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Inhibition of Lactoperoxidase-Catalyzed Oxidation by Imidazole-Based Thiones and Selones: A Mechanistic Study

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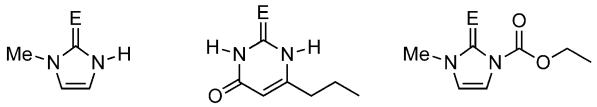
Abstract: Herein, we describe the synthesis and biomimetic activity of a series of N,N-disubstituted thiones and selones that contain an imidazole pharmacophore. The N,N-disubstituted thiones do not show any inhibitory activity towards LPO-catalyzed oxidation reactions, but their corresponding N,N-disubstituted selones exhibit inhibitory activity towards LPO-catalyzed oxidation reactions. Substituents on the N atom of the imidazole ring appear to have a significant effect on the inhibition of LPO-catalyzed oxidation and iodination reactions. Selones **16**, **17**, and **19**, which contain methyl, ethyl, and benzyl substituents, exhibit similar inhibition activities towards LPO-catalyzed oxidation reactions with IC_{50} values of 24.4, 22.5, and 22.5 μM, respectively. However, their activities are almost three-fold lower than that of the commonly used anti-thyroid drug methimazole (MMI). In contrast, selone **21**, which contains a N–CH_2CH_2OH substituent, exhibits high inhibitory activity, with an IC_{50} value of 7.2 μM, which is similar to that of MMI. The inhibitory activity of these selones towards LPO-catalyzed oxidation/iodination reactions is due to their ability to decrease the concentrations of the co-substrates (H_2O_2 and I_2), either by catalytically reducing H_2O_2 (anti-oxidant activity) or by forming stable charge-transfer complexes with oxidized iodide species. The inhibition of LPO-catalyzed oxidation/iodination reactions by N,N-disubstituted selones can be reversed by increasing the concentration of H_2O_2. Interestingly, all of the N,N-disubstituted selones exhibit high anti-oxidant activities and their glutathione peroxidase (GPx)-like activity is 4–12-fold higher than that of the well-known GPx-mimic ebselen. These experimental and theoretical studies suggest that the selones exist as zwitterions, in which the imidazole ring contains a positive charge and the selenium atom carries a large negative charge. Therefore, the selenium moieties of these selones possess highly nucleophilic character. The ^{77}Se NMR chemical shifts for the selones show large upfield shift, thus confirming the zwitterionic structure in solution.

Keywords: bioorganic chemistry · chalcogens · heterocycles · inhibitors · scavengers

Introduction

The overproduction of active thyroid hormone, which leads to hyperthyroidism, can either be controlled by blocking the biosynthesis of thyroid hormone (TH), catalyzed by thyroid peroxidase (TPO), or by inhibiting iodothyronine deiodinases 1 and 2 (ID-1 and ID-2), which are responsible for the conversion of prohormone T4 into biologically active hormone T3. Anti-thyroid drugs, such as methimazole (**1**, MMI), 6-*n*-propyl-2-thiouracil (**3**, PTU), and carbimazole (**5**, CBZ; Scheme 1), are typically employed for the treatment of hyperthyroidism. Several mechanisms by which these thiourea drugs can inhibit the biosynthesis of TH have been proposed.[1–3] Compounds **1** and **5** exert their inhibitory activity by interacting with the oxidized heme group of TPO or by binding to the heme group of the enzyme upon oxidation.[1,2] Because some of these compounds have been shown to form stable charge-transfer complexes with iodine, such compounds may block the iodination in vivo by diverting the reactive iodine species from the reaction site.[3] In another mechanism, it has been proposed that TPO inactivation may occur through a competitive coordination of the drug to the iron center of the enzyme, which is assisted by hydrogen bonding between the free N–H group of the drug and the distal histidine group.[4] Therefore, the presence of a free N–H moiety appears to be important for the anti-thyroid activity of MMI and its related compounds.



1, E = S, (MMI) **3**, E = S, (PTU) **5**, E = S, (CBZ)
2, E = Se (MSeI) **4**, E = Se (PSeU) **6**, E = Se (SeCBZ)

Scheme 1. Selected commonly used anti-thyroid drugs and their selenium analogues.

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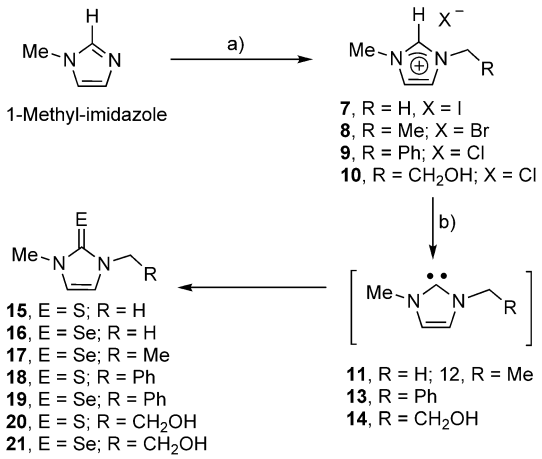
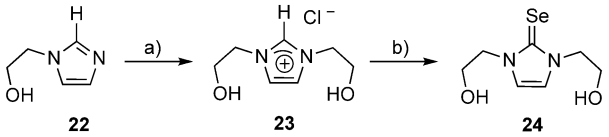
Hydrogen peroxide (H_2O_2), an essential co-substrate in the TPO-catalyzed biosynthesis of TH, is produced in high amounts by thyroid oxidases DUOX-1 and DUOX-2 in the thyroid gland. During its normal physiological activity, the thyroid gland is continuously exposed to relevant concentrations of H_2O_2 and H_2O_2-derived reactive oxygen species (ROS).[5] It has been reported that compounds **1** and **3** partially inhibit the Ca^{2+}/NADPH-dependent generation of H_2O_2 in the thyroid, owing to their antioxidant properties. The inhibition of the biosynthesis of TH by MMI in vivo may arise from both a direct effect on TPO activity and its ability to scavenge H_2O_2.[6,7] The selenium analogues of anti-thyroid drugs MSeI (**2**), PSeU (**4**), and SeCBZ (**6**) have attracted considerable attention. Because selones are typically better nucleophiles than their corresponding thiones, the selenium analogues of anti-thyroid drugs may exhibit better inhibitory activity towards ID-1 than their sulfur analogues. Recently, we reported that MSeI (**2**) and SeCBZ (**6**) inhibited the LPO-catalyzed oxidation of the ABTS reaction by depleting the H_2O_2 in a model system.[8–11] Herein, we report the synthesis and inhibition behavior of a series of N,N-disubstituted thiones and selones that do not contain a free N–H moiety. We also show that the selones exhibit significant antioxidant activity in the presence of thiols.

