeric rearrangements,[14] the electron delocalization in selenourea should be larger than that in thiourea and urea. On the other hand, the lower electronegativity of Se (2.4) as compared with those of S (2.5) and O (3.5) suggests that the electron delocalization in selenourea should be less than that of thiourea and urea. However, Glendening and Hrabal reported that the polarizability of the C−X (X = O, S, Se) bond rather than electronegativity of X plays an important role in allowing the chalcogen atom to accommodate more charge density.[15] In accordance with this, the C−Se bond length of 1.833 Å in selone is found to be much longer than that observed in compound **15** (1.775 Å), which does not have any nitrogen atom in the five-membered ring. This also confirms the tautomeric behavior of **2**. The ^{77}Se NMR chemical shift of 30 ppm for 2 with respect to Me_2Se suggests the existence of this compound in a selenolate form (**2c**). To further understand the nature of selenium moiety under in vivo conditions, we included the solvent effects in the calculations by using Tomasi's polarizable continuum model (PCM).[16] The structure of selone (**2a**) was optimized in water (ϵ = 78.39) using the PC model at the B3LYP/6-311++G(d,p) level, and the ^{77}Se NMR chemical shift was calculated by using GIAO method[17] at the same level of theory as with 6-311++G(2d,p) basis set. These calculations predict a ^{77}Se NMR chemical shift of 67 ppm, which is shifted upfield as compared with that of selenourea (350 ppm), confirming the existence of **2** in its selenolate form.

The reactivity and reaction patterns of selones, in general, vary considerably, depending upon the substituents adjacent to the selenocarbonyl group. Therefore, the heteroatom-substituted selones are more polar than selenoaldehydes and selenoketones.[8] In view of this, we have undertaken further studies to understand the effect of substituents on selenocarbonyl moiety. The replacement of the H atom in **2a** with a methyl substituent (compound **14**, Figure 4) also leads to the stabilization of this compound in its selenolate form as evidenced by a large upfield shift in the ^{77}Se NMR chemical shift (46 ppm). In contrast, an isosteric replacement of the −NH− moiety in **2a** with a $-CH_2-$ group stabilizes the compound (**12**) in its selone form (**12a**), indicating that the presence of a nitrogen atom in the 5-position is important for the conversion of selone to the more reactive selenolate. The calculated ^{77}Se NMR chemical shift showed a dramatic downfield shift for **12a** (659 ppm) as compared with that for **2a**, confirming the stability of compound **12** in its selone form (Table 1). On the other hand, the optimized geometry of the corresponding selenol (**12b**) and the ^{77}Se NMR chemical shift calculated for this species (−150 ppm) suggest that the −SeH group in this compound exists truly as a selenol moiety and not in a tautomeric form. This clearly indicates that the presence of the unsubstituted nitrogen in **2b** is responsible for the formation of the zwitterion **2c**. It should be mentioned that

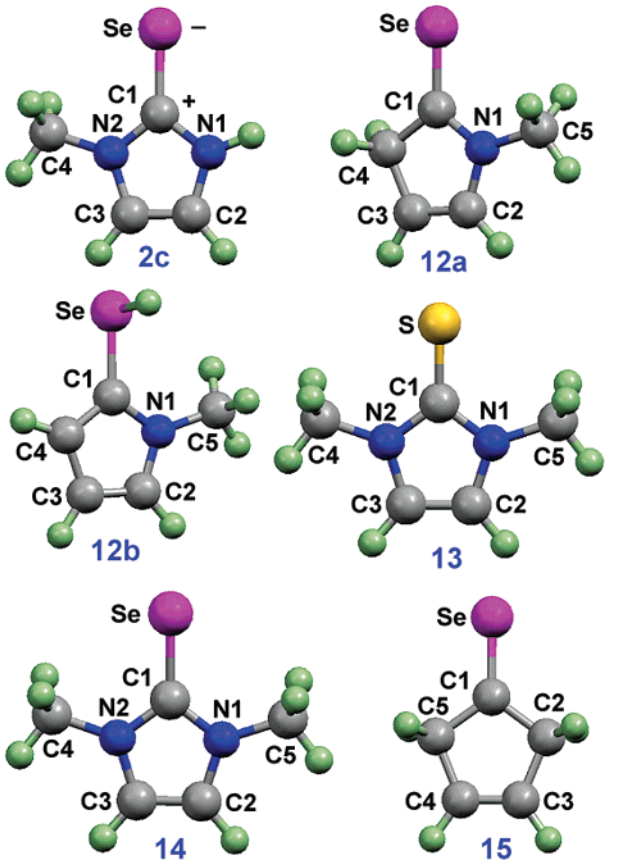


Figure 4. Optimized geometries of **2c**, **12**−**15**. The structures were optimized at the B3LYP level of theory using 6-311++G(d,p) basis set.

Table 1. Theoretical Data for **2**, **8**, **12**, **14**, and **15** Obtained by DFT Calculations at B3LYP/6-311++G(d,p) Level along with the GIAO ^{77}Se NMR Chemical Shifts

no.	compd	C−Se bond length (Å)	^{77}Se chemical shift (ppm)[b]
1	**2a**	1.835	30
2	**2b**	1.917	−101
3	**2c**	1.835	30
4	**8**	1.902[a]	386
5	**12a**	1.908	659
6	**12b**	1.811	−150
7	**14**	1.839	46
8	**15**	1.774	2100

[a] Calculated using B3LYP/6-31G(d) level. [b] NMR values were calculated using B3LYP/6-311++G(2d,p) level and referenced to Me_2Se.

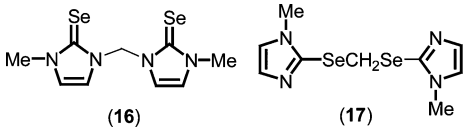
the replacement the N−H moiety in **1** with the N−Me group has been shown to abolish the inhibitory effect of **1** in in vivo experiments.[18] In agreement with this, the calculations on **13** show that the thione form is highly stabilized by the methyl substituents.

Although compound **2** readily oxidizes to diselenide **8**, the corresponding selenol (**2b**) can be conveniently obtained by reducing the diselenide by $NaBH_4$ or glutathione (GSH). The reaction of **8** with $NaBH_4$ followed by aqueous workup afforded the selenol as yellow solid, which was found to be stable under inert atmosphere and could be employed for in vitro biological assays without any noticeable oxidation. The treatment of diselenide **8** with 2 equiv of GSH has also produced the selenol

(14) (a) Sakaizumi, T.; Yasukawa, A.; Miyamoto, H.; Ohashi, O.; Yamaguchi, I. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1614−1616. (b) Leszczynski, J.; Kwiatkowski, J. S.; Leszczynska, D. *J. Am. Chem. Soc.* **1992**, *114*, 10089−10091. (c) Prasad, B. V.; Uppal, P.; Bassi, P. S. *Chem. Phys. Lett.* **1997**, *276*, 31−38. (d) Moudgil, R.; Bharatam, P. V.; Kaur, R.; Kaur, D. *Proc. Ind. Acad. Sci.* (*Chem. Sci.*) **2002**, *114*, 223−230.
(15) Glendening, E. D.; Hrabal, J. A., II. *J. Am. Chem. Soc.* **1997**, *119*, 12940−12946.
(16) Miertus, S.; Scrocco, E.; Tomasi, J. *J. Chem. Phys.* **1981**, *55*, 117−129.
(17) Nakanishi, W.; Hayashi, S. J. *J. Phys. Chem. A* **1999**, *103*, 6074−6081.
(18) Visser, T. J.; Van Overmeeren, E.; Fekkes, D.; Docter, R.; Hennemann, G. *FEBS Lett.* **1979**, *103*, 314−318.