Results and Discussion

N,N-disubstituted thiones and selones **15**–**21** were synthesized by treating 1-methylimidazole with appropriate halides to produce the corresponding imidazolium salts,[9a] followed by their reactions with elemental sulfur or selenium to afford the corresponding thiones and selones. In these reactions, deprotonation of the imidazolium salts by a base leads to an in situ generation of reactive carbenes, which, in turn, react with elemental sulfur or selenium to afford the corresponding thiones or selones (Scheme 2). Because compounds **15**–**21** are readily soluble in organic solvents, such as CH_2Cl_2, they can be easily separated from other salt-like impurities. These compounds were very stable in the presence of air and no decomposition was observed. To understand whether the replacement of the methyl group in MMI/MSeI and related compounds by other substituents affected their inhibition properties towards peroxidase-catalyzed oxidation and iodination reactions, we synthesized compounds **20, 21**, and **24**, which contained one or two CH_2CH_2OH moieties. This replacement was expected to increase the solubility of these compounds in aqueous buffer. For the synthesis of compound **24**, we employed the 2-(1*H*-imidazol-1-yl)ethanol (**22**) as the key starting material, which could be readily synthesized by treating imidazole with sodium hydride, followed by reaction with 2-chloroethanol in DMF (Scheme 3). Compound **24** could also be directly synthesized from imidazole as a white solid by using slightly more than two equivalents of 2-chloroethanol. In this reaction, we found that the use of sodium hydride was not necessary for the replacement of the hydrogen atom by a CH_2CH_2OH group.

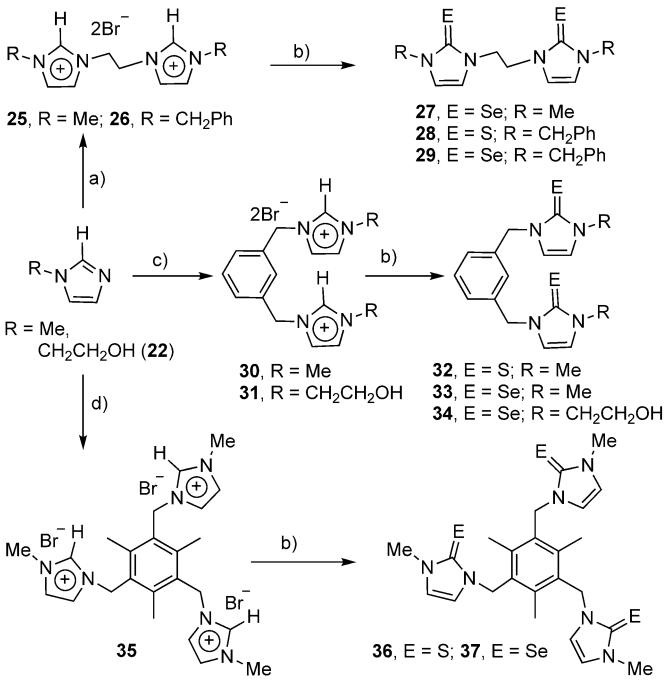


Scheme 2. Synthesis of compounds **15**–**21** by using heterocyclic carbenes that were generated in situ from their corresponding imidazolium salts. Reagents and conditions: a) ethyl bromide, NaI, acetone, 12 h, RT (for compound **8**); chloroethanol, 80 °C (for compound **10**); b) K_2CO_3, dry MeOH, S or Se powder, reflux, 24 h.



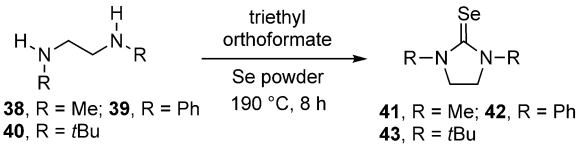
Scheme 3. Synthesis of compound **24** by using a heterocyclic carbene that was generated in situ from its corresponding imidazolium salt. Reagents and conditions: a) chloroethanol, 80 °C; b) K_2CO_3, dry MeOH, Se powder, reflux, 24 h.

To investigate the effect of the thione/selone moiety on the inhibition of peroxidase-catalyzed oxidation and iodination reactions, several N,N-disubstituted thiones and selones that contained more than one thione (C=S)/selone (C=Se) moiety (compounds **27**–**29**, **32**–**34**, **36**, and **37**) were synthesized. These compounds were synthesized from their corresponding N-substituted imidazole by using appropriate halides, as shown in Scheme 4. Compounds **27**–**29** were synthesized from the reactions of 1-methylimidazole or 1-benzyl imidazole with 2-dibromoethane at reflux in dry THF. The addition of elemental sulfur or selenium in dry MeOH under basic conditions to halide salts **25** and **26** afforded thione **28** and selones **27** and **29**.[12] The reactions of 1-methylimidazole or compound **22** with 1,3-bis(bromomethyl)benzene in dry THF, followed by the addition of sulfur or selenium powder in dry MeOH under basic conditions produced compounds **32**–**34**. Similarly, the reaction of 1-methylimidazole with 1,3,5-tris(bromomethyl)-2,4,6-trimethyl benzene in dry THF afforded the corresponding imidazolium salt (**35**), which, upon treatment with elemental sulfur and selenium, produced thione **36** and selone **37**, respectively. To understand the effect of the C=C double bond in the imidazole ring on the inhibition of peroxidase-catalyzed reactions, we synthesized compounds **41**–**43**, which contained a C–C



Scheme 4. Synthesis of N,N-disubstituted thiones and selones that contained multiple thione (C=S)/selone (C=Se) moieties (**27**–**29**, **32**–**34**, **36**, and **37**) by using heterocyclic carbenes that were generated in situ from their corresponding imidazolium salts. Reagents and conditions: a) 2-dibromoethane, THF; b) K_2CO_3, dry MeOH, S or Se powder; c) α,α′-dibromo-*m*-xylene, THF; d) 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene, THF.

single bond instead of a double bond. Compounds **41**–**43** could be synthesized by treating different amines (**38**–**40**) with triethylorthoformate and selenium in a sealed Teflon bomb and heating at 190 °C for 8 h (Scheme 5).[13]



Scheme 5. Synthetic route to compounds **41**–**43**.