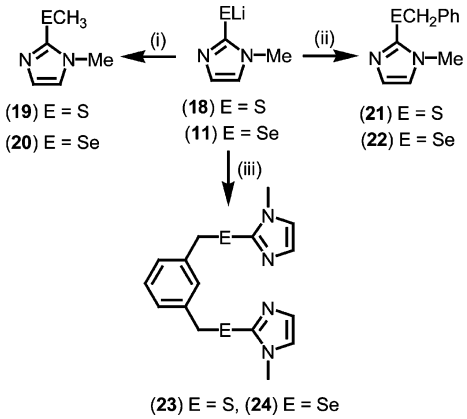
in nearly a quantitative yield. Interestingly, the formation of selone (**2a**) was not observed in any of these processes, supporting the theoretical calculations that the selenol is deprotonated (**2c**) by the nitrogen atoms present in the imidazole ring. The ^{1}H and ^{13}C NMR data and the large upfield shift in the ^{77}Se NMR chemical shift for the selenol (−5 ppm) supports this assumption. In agreement with the theoretical data, the ^{77}-Se NMR experiments show that the selenol is more dissociated in water (−53 ppm) than in organic solvent (−5 ppm) as evidenced by a large upfield shift when changing the solvent from $CDCl_3$ to D_2O. The facile reduction of **8** by GSH suggests that this compound may exist in its selenol form under in vivo conditions, because GSH is present in the thyroid gland in high concentrations. It has been shown that GSH is an important antioxidant in thyroid gland and the peroxide scavenging activity of MMI is considerably increased by GSH.[19]

Interestingly, the solvents employed for the workup appear to have some effect on the nature of products in the reaction depicted in Scheme 2. When CH_2Cl_2 was used for the workup procedure, an unexpected species with a ^{77}Se NMR chemical shift of 280 ppm was obtained as one of the major products. The isolation of this species by column chromatography and mass spectral studies showed the formation of a dimeric selenium compound having molecular mass (*m*/*z*) of 335.9. The ^{13}C NMR studies also did not give any conclusive evidence, but these studies suggested two possible structures (**16** and **17**) with a bridging methylene moiety. However, the ^{1}H NMR spectrum of this compound showed a signal at 4.67 ppm (in CD_3OD) for the $-CH_2-$ with ^{77}Se coupling, which is consistent with structure **17**.[20] The unexpected formation of this compound is probably due to the high reactivity of the selenol (or selenolate), which reacts with the solvent CH_2Cl_2 to produce **17**.



As previously mentioned, the N-methylation of MMI leads to a complete loss of its TPO inhibitory effect. Recent studies on the hepatotoxicity of MMI and the corresponding S-methylated derivative (**19**) in glutathione-depleted mice show that the presence of a free thiol or thione group is important for the hepatotoxicity of MMI.[21] To probe the role of selenol in the inhibition, we synthesized compounds **19**−**24**, which do not have any thione/thiol or selone/selenol moiety. These compounds were synthesized by following the low-temperature lithiation and chalcogen (S, Se) insertion reactions (Scheme 3). The reaction of lithium chalcogenolates with MeI afforded the corresponding methyl derivative in good yield. Although the tautomeric behavior of chalcogenolates having imidazole moiety are expected to produce both N-substituted and Se-substituted compounds, the facile formation of the Se-substituted derivatives

Scheme 3. Synthetic Routes to Compound **19**−**24**[a]



[a] Reagents and conditions: i) CH_3I, 0 °C, 4 h; ii) $PhCH_2Br$, 0 °C, 4 h; iii) α,α′-dibromo-*m*-xylene, 0 °C, 4 h.

suggests that the selenium moiety exists as a selenolate rather than selone. The reaction of lithium chalcogenolates with $PhCH_2Br$ and α,α'-dibromo-*m*-xylene produced the corresponding benzylic derivatives. It has been shown that aryl benzyl and aryl allyl selenides are generally unstable, but the stability of these compounds could be enhanced by intramolecular Se···N interactions.[22] The expected $^{1}H\cdots^{77}Se$ coupling observed in the ^{1}H NMR spectra of the selenium compounds confirms that the substitution takes place at the selenium center and not at the nitrogen.

The third class of compounds (**25**, **26**) that we used in our study belongs to the group of selenazoles, which do not have the methimazole moiety but contain a readily cleavable Se−N bond. Compound **25** was synthesized from commercially available benzanilide by following literature method and compound **26** was obtained unexpectedly during our attempts to synthesize diselenide **27**. Although the formation of compound **27** could not be detected during the synthesis, we presume that the formation of the five-membered heterocycle proceeds by a spontaneous disproportionation of the diselenide **27** (Scheme 4). This phenomenon can be rationalized by correlating the stability of this compound with the presence of the oxazoline nitrogen, which interacts noncovalently with the divalent selenium. When one of the selenium atoms in **27** participates in such interactions, the *ortho* donor group (N in this case) must be approximately collinear with the Se−X substituent on selenium (Se−Se in this case), because the donor atom interacts with the σ^* orbital of the Se−Se bond. The presence of a substituent at the 6-position prevents such an interaction through steric hindrance. As the second half of the molecule bearing the arylselenium moiety would have to approximately occupy the position of the 6-substituent, a strain is imposed in the molecule, which leads to the unexpected disproportionation. This is in agreement with the recent reports that organoselenium compounds bearing two benzamide groups ortho to selenium undergo disproportionation reactions, leading to the formation of selenazoles.[23] However, a number of diselenides having two identical or different substituents in the ortho positions have been shown to be stable.[24] This indicates that the presence of

(19) Kim, H.; Lee, T.-H.; Hwang, Y. S.; Bang, M. A.; Kim, K. H.; Suh, J. M.; Chung, H. K.; Yu, D.-Y.; Lee, K.-K.; Kwon, O.-Y.; Ro, H. K.; Shong, M. *Mol. Pharmacol.* **2001**, *60*, 972−980.

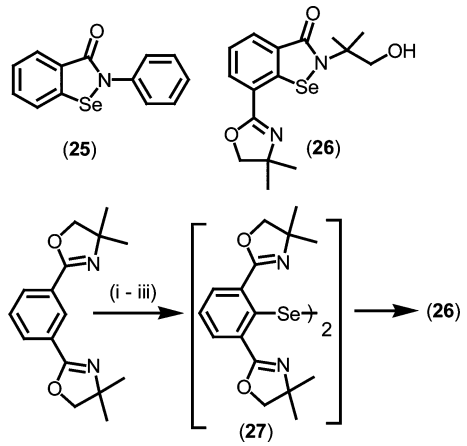
(20) ^{1}H NMR (CD_3OD) δ: 3.99 (s, 6H), 4.68 (s, 2H), 7.71 (s, 2H), 7.78 (s, 2H); ^{13}C NMR (CD_3OD) δ: 23.7, 35.9, 121.9, 126.2, 133.6; HRMS *m*/*z* (TOF) calcd for $C_9H_{12}N_4Se$ $[M + H]^+$ 336.9470, found 336.9464; ^{77}Se NMR δ: 292 (CD_3OD); 294 ($CDCl_3$).

(21) Mizutani, T.; Yoshida, K.; Murakami, M.; Shirai, M.; Kawazoe, S. *Chem. Res. Toxicol.* **2000**, *13*, 170−176.

(22) (a) Mugesh, G.; Panda, A.; Singh, H. B.; Butcher, R. J. *Chem. Eur. J.* **1999**, *5*, 1411−1421. (b) Mugesh, G.; Singh, H. B. *Acc. Chem. Res.* **2001**, *35*, 226−236.

(23) Kersting, B.; Delion, M. *Z. Naturforsch.* **1999**, *54b*, 1042−1047.