Single-crystal X-ray structural analysis shows that the C–Se bond lengths (1.841–1.854 Å) in the N,N-disubstituted selones (**16**, **19**, **21**, **24**, and **27**) are much longer than that of a typical C=Se double bond (1.74 Å) and are close to that of a C–Se single bond (1.88 Å, Table 1).[8] The C–N bond lengths (1.384–1.339 Å) in the imidazole ring are significantly shorter than that of a C–N single bond (1.48 Å) and slightly longer than that of a C=N double bond (1.29 Å). Furthermore, the C2–C3 bond lengths (1.326, 1.325, and 1.336 Å) are significantly longer than that of a typical C=C double bond (1.28 Å). These observations suggest that these N,N-disubstituted selones can be considered as zwitterions, with a negative charge on the selenium atom and a delocalized positive charge over the five-membered ring. The crystal structures of selones **21** and **24** show some interesting features: In compound **21**, an intermolecular hydrogen bond between the hydroxy group of one molecular unit and the selenium atom of another molecular unit leads to the formation of a chain-like structure, as shown in Figure 1A. Although the hydrogen bonding interactions do not appear to affect the nature of the C–Se bond in compound **24**, the NCH_2CH_2OH substituent in the heterocycle does alter the nature of the C–Se bond in compound **21**. The C–Se bond length (1.854 Å) is slightly longer than that of other selones

Table 1. Summary of the DFT calculations of selenium compounds at the B3LYP/6-311+G(d,p) level and GIAO ^{77}Se NMR chemical shifts at the B3LYP/6-311+G(d,p)//B3LYP/6-311++G(2d,p) level by using the Gaussian 98 suite of quantum chemical calculations, as well as experimental ^{77}Se NMR chemical shifts.

Compound	C–Se bond length [Å]		C–Se bond order	Charge on Se	^{77}Se NMR [ppm]
	Expt.	Calcd	Calcd	Calcd	Expt.[a] (calcd)[b]
2	1.848(4)[c]	1.835	1.371	−0.262	−5 (23)
16	1.843[d]	1.838	1.361	−0.269	−6 (39)
19	1.843(2)[c]	1.844	1.344	−0.272	−3 (−2)
21	1.854(4)	1.844	1.341	−0.286	−9 (19)
24	1.848(3)	1.848	1.335	−0.289	−45[b] (−25)
27	1.841(3)	1.844	1.339	−0.289	0 (0)
41	1.834(4)	1.837	1.405	−0.244	63 (113)
42	1.828(4), 1.840(4)	1.828	1.459	−0.182	161 (173)

[a] ^{77}Se NMR data for all compounds were recorded in $CDCl_3$, except for compound **24**, for which D_2O was used. [b] Chemical shifts (δ) are reported referenced to dimethyl selenide. The calculated ^{77}Se NMR chemical shift for dimethyl selenide at the B3LYP/6-311+G(d,p)//B3LYP/6-311++G(2d,p) level of theory is δ = 1637.6348 ppm. [c] Data taken from Ref. [9a]. [d] The C–Se bond length matches that reported previously in Ref. [9c].

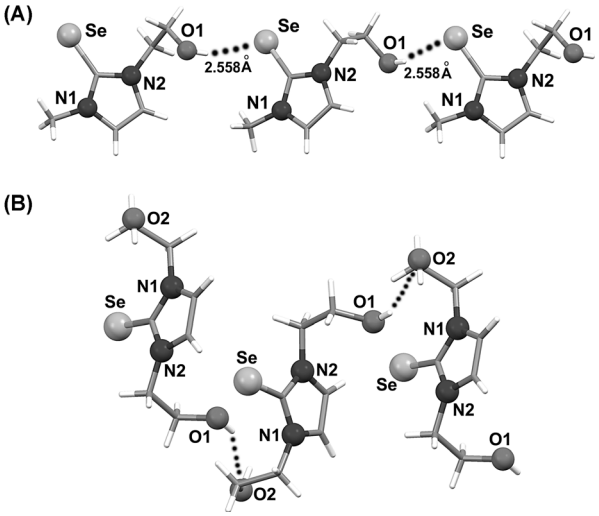


Figure 1. A) Intermolecular H-bonding interaction between the OH group of one molecular unit and the Se atom of another molecular unit in compound **21**; O···Se 3.345 Å, OH···Se 2.558 Å. B) Intermolecular H-bonding interaction in compound **24**; O–H···OH 2.138 Å, H–O···O–H 2.744 Å.

and it is very close to that of a C–Se single bond (1.88 Å).[8–11] The structure of compound **24** also indicates the formation of an intermolecular hydrogen bond between the hydroxy moieties of two adjacent molecular units (Figure 1 B). However, in contrast to compound **21**, the selenium atom in compound **24** does not participate in hydrogen bonding.