Scheme 4. Formation of Selenazole **26** by Disproportionation Reaction[a]



[a] Reagents and conditions: i) LDA, benzene, TMEDA, THF, 4 h; ii) Se powder, THF, 12 h; iii) H_2O, O_2.

Table 2. Experimental and Theoretical ^{77}Se NMR Chemical Shifts[a] for Some of the Selenium Compounds

no.	compd	^{77}Se (δ) ppm (Expt)	^{77}Se (δ) ppm (Calcd)[b]
1	**8**	397	386[c]
2	**20**	117	131
3	**22**	282	299
4	**25**	959	951[c]
5	**26**	820	752[c]
6	**32**	1101	1026[c]

[a] ^{77}Se NMR chemical shifts δ relative to Me_2Se. [b] B3LYP/6-311G++(d,p) and B3LYP/6-311++G(2d,p) basis sets were used for optimization and ^{77}Se NMR chemical shift calculations, respectively. [c] B3LYP/6-31G(d) and B3LYP/6-311++G(d,p) basis sets were used for optimization and ^{77}Se NMR chemical shift calculations, respectively.

an oxazoline moiety that can be hydrolyzed easily is responsible for the cyclization.

However, compound **26** turned out to be an interesting ebselen analogue due to its high stability and solubility in water. Another interesting feature of this compound is the presence of noncovalent Se···N interactions between selenium and the nitrogen atom present in the five-membered oxazoline ring.[25] These interactions would enhance the Se−N bond cleavage by thiols as previously shown for some ebselen analogues. The ^{77}Se NMR chemical shifts for this compound along with other selenium compounds used in this study are summarized in Table 2. The B3LYP optimized structure of **26** and its comparison with the crystal structure are shown in Figure 5. The Se···N distance calculated for **26** is found to be slightly shorter than that observed in the crystal structure, probably due to the presence of intermolecular interactions in the crystal lattice. For example,

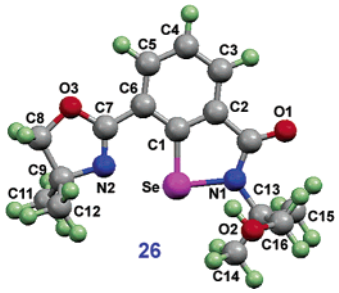


Figure 5. The optimized structure of **26**, calculated with the B3LYP/6-31G(d) method, showing Se···N noncovalent interactions. Se···N 2.547 Å, C1−Se 1.870 Å, Se−N1 1.949 Å, ∠C1−Se−N1 84.8° (X-ray data: Se···N 2.601 Å, C1−Se 1.860 Å, Se−N1 1.912 Å, ∠C1−Se−N1 84.7°).[7]

Table 3. Inhibition of LPO Activity by **1**−**3** and **5**

no.	compd[a]	IC_{50} (μM)[a]
1	MMI (**1**)	7.0 ± 1.1
2	MSeI(**2**)	16.4 ± 1.5
3	PTU (**3**)	45.0 ± 2.1
4	MTU (**5**)	47.8 ± 0.1

[a] Concentration of the compound causing 50% inhibition. Each IC_{50} value was calculated from at least three independent experiments.

the −OH and carbonyl groups in this compound participate in intermolecular hydrogen bonding, leading to the formation of a dimeric structure.

The enzyme inhibition experiments were carried out with Fe-containing lactoperoxidase (LPO) since it is readily available in purified form. Furthermore, LPO has been shown to behave very similarly to TPO with respect to iodination of thyroglobulin, the natural substrate, and other iodide acceptors.[26] Edelhoch et al. have reported the inactivation of LPO by thiourea-based drugs using the LPO-*N*-acetyltyrosylamide assay.[27] We have employed 2,2′-azio-bis-3-ethyl-benthiazoline-6-sulfonic acid (ABTS) and H_2O_2 as substrates[28] to determine the half-maximal inhibitory concentration (IC_{50}) of test compounds. The IC_{50} values for the inhibition of LPO-catalyzed oxidation of ABTS by **1**−**3** and **5** are summarized in Table 3.

To obtain reliable IC_{50} values for compound **2** and to make a direct comparison with those of the sulfur analogue, it is important to carry out the inhibition experiments with the completely reduced species. We have observed that the selenium compound obtained directly from the reaction (Scheme 2) does not give any reproducible results. Therefore, we carried out the experiments with the reduced species (selenol, **2b**), which was obtained by reducing the diselenide (**8**) with $NaBH_4$ in an aqueous solution. As expected, MMI inhibited the LPO activity with an IC_{50} value of 7.0 μM, which is much lower than those observed with PTU and MTU. The selenium analogue (**2**) also inhibited LPO, and the IC_{50} value was found to be almost 4−5 times lower than those of PTU and MTU. The higher activity of MMI as compared with those of PTU and MTU is in agreement with the previous studies on the inhibition of TPO. Since the activation of the iron center in TPO must proceed through an interaction of Fe(III) with H_2O_2, TPO inactivation may occur through a competitive coordination of the drug to iron, assisted by hydrogen bonding with a histidine residue of

(24) (a) Wirth, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1726−1728. (b) Wirth, T.; Fragale, G. *Chem. Eur. J.* **1997**, *3*, 1894−1902. (b) Fragale, G.; Neuburger, M.; Wirth, T. *J. Chem. Soc., Chem. Commun.* **1998**, 1867−1868. (c) Déziel, R.; Goulet, S.; Grenier, L.; Bordeleau, J.; Bernier, J. *J. Org. Chem.* **1993**, *58*, 3619−3621. (d) Déziel, R.; Malenfant, E. *J. Org. Chem.* **1995**, *60*, 4660−4662. (c) Déziel, R.; Malenfant, E.; Bélanger, G. *J. Org. Chem.* **1996**, *61*, 1875−1876. (e) Déziel, R.; Malenfant, E.; Thibault, C.; Fréchette, S.; Gravel, M. *Tetrahedron Lett.* **1997**, *38*, 4753−4756.
(25) The nature of these interactions is not discussed here. For excellent articles on Se···X (X = N, O, etc.) interactions, see: (a) Iwaoka, M.; Tomoda, S. *J. Am. Chem. Soc.* **1996**, *118*, 8077−8084. (b) Wirth, T.; Fragale, G.; Spichty, M. *J. Am. Chem. Soc.* **1998**, *120*, 3376−3381. (c) Spichty, M.; Fragale, G.; Wirth, T. *J. Am. Chem. Soc.* **2000**, *122*, 10914−10916. (d) Iwaoka, M.; Komatsu, H.; Katsuda, T.; Tomoda, S. *J. Am. Chem. Soc.* **2002**, *124*, 1902−1909. (e) Iwaoka, M.; Komatsu, H.; Katsuda, T.; Tomoda, S. *J. Am. Chem. Soc.* **2004**, *126*, 5309−5317. (f) Iwaoka, M.; Katsuda, T.; Komatsu, H.; Tomoda, S. *J. Org. Chem.* **2005**, *70*, 321−327.
(26) Taurog, A.; Dorris, M. L.; Lamas, L. *Endocrinology* **1974**, *94*, 1286−1294.
(27) Edelhoch, H.; Irace, G.; Johnson, M. L.; Michot, J. L.; Nunez, J. *J. Biol. Chem.* **1979**, *254*, 11822−11830.
(28) Childs, R. E.; Bardsley, W. G. *Biochem. J.* **1975**, *145*, 93−103.