To further investigate the nature of the C–Se bond and the charge on the selenium atom in these N,N-disubstituted selones, we performed quantum chemical calculations. So far, theoretical investigations of selones have been highly limited to compounds that contain simple substituents, mainly owing to the requirements of large basis sets for the calculations.[14] The bond order, the charges on the atoms, and the ^{77}Se NMR data of all of the compounds were calculated at the B3LYP level of theory by using the 6-311++G-(2d,p) basis set after optimization of all compounds at the B3LYP/6-311+G(d,p) level of theory.[15,16] The ^{77}Se NMR chemical shifts were calculated by using the gauge invariant atomic orbitals (GIAO) method.[17] Experimental (X-ray) and theoretical (DFT calculations) results of the C–Se bond length, bond order, charge on the Se atom, and ^{77}Se NMR data of the selones are summarized in Table 1. Crystal-structure determination, as well as theoretical calculations, show that the C–Se bond lengths of these selones are intermediate between the values as predicted by the respective sum of the covalent radii of carbon and selenium atom single and double bonds (d(C–Se) = 1.94 Å for a single C–Se bond and d(C–Se) = 1.74 Å for a C=Se double bond). The C–Se bond orders of all of the selones lie in between single and double bonds (1.33–1.46), in agreement with the X-ray crystal-structure analysis. The selenium moiety of these N,N-disubstituted selones contains a large negative charge, ranging from −0.262 to −0.289, where the selenium moiety behaves as a nucleophile (Table 1). The occupied π molecular orbitals (MOs) of selone **21** indicate that there is relatively little π overlap between the Se 4p orbital and the adjacent carbon atom of the aromatic ring (Figure 2). Therefore, both the crystal-structure analysis and theoretical calculations clearly suggest that the N,N-disubstituted selones can be best ascribed as zwitterions with a negative charge on the selenium atom and a delocalized positive charge over the five-membered ring (see above). The C–Se double bond in these selones is very weak and they do not have pure C–Se double-bond character. However, in the case of selones that lack the C–C double bond in the ring (**41** and **42**), the C–Se bond lengths are a little shorter and the charge on the selenium atom in these compounds is smaller compared to the other selones (from −0.182 to −0.244). These observations suggest that the zwitterionic character in compounds **41** and **42** is less compared to that of the other selones that have a C–C double bond in the imidazole ring.

The mechanism by which anti-thyroid drugs inhibit the biosynthesis of thyroid hormone is currently an active area of research. We have previously shown that the selenium analogue of MMI inhibits the peroxidase-catalyzed reaction through a different mechanism to that of MMI. The effect

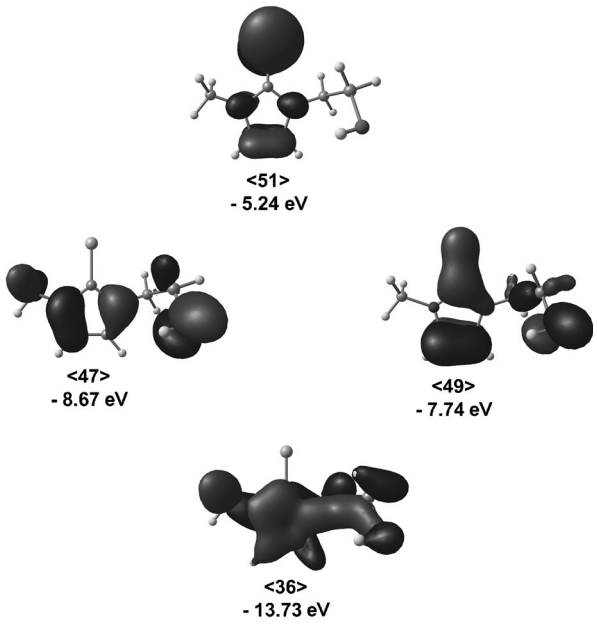


Figure 2. Occupied π MOs of selone **21**, which contain three bonding orbitals of the imidazole ring and one Se 4p orbital. The C atom and the Se 4p orbital have poor π-overlap.

of various thiones and selones on peroxidase-catalyzed oxidation reactions was studied in vitro by using spectroscopic techniques. The enzyme-inhibition experiments were carried out with heme-containing lactoperoxidase (LPO) because it is readily available in its pure form. Furthermore, LPO has been shown to behave very similarly to TPO with respect to the oxidation of organic substrates and the iodination of thyroglobulin (a protein that is required for the synthesis of thyroxine) and other iodide acceptors.[3b] In the LPO-catalyzed oxidation reaction, we employed 2,2′-azio-bis-3-ethylbenthiazoline-6-sulfonic acid (ABTS) and H_2O_2 as substrates to determine the half-maximal inhibitory concentration (IC_{50}) of the test compounds. The IC_{50} values of all of the thiones/selones that were tested for the inhibition of the LPO-catalyzed oxidation of ABTS are listed in Table 2.

Table 2. Inhibition of the LPO-catalyzed oxidation of ABTS by various thiones and selones.

Compound	IC_{50} [μM][a]	Compound	IC_{50} [μM][a]
1	7.0(±1.1)	**24**	18.8(±1.9)
2	16.4(±1.5)	**27**	7.3(±0.7)
15	inactive	**28**	inactive
16	24.4(±2.0)[b]	**29**	7.7(±0.5)
17	22.5(±1.5)	**32**	inactive
18	inactive	**33**	5.2(±0.8)
19	22.6(±1.9)[b]	**34**	3.2(±1.2)
20	inactive	**41**	84.3(±2.9)
21	7.2(±0.9)	**42**	90.0(±4.0)

[a] The reactions were performed in a 1 mL cuvette and the change in UV absorption owing to the oxidation of ABTS was followed at 411 nm. The assay mixture contained 12.9 nM LPO, 28.7 μM H_2O_2, 1.4 mM ABTS, and 1–200 μM thione/selone. [b] Data taken from Ref. [9a].

It has been proposed that the activation of the iron center in TPO/LPO must proceed through an interaction of the Fe^{III} atom with H_2O_2 and that the inactivation of TPO by MMI may occur through a competitive coordination of the drug to the iron center, assisted by a hydrogen-bonding interaction between the free N–H group of MMI and a histidine residue of the TPO enzyme. In agreement with this assumption, MMI, which contains a free N–H group, inhibited the LPO-catalyzed oxidation of ABTS with an IC_{50} value of 7.0 μM, whereas all of the N,N-disubstituted thiones (**15**, **18**, **20**, **28**, and **32**), which lacked any free N–H groups on the heterocycle, were found to be inactive towards LPO-catalyzed oxidation. In contrast, the N,N-disubstituted selones exhibited excellent inhibitory activity towards LPO-catalyzed oxidation (Table 2). The substituents that are attached on the nitrogen atom in the heterocycle (imidazole ring) play an important role in the inhibition of the LPO-catalyzed oxidation of ABTS. Among the compounds with one selone (C=Se) moiety, compound **21**, which contained a 2-hydroxyethyl (CH_2CH_2OH) group at the N1 position, exhibited an almost-three-times larger inhibitory activity (IC_{50} = 7.2 μM) than that of the methyl- (**16**), ethyl- (**17**), and benzyl-substituted compounds (**19**). The activity of selone **21** is comparable to that of the most-potent commercially available anti-thyroid drug, MMI (7.0 μM). However, selone **24**, which contained a 2-hydroxyethyl group on both nitrogen atoms of the imidazole ring, exhibited much lower inhibitory activity (18.8 μM) than that of compound **21**, thus indicating that the increased hydrophilic nature of selone **24** did not enhance its inhibitory activity.

Compounds **2** and **24**, which had similar C–Se bond lengths (1.848 Å), exhibited similar inhibitory activities towards the LPO-catalyzed oxidation reaction, with IC_{50} values of 16.4 and 18.8 μM, respectively; the inhibitory activity of selone **34** (3.2 μM), which contained a 2-hydroxyethyl group, was almost twice that of selone **21**. This result is probably due to the fact that compound **34** contains two imidazole moieties, whereas selone **21** only contains a single selone unit. Similarly, compounds **27**, **29**, and **33**, which contain two selone units with methyl and benzyl substituents on the nitrogen atoms, exhibited excellent inhibitory activities towards the LPO-catalyzed oxidation reaction. The inhibitory activities of these compounds are almost 2–3-times higher than those of compounds **16** and **19**. The IC_{50} values for compounds **27** and **29** are 7.3 and 7.7 μM, respectively, which are similar to that of MMI. This result indicates that the selone moieties in these compounds are responsible for the inhibitory activity towards the LPO-catalyzed oxidation reaction. Reliable inhibition data could not be obtained for compound **37**, owing to its poor solubility in the assay solvent.

To understand the nature of the inhibition of LPO-catalyzed oxidation reactions by N,N-disubstituted selones, we performed further experiments at different concentrations of H_2O_2 because the selenium moiety in the selones are known to react with peroxides. The initial rates (v_o), which were obtained at various concentrations of H_2O_2, were plotted against the concentration of H_2O_2. As expected for irreversible inhibitors, the LPO activity was completely inhibited by MMI at a concentration of 12 μM and the enzyme activity could not be recovered by increasing the concentration of H_2O_2. The enzymatic activity was also almost completely inhibited at a 12 μM concentration of selone **34**. This particular compound exhibited the highest inhibitory activity (IC_{50}: 3.2 μM) among all of the selones in this study. However, the enzyme activity could be completely recovered by increasing the concentration of H_2O_2, although the inhibitory activity of selone **34** was found to be almost twice as high as that of MMI. The effect of H_2O_2 concentration on the inhibition of LPO-catalyzed oxidation of ABTS at different concentrations of selone **34** is shown in Figure 3. We observed

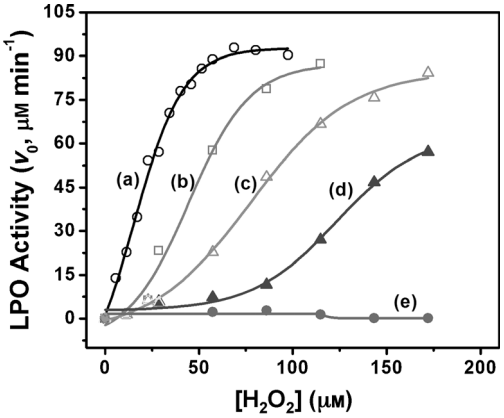
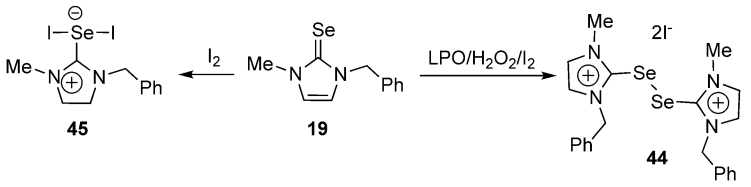


Figure 3. Effect of H_2O_2 on the inhibition of the LPO-catalyzed oxidation of ABTS by compound **34**: a) control sample (0 μM); b) 12 μM of compound **34**; c) 15 μM of compound **34**; d) 20 μM of compound **34**; and e) 12 μM of MMI (**1**).

that the enzyme activity could be recovered to a large extent by increasing the concentration of H_2O_2 up to a certain inhibitor concentration (20 μM for selone **34**). The amount of H_2O_2 that was required for complete recovery of the enzyme activity correlates well with the amount of inhibitor used. The control experiments indicate that it is the depletion of H_2O_2 and not the enzyme inactivation that is responsible for the deviations from the control rates. The sigmoidal behavior of the graph for compound **34** is probably due to the utilization of H_2O_2 for the oxidation of selenenic acid into other oxidized products at lower concentrations of the peroxide. Similar effects of H_2O_2 were also observed for other selones. These observations strongly suggest that MMI does not act on H_2O_2, but rather that it acts on the enzyme itself, thus leading to irreversible inhibition, as previously proposed. On the other hand, the inhibition by various selones is mainly due to their ability to react with H_2O_2 that is present in the assay mixture.[1i,18]

As discussed in the Introduction, one of the possible mechanisms by which anti-thyroid drugs inhibit the biosynthesis of thyroid hormone is through the diversion of the oxidized iodides away from thyroglobulin (Tg) by forming

stable electron-donor/electron-acceptor complexes with diiodine (I_2).[3a,b] Anti-thyroid drugs, which are oxidized by the TPO/H_2O_2 system, may form stable donor–acceptor complexes with either diiodine (I_2) or activated iodine (I^+, produced by TPO/H_2O_2/I^-). Because the oxidized drug molecules may react further with TPO/H_2O_2/I^- in vivo to form some other metabolites, it is important to identify the species that are produced in the reaction of anti-thyroid drugs with TPO/H_2O_2/I^-. To this end, selone **19** was treated with the LPO/H_2O_2/I^- system in phosphate buffer at pH 7.4, similar to the assay conditions that are employed for LPO-catalyzed iodination reactions. The treatment of selone **19** with LPO/H_2O_2/I^- produced a bright-orange-colored compound. Single crystals suitable for X-ray analysis were obtained by the slow evaporation of the solvent. X-ray crystal-structure analysis indicated the formation of complex **44**, which contained a diselenide dication and two I^- ions as counterions (Scheme 6, Figure 4). In this reaction, selone **19** was oxidized by the LPO/H_2O_2/I_2 system into its corresponding diselenide, which produced a charge-transfer complex (**44**) with



Scheme 6. Charge-transfer complexes of selone **19** with the LPO/H_2O_2/I_2 system (**44**) and with molecular iodine (**45**).