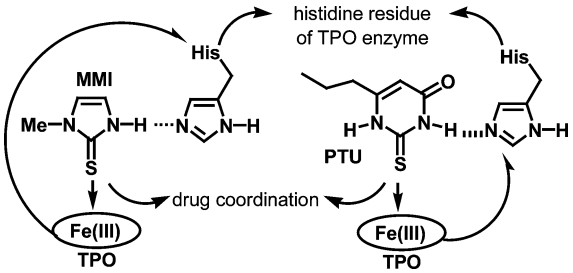


Figure 6. A hypothetical model for the coordination of thiourea drugs to the Fe-center of TPO.

the TPO enzyme (Figure 6).[11b] Under these conditions, MMI might compete more successfully than PTU with H_2O_2, because the hydrogen-bond (hard) basicity pk_{HB} value of MMI (2.11) is much higher than that of PTU (~1.32). Similar to PTU, the methyl derivative **5** is also expected to be a weak inhibitor of TPO. On the other hand, compound **2**, which exists predominantly in its selenol form, does not have the ability to coordinate to the iron center; therefore, this compound must inhibit the LPO activity by a different mechanism.

Taurog et al. have shown, in their pioneering work, that MMI and related derivatives irreversibly inhibit LPO and TPO, leading to a complete inactivation of the enzymes.[29] Doerge and other have shown that mammalian peroxidases including LPO may activate the anti-thyroid drugs through S-oxygenation to produce the corresponding sulfoxides or sulfenic acids.[30] They have also shown that the suicide inactivation of LPO and TPO by MMI proceeds through the S-oxygenation of the thione moiety to form a reactive sulfenic acid, which binds covalently to the prosthetic heme and irreversibly blocks enzyme activity.[31] Given the higher reactivity of selenium compounds as compared with the sulfur derivatives toward oxidation, it is possible that the facile oxidation of the selenium compounds may lead to an efficient inhibition of LPO activity. With this in mind, we treated all the compounds in this study with H_2O_2 before adding LPO and ABTS. The LPO activity was measured several times by increasing the time for the reaction of the test compounds with H_2O_2. The reaction time required to inhibit 50% of the LPO activity are expressed in terms of t_{50} values, which are summarized in Table 4.

All the selenium compounds were oxidized much faster that than their sulfur analogues (**19**, **21**, **23**), which is responsible for the lower t_{50} values of the selenium derivatives. The isolation and characterization of the oxidized products reveal that the selenium compounds were oxidized by H_2O_2 to produce the corresponding selenoxides. For example, compound **22** was oxidized to selenoxide **28,** which was stable enough to be purified by column chromatography. Addition of an excess amount of H_2O_2 to **28** produced an additional signal at 1293 ppm, which could not be characterized. Similarly, compound

Table 4. Inhibition of LPO by PTU, MTU and Methimazole Analogues

no.	compd	t_{50} (h)	no.	compd	t_{50} (h)
1	PTU (**3**)	3.8	7	**22**	3.3
2	MTU (**5**)	4.6	8	**23**	26.0
3	**8**	2.2	9	**24**	0.6
4	**19**	32.6	10	**25**	0.6
5	**20**	0.1	11	**26**	2.7
6	**21**	17.0			

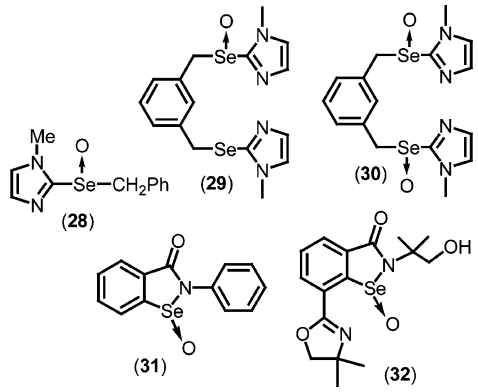
Conditions: LPO: 6.5 nM; H_2O_2:22.9 μM; test compound: 40 μM.

Table 5. Inhibition[a] of LPO by Selenoxides **28**, **29** and **32**

no.	compd	t_{50} (h)
1	**28**	27.0
2	**29**	19.5
3	**32**	45.7

[a] Conditions: LPO: 6.5 nM; H_2O_2: 22.9 μM; test compound: 40 μM.

24 underwent oxidation at the selenium center to produce the monooxo derivative (**29**), which underwent further oxidation to produce the corresponding dioxo derivative **30**. In contrast to the monooxo derivative, the Se(IV) species (**30**) was found to be unstable and decomposed over a period of 2 h to give red selenium. Ebselen (**25**) and selenazole **26** were also oxidized to the corresponding selenoxides **31** and **32**, respectively, as stable products.



Although the selenium compounds were readily oxidized to the corresponding selenoxides, this nonenzymatic oxidation may not play any significant role in the inhibition of LPO except for compound **20** and 2**5**, which showed t_{50} values of 6 and 35 min, respectively. However, given the time interval used for the LPO inhibition assay, the oxidation of these compounds may have only minor effect on the inhibition. This is in agreement with the report of Taurog et al.[6e] that the nonenzymatic oxidation of MSeI by H_2O_2 does not interfere appreciably with the guaiacol and iodination assays. In contrast to the selenides, selenoxides do not show any appreciable inhibition (Table 5), confirming that the lower oxidation state of the selenium center in the selenides is responsible for the weak inhibition. In contrast to the s-oxygenation of the sulfur compounds by LPO, these selenium derivatives do not seem to undergo any enzymatic oxidation by LPO/H_2O_2 system as evidenced by ^{77}Se NMR studies. The rates of oxidation of these selenides to the corresponding selenoxides in the presence of LPO were found to be almost identical with the rate in the absence of LPO.

In the presence of GSH, the selenoxides exhibit redox shuttles between oxidized and reduced forms. For example, the selena-

(29) (a) Englar, H.; Taurog, A.; Luthy, C.; Dorris, M. L. *Endocrinology* **1983**, *112*, 86−95. (b) Taurog, A. *Endocrinology* **1976**, *98*, 1031−1046. (c) Nogimori, T.; Braverman, L. E.; Taurog, A. Fang, S.-L.; Wright, G.; Emerson, C. H. *Endocrinology* **1986**, *118*, 1598−1605. (d) Taurog, A.; Dorris, M. L.; Guziec, F. S., Jr. *Endocrinology* **1989**, *124*, 30−39 and references therein.

(30) (a) Doerge, D. R. *Arch. Biochem. Biophys.* **1986**, *244*, 678−685. (b) Kobayashi, S.; Nakano, M.; Goto, T.; Kimura, T.; Schaap, A. P. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 166−171.

(31) (a) Doerge, D. R. *Biochemistry* **1986**, *25*, 4724−4728. (b) Doerge, D. R.; Pitz, G. L.; Root, D. P. *Biochem. Pharmacol.* **1987**, *36*, 972−974. (c) Doerge, D. R.; Takazawa, R. S. *Chem. Res. Toxicol.* **1990**, *3*, 98−101. (d) Doerge, D. R.; Cooray, N. M.; Brewster, M. E. *Biochemistry* **1991**, *30*, 8960−8964.

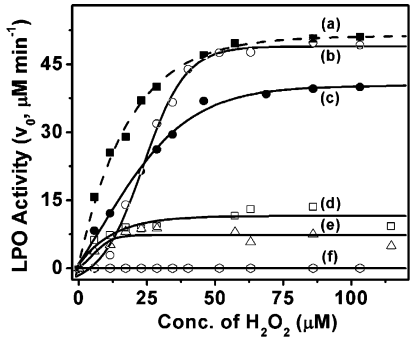
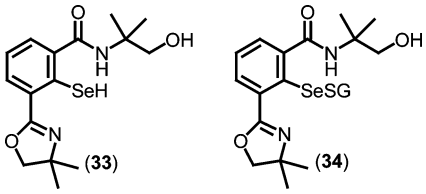


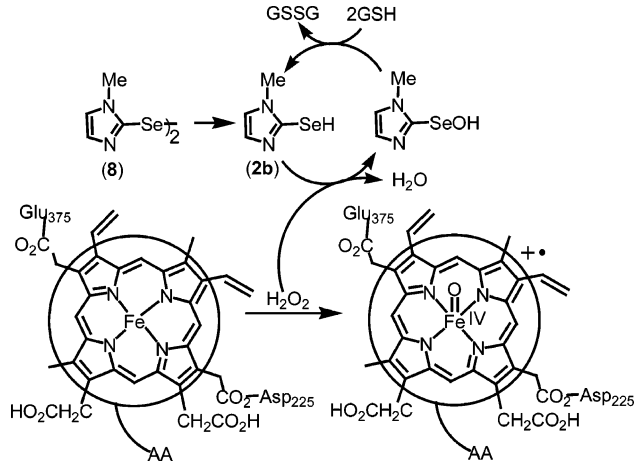
Figure 7. Plot of initial rates (v_o) for the LPO-catalyzed oxidation of ABTS vs concentration of H_2O_2. (a) Control activity, (b) 40 μM of **2b**, (c) 40 μM of **8**, (d) 80 μM of **PTU**, (e) 80 μM of **MTU**, (f) 40 μM of **MMI**. Conditions: LPO: 6.5 nM; H_2O_2: 22.9 μM.

zole **26** was oxidized to selenoxide **32** by H_2O_2 and the addition of GSH to **32** reduced this species back to **26** in nearly quantitative yield.



In the absence of H_2O_2, the Se−N bond in **26** is cleaved by GSH to produce the corresponding selenol (**33**). In contrast to ebselen, the formation of the corresponding selenenyl sulfide (**34**) was not detected, indicating that the presence of oxazoline ring at 6-position destabilizes the selenenyl sulfide species. The ^{77}Se NMR signal at 820 ppm for the selenazole **26** did not disappear completely even with an excess amount of GSH. However, the reduction of **32** to **26** may proceed through a ring opening, followed by a cyclization as proposed for ebselen.[32] The oxidation of selenium center in ebselen and **26** may not have any direct effect on the LPO activity, because this oxidation is very slow as compared with the enzymatic oxidation of ABTS. Remarkably, MSeI (**2**) inhibited the enzyme within few seconds even at lower concentrations, which can be ascribed to the facile oxidation of the reactive selenol group in **2** (MSeI) by H_2O_2. Because MMI also inhibits the enzyme very efficiently, we have carried out further experiments to prove that the mechanisms by which MMI and MSeI exert their inhibitory action are different. The initial rates (v_o) derived from various concentrations of H_2O_2 were plotted against the concentration of H_2O_2. The LPO activity was completely inhibited by 40 μM MMI, and the enzyme's activity could not be recovered by increasing the H_2O_2 concentration (Figure 7, f). The LPO activity could not be recovered even at lower concentration of MMI (10 μM) and higher concentration of H_2O_2 (230 μM). This suggests that MMI does not act on H_2O_2 but acts on the enzyme itself, leading to an irreversible inhibition as previously proposed. On the other hand, **2** also inhibited the LPO activity as efficiently as MMI, but in this case, the enzyme's activity could be completely recovered by increasing H_2O_2 concentration (Figure 7, b). The sigmoidal behavior of the graph for this compound (Figure 7, b) is probably due to the utilization of H_2O_2 for the oxidation of selenenic acid (vide infra) to other oxidized products at lower concentrations of the peroxide.

Scheme 5. Hypothetical Model Representing the Inhibition of LPO[a] by **2**



[a] The porphyrin core inside the circle represents the active center of LPO. AA: amino acid residues.

These observations strongly support the assumption that MSeI, in contrast to MMI, does not interfere with the enzyme directly but inhibits the LPO activity by reducing the H_2O_2, which is required for the oxidation of the iron center in LPO (Scheme 5). When coupled with a suitable thiol such as GSH, compound **2** may constitute a redox cycle involving a catalytic reduction of H_2O_2 (glutathione peroxidase (GPx) activity).[33] In this way, compound **2** mimics the action of GPx, a selenoenzyme that protects the cellular components from oxidative damage by reducing H_2O_2 with the help of GSH.[34] Recently, the GPx enzyme present in thyroid gland has been shown to inhibit the iodination reactions by degrading the intracellular H_2O_2.[35] In fact, the key compound **2** exhibited interesting GPx activity, leading to an assumption that some of the anti-thyroid drugs may act as antioxidants in addition to their inhibition behavior. The cyclic selenazole **26** also showed higher GPx activity, but this activity does not seem to correlate with its LPO inhibitory activity. The sulfur compounds (**19**, **21**, **23**) and selenides (**20**, **22**, **24**), however, did not show any significant GPx activity, confirming that the inhibition of LPO by these compounds is completely different from that of **2**. Similar to the selenol-mediated inhibition, the inhibition by diselenide **8** could also be reversed by increasing the H_2O_2 concentration (Figure 7, c). Although compound **8** did not give any new ^{77}Se

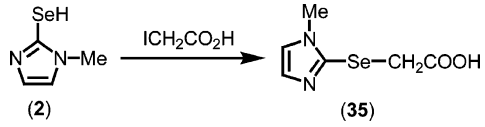
(32) Fischer, H.; Dereu, N. *Bull. Soc. Chim. Belg.* **1987**, *96*, 757−768.

(33) For GPx activity of selenium compounds, see: (a) Reich, H. J.; Jasperse, C. P. *J. Am. Chem. Soc.* **1987**, *109*, 5549−5551. (b) Wilson, S. R.; Zucker, P. A.; Huang, R.-R. C.; Spector, A. *J. Am. Chem. Soc.* **1989**, *111*, 5936−5939. (c) Tomoda, S.; Iwaoka, M. *J. Am. Chem. Soc.* **1994**, *116*, 6, 2557−2561. (d) Back, T. G.; Dyck, B. P. *J. Am. Chem. Soc.* **1997**, *119*, 2079−2083. (e) Wirth, T. *Molecules* **1998**, *3*, 164−166. (f) Mugesh, G.; Singh, H. B. *Chem. Soc. Rev.* **2000**, *29*, 347−357. (g) Mugesh, G.; du Mont, W.-W.; Sies, H. *Chem. Rev.* **2001**, *101*, 2125−2179. (h) Mugesh, G.; Panda, A.; Singh, H. B.; Punekar, N. S.; Butcher, R. J. *J. Am. Chem. Soc.* **2001**, *123*, 839−850. (i) Jauslin, M.; Wirth, T.; Meier, T.; Schoumacher, F. *Hum. Mol. Gen.* **2002**, *11*, 3055−3063. (j) Back, T. G.; Moussa, Z. *J. Am. Chem. Soc.* **2002**, *124*, 12104−12105. (k) Back, T. G.; Moussa, Z. *J. Am. Chem. Soc.* **2003**, *125*, 13455−13460. (l) Sun, Y.; Li, T.; Chen, H.; Zhang, K.; Zheng, K.; Mu, Y.; Yan, G.; Li, W.; Shen, J.; Luo, G. *J. Biol. Chem.* **2004**, *279*, 37235−37240.

(34) (a) *Selenium in Biology and Human Health*; Burk, R. F., Ed.; Springer-Verlag: New York, 1994. (b) Tappel, A. L. *Curr. Top. Cell Regul.* **1984**, *24*, 87−97. (c) Flohé, L. *Curr. Top. Cell Regul.* **1985**, *27*, 473−478. (d) Epp, O.; Ladenstein, R.; Wendel, A. *Eur. J. Biochem.* **1983**, *133*, 51−69.