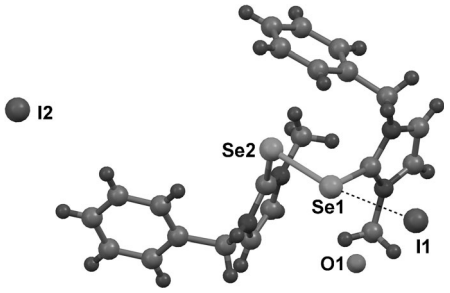


Figure 4. X-ray crystal structure of compound **44**, which shows an intramolecular Se···I interaction.

oxidized iodine. It should be noted that the reaction of compound **19** with molecular iodine produces a completely different complex (**45**, Scheme 6).[19]

Recent biochemical studies on the metabolism of thyroid hormone suggest that glutathione peroxidases (GPx), a selenoenzyme that is present in the thyroid gland, degrades intracellular H_2O_2 and inhibits the biosynthesis of thyroid hormones.[6c] Hydrogen peroxide is an essential co-substrate for the biosynthesis of thyroid hormone as produced by the NADPH-dependent flavoproteins thyroid oxidase DUOX (dual oxidases) 1 and 2. Thus, as a consequence of its normal physiological activity, the thyroid gland is continuously exposed to relevant concentrations of H_2O_2, which can freely diffuse into the cytoplasm and the nucleus, where it may lead to aberrant oxidation and iodination of the proteins and lipids, trigger apoptosis, and might induce DNA damage.[5a] However, the thyroid gland, which contains the highest amount of selenium among other endocrine tissues, contains at least 11 different selenoproteins, including glutathione peroxidases GPx1, GPx3, and GPx4, thioredoxin reductase (TrxR), the Se-transport protein SePP, and selenoprotein 15 (SeP15). These proteins catalyze redox reactions in various physiological processes, such as controlling the H_2O_2 level in the thyroid gland and the scavenging of reactive oxygen species (ROS), TH metabolism, and others.[5] To understand the antioxidant activities of thiones and selones and to understand the mechanism by which the N,N-disubstituted selones exhibit an inhibitory effect towards the LPO-catalyzed oxidation and iodination reactions, we investigated the glutathione peroxidase (GPx)-like activity of the thiones and selones. The GPx-like activity was studied by using H_2O_2 as a substrate and PhSH as a cofactor. The amount of diphenyl disulfide (PhSSPh) that was formed during the course of reaction was monitored by HPLC and the activities were compared with the well-known GPx-mimic ebselen.[20,21] Interestingly, all of the N,N-disubstituted selones, which are potent inhibitors of LPO-catalyzed reactions, exhibit high GPx-like activity. The activity of all of the N,N-disubstituted selones were 4–12-times higher than that of ebselen (Table 3). Among the selones that contained one C=Se moiety, compound **17**, which contained an ethyl substituent, exhibited the highest GPx activity (29.1 μM min^{-1}). The GPx activity of compound **21** (20.8 μM min^{-1}) was almost seven-times higher than that of ebselen (2.9 μM min^{-1}). Substituents on the nitrogen atom of the imidazole ring play an important role in their antioxidant activities. Compounds **16** and **24** exhibited almost-similar GPx-like activities (16.3 and 15.2 μM min^{-1}, respectively). The introduction of a benzyl group appeared to lower the GPx activity because the activity of compound **19** (11.2 μM min^{-1}) was much lower than that of alkyl-based compounds **16**, **17**, and **21**. However, this compound was found to be almost four-times more active than ebselen.

Table 3. GPx-like activity for ebselen, thiones, and selones.[a]

Compound	Rate [μM min^{-1}]	Compound	Rate [μM min^{-1}]
ebselen	2.9(±0.4)	**27**	19.9(±2.1)
MMI (**1**)	–	**28**	–
MSeI (**2**)	231.7(±8.8)	**29**	14.3(±0.3)
15	–	**32**	–
16	16.3(±1.1)	**33**	26.5(±3.2)
17	29.1(±1.5)	**34**	25.3(±1.4)
18	–	**36**	–
19	11.2(±1.7)	**37**	35.5(±0.2)
20	–	**41**	12.7(±0.3)
21	20.8(±1.8)	**42**	13.9(±1.7)
24	15.2(±1.5)	**43**	14.9(±0.6)

[a] The concentration of the test compounds was 100 μM.

The GPx-like activities of compounds that contained two selone moieties (**27**, **29**, **33**, and **34**) were also found to be 7–9-times higher than that of ebselen. Compound **34**, which exhibited promising LPO-inhibitory activity, was about nine-fold more active (25.3 $\mu M\,min^{-1}$) than ebselen. Compound **37**, which contained three selone moieties, also exhibited high GPx-like activity, with an initial rate of 35.5 $\mu M\,min^{-1}$. The activities of compounds **41**, **42**, and **43** were found to be slightly lower than those of the selones that contained a C=C double bond in the imidazole ring. It should be noted that compounds **41** and **42** exhibited very weak inhibitory activity toward LPO-catalyzed reactions. In contrast to the selones, all of the N,N-disubstituted thiones did not show GPx activity under identical experimental conditions. Interestingly, MSeI (**2**), which has a free N–H group in the imidazole ring, is almost 80-times more active than ebselen (Table 3). These observations suggest that the mechanism for the GPx-like activity of MSeI is different from that of the N,N-disubstituted selones. The mechanism for the reduction of H_2O_2 by MSeI may involve the formation of selenenic acid (SeOH) and selenenyl sulfide intermediates, as previously shown for other GPx mimics.[21] Unfortunately, our attempts to detect these intermediates by ^{77}Se NMR spectroscopy were unsuccessful. However, in the absence of PhSH, the reactions of the selones with H_2O_2 produced the corresponding seleninic acids (SeO_2H), thus indicating that the rapid reaction of the selenenic acid with PhSH may prevent the further oxidation of SeOH into SeO_2H.