(35) (a) Björkman, U.; Ekholm, R. *Mol. Cell. Endocrinol.* **1995**, *111*, 99−107. (b) Ekholm, R.; Björkman, U. *Endocrinology* **1997**, *138*, 2871−2878.

Scheme 6. Reaction of Selenol **2** with Iodoacetic Acid, Producing Selenide **35**



(**2**) (**35**)

NMR signal with one equiv of H_2O_2, addition of an excess amount of H_2O_2 to **8** produced a new signal at 1045 ppm, which cannot be ascribed to the selenenic acid[36] and this signal appears to be different from that obtained from the reaction of **2** with H_2O_2, which showed a signal at 1207 ppm.

As expected, the plot of initial rates (υ_o) vs concentration of MSeI shows that the rate of the reaction decreases with increasing concentration of MSeI (see Supporting Information, Figure S15). In all these cases, the LPO activity could be recovered by increasing the hydrogen peroxide concentration. These experimental observations support the conclusions made by Taurog et al. that MSeI, unlike MMI, cannot act as an irreversible inhibitor of TPO. These observations also support the in vivo experiments, which showed that MMI is at least 50 times more potent than MSeI as an inhibitor of organic iodine formation in the thyroid. Crucially, the treatment of **2** with the selenol specific reagent, iodoacetic acid, abolished the inhibitory potency of **2**, confirming that the oxidation of the selenol group by H_2O_2 is responsible for the inhibition. In contrast, the sulfur analogue MMI was found to be less sensitive to the iodoacetic acid treatment, and this also confirms that the thiol form of MMI is not only less predominant in solution but also less reactive as compared with the thione form (see Supporting Information, Figure S16). The decrease in the inhibitory activity of **2** upon iodoacetic acid treatment can be ascribed to the formation of selenide **35** as a dead-end product. The formation of **35** in the reaction of **2** with iodoacetic acid was further confirmed by its independent synthesis (Scheme 6).

Conclusion

Experimental and theoretical studies show that the selenium analogue of methimazole (MSeI) exists predominantly in its selenol form, whereas the sulfur compound exists in its thione form and the oxidation of the selenol to the corresponding diselenide is energetically more favored than the conversion of thione/thiol to the corresponding disulfide. Although MSeI readily oxidizes to produce the diselenide, the oxidized form can be easily reduced by reducing agents such as $NaBH_4$ or glutathione (GSH). The ^{77}Se NMR studies show that the selenol form of MSeI dissociates in solution to form a more reactive selenolate, which could be trapped by selenol-specific reagents such as iodoacetic acid. In its reduced form, MSeI effectively and reversibly inhibits the iron-containing lactoperoxidase (LPO). In contrast to methimazole, MSeI does not interfere with the enzyme directly, but it inhibits LPO by reducing the H_2O_2 that is required for the oxidation of the iron center in LPO. In the presence of GSH, MSeI constitutes a redox cycle involving a catalytic reduction of H_2O_2 and thereby mimics the glutathione peroxidase (GPx) activity in vitro. These studies reveal that the degradation of the intracellular H_2O_2 by the selenium analogues of anti-thyroid drugs may be beneficial to the thyroid gland as these compounds may act as antioxidants and protect thyroid cells from oxidative damage. Because the drugs with an action essentially on H_2O_2 can reversibly inhibit thyroid peroxidase, such drugs with a more controlled action could be of great importance in the treatment of hyperthyroidism.

(36) Although reactions of diselenides with H_2O_2 generally afford the corresponding selenenic acid in the first step, the reaction of **8** with H_2O_2 does not give any isolable selenenic acid. This reaction produced a white solid for which the following NMR and mass spectral data were obtained [^{1}H NMR (D_2O) δ: 3.54 (s, 3H), 7.06 (s, 1H), 8.32 (s, 1H); ^{13}C NMR (D_2O) δ: 35.3, 119.3, 122.7, 134.76; LRMS *m*/*z* (TOF): 243; ^{77}Se NMR (D_2O): δ = 1045]. However, this species reacts further with H_2O_2 to produce another compound [^{77}Se NMR (D_2O): δ = 1316] that could not be characterized.

Experimental Section

General Procedure. Lactoperoxidase from bovine milk and ABTS were purchased from Fluka Chemical Co. *n*-Butyllithium was purched from Acros Chemical Co. (Belgium). All other chemicals were of the highest purity available. All chemical reactions were carried out under nitrogen or argon using standard vacuum-line techniques. Solvents were purified by standard procedures and were freshly distilled prior to use. ^{1}H (400 MHz), ^{13}C (100 MHz) and ^{77}Se (76.3 MHz) NMR spectra were obtained on a Bruker Avance 400 NMR Spectrometer. Chemical shifts are cited with respect to $SiMe_4$ as internal (^{1}H and ^{13}C) and Me_2Se (^{77}Se) as external standard.

Synthesis of Compound 2. To a solution of compound **8** (65.0 mg, 0.2 mmol) in water was added $NaBH_4$ (15.4 mg, 0.4 mmol) at room temperature. After stirring for 10 min, the product was extracted with CH_2Cl_2, and the organic layer was concentrated to give the expected selenol as yellow solid. This was used for the biological studies without any further purification. Yield: 80 mg (82%). ^{1}H NMR ($CDCl_3$): δ = 3.69. (s, 3H), 6.89 (d, J = 2 Hz, 1H), 6.95 (d, J = 2 Hz, 1H), 8.75 (br, 1H); ^{13}C NMR ($CDCl_3$): δ = 36.1, 118.6, 121.2, 148.8;^{77}Se NMR ($CDCl_3$/MeOH): δ = −32.

Synthesis of Compound 8. To a cooled (−78 °C) solution of 1-methylimidazole (0.48 mL, 6.09 mmol) in freshly distilled THF (50 mL) was added via syringe *n*-butyllithium (3.8 mL, 1.6 M in hexanes). The mixture was stirred at this temperature for 35 min and then allowed to come to room temperature at which time elemental selenium (0.72 g, 9.12 mmol) was added. The resulting mixture was stirred at room temperature for an additional 12 h. The mixture was quenched with cold water and neutralized with 1 N HCl. The aqueous mixture was extracted with $CHCl_3$, and the organic layer was washed with brine and dried over Na_2SO_4. Removal of solvent afforded an orange solid. The product was recrystallized from $CHCl_3$ to give orange crystals of the desired compound. Yield 0.5 g (50%); mp 142−144 °C; ^{1}H NMR ($CDCl_3$) δ: 3.52 (s, 3H), 7.00 (d, 1H), 7.09 (d,1H); ^{13}C NMR ($CDCl_3$) δ: 35.2, 124.7, 131.3, 133.0; HRMS *m*/*z* (TOF) calcd for $C_8H_{10}N_4Se_2$ $[M + H]^+$ 322.9314, found 322.9319; ^{77}Se NMR ($CDCl_3$) δ: 397.

Synthesis of Compound 19. To a cooled (−78 °C) solution of 1-methylimidazole (1.0 g, 12.18 mmol) in freshly distilled dry THF (100 mL) was added *n*-butyllithium (9.1 mL, 1.6 M in hexanes) via syringe dropwise. The mixture was stirred at −78 °C for 35 min and then allowed to come to r.t at which time elemental sulfur (0.43 g, 13.4 mmol) was added. The solution was heated at reflux 12 h and then methyl iodide (1.5 mL, 24.36 mmol) was added dropwise at 0 °C and the stirring was continued for an additional 4 h at r.t. The resulting turbid solution was filtered through Celite and the solvent was evaporated. The resulting yellow oil was dissolved in $CHCl_3$ and again filtered through Celite and concentrated to give expected product as viscous oil. Yield: 1.24 g (79%). ^{1}H NMR ($CDCl_3$): δ = 2.58 (s, 3H), 3.59 (s, 3H), 6.91 (s, 1H), 7.03 (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 16.2, 33.0, 122.1, 128.9, 142.9; HRMS *m*/*z*(TOF) calcd for $C_5H_8N_2S$ $[M+H]^+$ 129.0486, found: 129.0506.