Conclusions

Herein, we have described the synthesis, characterization, and biomimetic activity of a series of N,N-disubstituted thiones and selones. Whereas methimazole (MMI) strongly and irreversibly inhibits lactoperoxidase (LPO)-catalyzed oxidation reactions, the replacement of the N–H group in MMI by alkyl or benzyl substituents abolishes this inhibitory effect. In contrast, the N,N-disubstituted selones exhibit significant inhibitory effect on LPO-catalyzed oxidation reactions and the IC_{50} values for these compounds not only depend on the number of selone moieties, but also on the nature of the C=Se bond in the molecule. Experimental and theoretical studies have indicated that the selones exist as zwitterions, in which the heterocyclic ring contains a partial positive charge and the selenium atom carries a large negative charge. Owing to their highly nucleophilic character, the selone moieties readily react with H_2O_2 and diiodine (or oxidized iodide). The ^{77}Se NMR chemical shifts for the selones show large upfield shifts with respect to their related selenocarbonyl compounds, thus confirming the zwitterionic structure in solution. In addition to the LPO inhibition, the N,N-disubstituted selones exhibit good glutathione peroxidase (GPx)-like activity and the activities of all of the selones are higher than that of ebselen, a well-known GPx mimic. These observations suggest that the inhibition of LPO-catalyzed oxidation/iodination reactions by N,N-disubstituted selones is mainly due to their ability to react with co-substrates H_2O_2 (antioxidant activity) and diiodine (or oxidized iodide). In contrast to the selones, the N,N-disubstituted thiones do not exhibit GPx-like activity, which is in agreement with their effect on LPO-catalyzed reactions.

Experimental Section

General Procedure

Lactoperoxidase from bovine milk and ABTS (2,2′-azino-bis-3-ethylbenzthiazoline sulfonic acid) were purchased from Fluka Chemical Co. *n*-Butyl lithium was purchased from Acros Chemical Co. (Belgium) and selenium powder was purchased from Sigma–Aldrich. Ethylene chlorohydrine, sodium hydride (NaH), sodium borohydride ($NaBH_4$), benzyl chloride, and other chemicals were obtained from local companies. All experiments were performed under anhydrous and anaerobic conditions by using standard Schlenk techniques. Melting points were determined in open tubes on a Buchi melting point B-540 apparatus and are uncorrected. Mass spectroscopy was performed on a Q-TOF Micro mass spectrometer with electrospray ionization (ESI). In the case of isotopic patterns, the reported values are of the most-intense peaks. Elemental analysis was performed on a Thermo Finigan FLASH EA 1112 CHNS analyser. Solution-state NMR spectra were recorded in $CDCl_3$, [D_4]MeOH, D_2O, or [D_6]DMSO. ^1H (400 MHz), ^{13}C (100 MHz), and ^{77}Se NMR (76.3 MHz) spectra were recorded on a Bruker Avance 400 NMR Spectrometer. ^1H and ^{13}C NMR spectra are referenced to the solvent peak as an internal standard and the chemical shifts are reported relative to tetramethylsilane (TMS). ^{77}Se NMR spectra were referenced to diphenyl diselenide as an external standard and the chemical shifts are reported relative to dimethyl selenide ($\delta = 0$ ppm) by assuming that the resonance of the standard is $\delta = 461.0$ ppm. In most cases, the ^{77}Se NMR experiments were run overnight to obtain good-quality spectra. UV/Vis experiments were performed on a Varian CARY 300 Bio spectrophotometer that was equipped with a temperature-controlled multi-cell assembly. Thin-layer chromatography analysis was performed on pre-coated silica gel plates (Merck) and spots were visualized by using UV irradiation. Column chromatography was performed on glass columns that were loaded with silica gel or on an automated flash chromatography system (Biotage) by using preloaded silica cartridges. High-performance liquid chromatography (HPLC) experiments were performed on a Waters Alliance System (Milford, MA), which consisted of a 2690 separation module, a 2690 photodiode-array detector, and a fraction collector. The assays were performed in 1.8 mL sample vials and a built-in autosampler was used for sample injection. The Alliance HPLC System was controlled with EMPOWER software (Waters Corporation, Milford, MA). Compounds **15**, **16**, **18**, and **19** were synthesized according to a literature procedure.[9a]

*Synthesis of 1-Ethyl-3-methyl-1H-imidazole-2(3H)-selone (**17**)*

Step 1: A solution of ethyl bromide (7.29 g, 66.98 mmol) in acetone (30 mL) was added to a two-necked flask that contained sodium iodide (10.04 g, 66.98 mmol) and 1-methylimidazole 5.00 g, 60.89 mmol) and the reaction mixture was stirred for 12 h at RT. The solvent was removed under vacuum and the resulting residue was treated with CH_2Cl_2. The mixture was stirred for 5 min and the precipitated sodium chloride was removed by filtration through a pad of Celite. The solvent CH_2Cl_2 was evaporated under reduced pressure to yield a pale-yellow powder, which was used in the next step without any further purification. Step 2: The yellow solid as obtained from the first step was placed in a 250 mL two-necked round-bottomed flask that was fitted with a reflux condenser and dry MeOH (75 mL) was added. The resulting slurry was treated with selenium powder (4.8 g, 60.89 mmol) and anhydrous potassium carbonate (8.42 g, 60.89 mmol) and the reaction mixture was heated at reflux for 24 h. The brown solution was hot filtered through a pad of Celite and washed twice with dry MeOH. The desired compound was obtained as a white crystalline solid upon cooling at 4 °C. The small impurities in the sample could be removed by column chromatography on silica gel

(EtOAc/petroleum ether, 1:2). Yield: 7.5 g (65%); M.p. 52–55°C; ^{1}H NMR ($CDCl_3$): δ = 1.41 (t; 3H of NCH_2CH_3), 3.71 (s; 3H of NCH_3), 4.18 (q; 2H of NCH_2CH_3), 6.87 ppm (s, 2H; CH of imidazole ring); ^{13}C NMR ($CDCl_3$): δ = 14.5, 37.0, 44.9, 118.0, 120.0, 154.7 ppm; ^{77}Se NMR ($CDCl_3$): δ = −14 ppm; HRMS (TOF): *m/z* calcd for $C_6H_{10}N_2Se$: 191.0087 $[M+H]^+$; found: 191.0094.