Synthesis of Compound 20. To a cooled (−78 °C) solution of 1-methylimidazole (1.0 g, 12.18 mmol) in freshly distilled THF (100 mL) was added *n*-butyllithium (9.1 mL, 1.6 M in hexanes) via syringe dropwise. The mixture was stirred at −78 °C for 35 min and then allowed to come to r.t. at which time elemental selenium (1.44 g, 18.27

mmol) was added. The resulting mixture was stirred at r.t. for 12 h. To the solution was cooled to 0 °C and then was added Methyl iodide (1.5 mL, 24.36 mmol) dropwise. The reaction mixture was allowed to come to room temperature and the stirring was continued for an additional 4 h. The resulting turbid solution was filtered through Celite and concentrated under reduced pressure. The resulting orange color oil was dissolved in CH_2Cl_2 and again filtered through Celite and concentrated to give the expected product as orange oil. Yield: 1.05 g (60%). ^{1}H NMR ($CDCl_3$): δ = 2.45 (s, 3H), 3.63 (s, 3H), 6.97 (s, 1H), 7.08 (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 8.0, 34.0, 122.7, 129.9, 135.7; HRMS *m*/*z*(TOF) calcd for $C_5H_8N_2Se$ $[M+H]^+$ 176.9931, found: 176.9928;^{77}Se NMR ($CDCl_3$): δ = 117.

Synthesis of Compound 21. To a cooled (−78 °C) solution of 1-methylimidazole (1.0 g, 12.18 mmol) in freshly distilled dry THF (100 mL) was added *n*-butyllithium (9.1 mL, 1.6 M in hexanes) via syringe dropwise. The mixture was stirred at −78 °C for 35 min and then allowed to come to r.t at which time elemental sulfur (0.43 g, 13.4 mmol) was added. This mixture was reflux for 12 h. To the above solution was added benzyl chloride (3.0 g, 24.35 mmol) dropwise at 0 °C. The reaction mixture was allowed to come to room temperature and the stirring was continued for an additional 5 h and then was followed the above procedure (for compound **19**). Yield: 0.65 g (42%). ^{1}H NMR ($CDCl_3$): δ = 3.22 (s, 3H), 4.14 (s, 2H), 6.85 (s, 1H), 7.09−7.12 (m, 3H), 7.22−7.24 (m, 3H); ^{13}C NMR ($CDCl_3$): δ =33.0, 40.0, 122.4, 127.4, 128.5, 128.8, 129.8, 137.9, 140.5; HRMS *m*/*z*(TOF) calcd for $C_{11}H_{12}N_2S$ $[M+H]^+$ 205.0799, found: 205.0807.

Synthesis of Compound 22. To a cooled (−78 °C) solution of 1-methylimidazole (1.0 g, 12.18 mmol) in freshly distilled THF (100 mL) was added via syringe *n*-butyllithium (9.1 mL, 1.6 M in hexanes). The mixture was stirred at −78 °C for 35 min and then allowed to come to r.t. at which time elemental selenium (1.44 g, 18.27 mmol) was added. The resulting mixture was stirred at r.t. for 12 h. To the above solution was added benzyl chloride (3.0 g, 24.35 mmol) dropwise at 0 °C. The reaction mixture was allowed to come to room temperature and the stirring was continued for an additional 5 h. The workup was carried out by a similar method given for compound **20**. Yield: 0.647 gm (42%). ^{1}H NMR ($CDCl_3$): δ = 3.22 (s, 3H), 4.16 (s, 2H), 6.89 (s, 1H), 7.05−7.07 (m, 2H), 7.15 (s, 1H), 7.19−7.22 (m, 3H); ^{13}C NMR ($CDCl_3$): δ =33.0, 34.0, 123.0, 127.0, 128.5, 128.7, 130.6, 134.6, 138.8; HRMS *m*/*z*(TOF) calcd for $C_{11}H_{12}N_2Se$ $[M+Na]^+$ 275.0063, found: 275.0078; ^{77}Se NMR ($CDCl_3$): δ = 282.

Synthesis of Compound 23. To a cooled (−78 °C) solution of 1-methylimidazole (1.54 g, 18.82 mmol) in freshly distilled THF (100 mL) was added via syringe *n*-butyllithium (15.1 mL, 1.6 M in hexanes) The mixture was stirred at −78 °C for 35 min and then allowed to come to r.t at which time elemental sulfur (0.72 g, 22.5 mmol) was added. This mixture was heated at reflux overnight under nitrogen. To the above solution was added α, α′-dibromo-*m*-xylene (2.48 g, 9.4 mmol) in ether (25 mL) dropwise and stirring was continued for an additional 1 h at 0 °C followed by 4 h. The workup procedure used was similar to that of compound **19**. Yield 5.2 g (84%). ^{1}H NMR ($CDCl_3$): δ = 3.26 (s, 6H), 4.07 (s, 4H), 6.85 (s, 2H), 6.89 (s, 1H), 6.99−7.01 (m, 2H), 7.08−7.11 (m, 3H); ^{13}C NMR ($CDCl_3$): δ = 33.1, 39.7, 122.5, 127.8, 128.7, 129.2, 129.7, 138.1, 140.4; HRMS *m*/*z*(TOF)-calcd for $C_{16}H_{18}N_4S_2$ $[M+H]^+$ 331.1051, found: 331.1064.

Synthesis of Compound 24. To a cooled (−78 °C) solution of 1-methylimidazole (1.0 g, 12.18 mmol) in freshly distilled THF (100 mL) was added via syringe *n*-butyllithium (9.1 mL, 1.6 M in hexanes). The mixture was stirred at −78 °C for 35 min and then slowly allowed to come to r.t. Elemental selenium (1.44 g, 18.27 mmol) was added to the above reaction mixture and the stirring was continued for 12 h at r.t. To the above solution was added α, α′-dibromo-*m*-xylene (1.6 g, 6 mmol) in ether (15 mL) dropwise at 0 °C and stirring was continued for an additional 1 h. at this temperature. The reaction mixture was allowed to come to r.t. and the stirring was continued for an additional 4 h and then the workup procedure was followed similar to that of compound **20**. Yield 3.9 g (76%). ^{1}H NMR ($CDCl_3$): δ = 3.30 (s, 6H), 4.08 (s, 4H), 6.80 (s, 1H), 6.92 (s, 2H), 6.94−6.96 (m, 2H), 7.08-(m, H); 7.15 (s, 2H); ^{13}C NMR ($CDCl_3$): δ = 32.8, 34.3, 123.0, 127.5, 128.7, 128.9, 130.6, 134.7, 139.0; HRMS *m*/*z* (TOF) calcd for $C_{16}H_{18}N_4$-Se_2 $[M+H]^+$ 426.9940, found: 426.9944; ^{77}Se NMR ($CDCl_3$): δ = 280.

Synthesis of Compound 26. To a solution of 0.82 g (3 mmol) of 2,6-bis(4,4-dimethyl-2-oxazoline-2-yl)benzene in 20 mL of dry benzene were added 0.89 mL (9 mmol) of TMEDA and 9.0 mmol of LDA. The mixture was stirred for 4 h and the resulting precipitate was dissolved in dry THF (30 mL). The solution was cooled to −15 °C, and then elemental selenium (0.24 g, 3 mmol) was added. The stirring was continued for 12 h at r.t., and then the mixture was poured into a beaker containing saturated solution of $NaHCO_3$. Oxygen was bubbled for 15 min and the solution was extracted with ether. The organic phase was dried over anhydrous sodium sulfate, and the solvent was removed in *vacuo* to afford dark oil. The unusually cleaved product **26** was separated by column chromatography as a stable compound on silica gel with PE/ethyl acetate (1:1) as eluent. The product was recrystallized from diethyl ether to give yellow crystals. Yield 0.44 g (40%); mp 164−166 °C; 1H NMR (CDCl3) δ: 1.46 (s, 6H), 1.63 (s, 6H), 3.86 (d, 2H), 4.33 (s, 2H), 6.0 (t, 1H), 7.52 (t, 1H), 7.91 (d, 1H), 8.11 (d, 1H); 13C NMR (CDCl3) δ: 167.82, 162.69, 141.87, 131.33, 130.48, 129.10, 126.30, 121.13, 81.66, 77.26, 71.28, 67.26, 62.69, 28.77, 25.99, 25.93; ^{77}Se NMR (CDCl3, Me2Se) δ: 822; HRMS *m*/*z*(TOF) calcd for $C_{16}H_{20}N_2O_3Se$ $[M+Na]^+$ 391.0537, found: 391.0481.

Synthesis of Compound 28. Hydrogen peroxide (135 μl, 30% solution) was added to a solution of **22** (0.3 g, 1.19 mmol) in CH_2Cl_2. The reaction mixture was stirred about 4 h and evaporated to dryness. The crude product was chromatographed on a silica gel column. Yield: 0.240 g (75%). ^{1}H NMR ($CDCl_3$): δ = 3.37 (s, 3H), 4.31 (d, *J* = 11.2 Hz 1H), 4.42 (d, *J* = 11.2 Hz 1H), 6.86 (s, 1H), 7.03 (d, *J* = 7.6 Hz 2H), 7.17 (s, 1H), 7.26−7.35 (m, 3H); ^{13}C NMR ($CDCl_3$): δ =32.6, 57.2, 125.45, 128.6, 128.9, 129.9, 130.0, 130.2, 138.2; HRMS *m*/*z*(TOF) calcd for $C_{11}H_{12}N_2OSe$ $[M+H]^+$ 269.0193, found: 269.0181; ^{77}Se NMR ($CDCl_3$): δ = 926.

Synthesis of Compound 32. To a solution of selenazole **26** (0.1 g, 1.19 mmol) in MeOH was added a 30% solution of hydrogen peroxide (30 μl). The reaction mixture was stirred for 12 h and the solvent was evaporated to give compound **32** as white solid in nearly quantitative yield. ^{1}H NMR ($CDCl_3$): δ = 1.41 (s, 3H), 1.47 (s, 3H), 1.69 (s, 3H), 1.73 (s, 3H), 3.58 (d, *J* = 11.6 Hz 1H), 4.00 (d, *J* = 11.6 Hz, 1H), 4.44−4.28 (m, 2H) 7.75 (t, *J* = 7.2 Hz, 1H), 7.97 (d, *J* = 7.6 Hz, 1H), 8.02 (d, *J* = 7.6 Hz 1H); ^{13}C NMR ($CDCl_3$): δ = 25.2, 26.3, 28.0, 28.5, 63.3, 67.8, 68.2, 81.0, 125.3, 130.2, 131.2, 133.4, 134.0, 143.8, 160.2, 168.3; HRMS *m*/*z*(TOF) calcd for $C_{16}H_{20}N_2O_4Se$ $[M+Na]^+$ 407.0486, found: 407.0506. ^{77}Se NMR ($CDCl_3$): δ = 1102.

Synthesis of Compound 35. To a solution of selenol **2** (75.0 mg, 0.47 mmol) in CH_3OH was added iodoacetic acid (87.0 mg, 0.47 mmol) at r.t. After stirring 30 min, the solvent was evaporated to dryness to give the expected compound as orange oil. Yield: 77 mg (75%). ^{1}H NMR ($CDCl_3$): δ = 3.48 (s, 2H), 3.72 (s, 3H), 7.28 (s, 1H), 7.41 (s, 1H); ^{13}C NMR ($CDCl_3$/MeOH): δ = 29.9, 36.9, 121.9, 125.7, 132.9, 170.8;^{77}Se NMR ($CDCl_3$/MeOH): δ = 220.

GPx assay: The GPx activity was followed spectrophotometrically at 340 nm as described by Roveri *et al.*[37] with minor modifications. The test mixture contained GSH (1 mM), EDTA (1 mM), glutathione disulfide reductase (0.6 unit/ml), and NADPH (0.2 mM) in 0.1 M potassium phosphate buffer, pH 7.3. GPx samples were added to the test mixture at room temperature and the reaction was started by the addition of H_2O_2 (1 mM). The initial reduction rates were calculated from the rate of NADPH oxidation at 340 nm. Each initial rate was measured at least 3 times and calculated from the first 5−10% of the

(37) Roveri, A.; Maiorino, M.; Ursini, F. *Methods Enzymol*. **1994**, *233*, 202−212.

reaction by using 6.22 $mM^{-1}cm^{-1}$ as the extinction coefficient for NADPH. For the peroxidase activity, the rates were corrected for the background reaction between H_2O_2 and GSH.

LPO Assay: The LPO inhibition experiments were performed in phosphate buffer (pH 7) at 25 °C. The spectral measurements were carried out in a Perkin-Elmer spectrophotometer. Assay of LPO enzyme activity was followed by catalysis of the oxidation of ABTS. The initial rate was calculated by following UV absorption increase at 411 nm. Enzyme activity after the addition of various inhibitors was expressed as the percentage of that observed in the absence of inhibitors. The peroxide concentration was always present in excess with respect enzyme. The inhibition plots were obtained by using Origin 6.1 software and these plots are used for the calculation of the IC_{50} values. The t_{50} values were obtained by treating the test compounds with H_2O_2 before adding LPO and ABTS to the reaction mixture. Each initial rate was calculated by increasing the time for the reaction between the test compound and H_2O_2 and the time required to reduced the catalytic activity of enzyme to half its value represents the t_{50} value of a particular compound.

Computational Methods

All calculations were performed using Gaussian98 suite of quantum chemical programs.[38] The hybrid Becke 3-Lee−Yang−Parr (B3LYP) exchange correlation functional was applied for DFT calculations.[39] Geometries were fully optimized at B3LYP level of theory using the 6-31G(d) basis sets. All stationary points were characterized as minima by corresponding Hessian indices. The NMR calculations were done at B3LYP/6-311+G(d,p) level on B3LYP/6-31G(d) level optimized geometries using the GIAO method.[17] Orbital interactions were analyzed using the Natural Bond Orbital (NBO) method at the B3LYP/6-31G-(d) level, and charges were calculated from Natural Population Analysis (NPA).[40] Single-point energies with zero-point energies (ZPE) were calculated at B3LYP/6-311++G(d,p) level in vacuo. The solvent effect was included in the calculations at the same level using Tomasi's polarizable continuum model (PCM) in the water solution.[16]

Acknowledgment. This study was supported by the Department of Science and Technology (DST), New Delhi, India.

Supporting Information Available: Details of theoretical calculations (coordinates for the optimized structures and NBO analysis), LPO inhibition plots and complete ref 38. This material is available free of charge via the Internet at http://pubs.acs.org.

JA054497U

(38) Frisch, M. J. et al. *Gaussian98*; Gaussian, Inc.: Pittsburgh, PA, 1998. The full reference is given in Supporting Information.

(39) (a) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785−789. (b) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652.

(40) Glendening, E. D.; Reed, J. E.; Carpenter, J. E.; Weinhold, F. *NBO Program Manual*, version 3.1; University of Wisconsin, 1992.