*Synthesis of 1-(2-Hydroxyethyl)-3-methyl-(1H)-imidazole-2(3H)-thione (**20**)*

A 250 mL two-necked flask that was fitted with a reflux condenser and septum was charged with 1-methylimidazole (5.0 g, 60.9 mmol), ethylene chlorohydrine (4.83 g, 60.9 mmol) was added, and the reaction mixture was heated at 80°C for 96 h to produce a white solid, which was filtered and used in the next step without any further purification. The solid compound was placed in a 250 mL two-necked round-bottomed flask that was fitted with a reflux condenser and treated with dry MeOH (70 mL), sulfur powder (1.9 g, 60 mmol), and anhydrous potassium carbonate (7.6 g, 55 mmol). Then, the reaction mixture was heated at reflux for 20 h before being hot filtered through Celite. The desired compound was obtained from the filtrate as a white crystalline solid upon cooling at 4°C. The ^{1}H NMR spectrum of this sample indicated the presence of some impurities, which were removed by column chromatography on silica gel (EtOAc/petroleum ether, 1:2). Yield: 6.0 g (62%); M.p. 82–84°C; ^{1}H NMR ($CDCl_3$): δ = 3.43 (br s, 1H; OH), 3.61 (s; 3H of NCH_3), 3.94 (t, J(H,H) = 4.8 Hz, 2H; NCH_2), 4.22 (t, J(H,H) = 4.8 Hz, 2H; CH_2OH), 6.71 (d, J(H,H) = 1.6 Hz, 1H; CH of the imidazole ring), 6.85 ppm (d, J-(H,H) = 2.4 Hz, 1H; CH of imidazole ring); ^{13}C NMR ($CDCl_3$): δ = 35.2 (NCH_3), 50.32 (N-CH_2), 61.1 (CH_2OH), 117.8 (CH of the imidazole ring), 118.2 (CH of the imidazole ring), 161.7 ppm (C=S of the imidazole ring); HRMS (TOF): *m/z* calcd for $C_6H_{10}N_2OS$: 181.0412 $[M+Na]^+$; found: 181.0415.

*Synthesis of 1-(2-Hydroxyethyl)-3-methyl-(1H)-imidazole-2(3H)-selone (**21**)*

This compound was synthesized according to the procedure described for the corresponding thione. For this reaction, 1-methylimidazole (5.0 g, 60.9 mmol), ethylene chlorohydrine (4.83 g, 60.9 mmol), selenium powder (4.74 g, 60 mmol), and anhydrous potassium carbonate (7.6 g, 55 mmol) were used. The final product was purified by column chromatography on silica gel (EtOAc/petroleum ether, 1:2). Yield: 7.5 g (60%); M.p. 64–66°C; ^{1}H NMR ($CDCl_3$): δ = 3.15 (br s, 1H; OH), 3.65 (s; 3H of NCH_3), 3.91 (t, J(H,H) = 5.0 Hz, 2H; NCH_2), 4.26 (t, J(H,H) = 5.2 Hz, 2H; CH_2OH), 6.84 (d, J(H,H) = 2.0 Hz, 1H; CH of the imidazole ring), 6.97 ppm (d, J(H,H) = 2.0 Hz, 1H; CH of the imidazole ring); ^{13}C NMR ($CDCl_3$): δ = 37.2 (NCH_3), 51.9 (NCH_2), 60.8 (CH_2OH), 119.8 (CH of the imidazole ring), 120.4 (CH of the imidazole ring), 154.7 ppm (^1J(Se,C) = 227 Hz; C=Se of the imidazole ring); ^{77}Se NMR ($CDCl_3$): δ = −9 ppm; HRMS (TOF): *m/z* calcd for $C_6H_{10}N_2OSe$: 228.9856 $[M+Na]^+$; found: 228.9857.

*Synthesis of 1,3-Bis(2-hydroxyethyl)-1H-imidazole-2(3H)-selone (**24**)*

Method A Step 1: *Preparation of 2-(1H-imidazol-1-yl)ethanol (**22**)*. A solution of sodium hydride (2.98 g, 1.28 mol) in dry DMF (150 mL) was added into a 500 mL two-necked round-bottomed flask that was fitted with a sidearm. To this solution was slowly added imidazole (7.67 g, 1.13 mol) in dry DMF (25 mL) and the mixture was warmed to 90°C for 1 h and then cooled. Then, a solution of chloroethanol (9.02 g, 1.13 mol) in DMF (25 mL) was slowly added to the reaction mixture, which was stirred at 90°C for 24 h. After cooling to RT, the mixture was filtered and the filtrate was evaporated under reduced pressure to yield crude compound **22**, which was purified by column chromatography on neutral aluminum oxide ($CHCl_3$/MeOH, 5:1) and then further used in the next step. Step 2: A mixture of compound **22** (1.00 g, 8.92 mmol) and chloroethanol (0.79 g, 9.81 mmol) in a 250 mL two-necked flask that was fitted with a reflux condenser and a septum was warmed to 80°C for 96 h to produce a brown solid. This solid compound was placed in a 250 mL two-necked round-bottom flask that was fitted with a reflux condenser and treated with dry MeOH (25 mL), selenium powder (0.71 g, 8.92 mmol), and anhydrous potassium carbonate (1.23 g, 8.92 mmol). Then, the reaction mixture was heated at reflux for 24 h before being hot filtered through Celite. The desired compound was obtained from the filtrate as a white solid and it was crystallized by the slow evaporation of its solution in MeOH. The ^{1}H NMR spectrum of this sample indicated the presence of some impurities, which were removed by column chromatography on silica gel (EtOAc/petroleum ether, 1:2). Yield: 1.45 g (69%); M.p. 100–102°C; ^{1}H NMR (D_2O): δ = 3.89 (t, 4H; CH_2OH), 4.23 (t, 4H; NCH_2), 7.23 ppm (2H; CH of the imidazole ring); ^{13}C NMR (D_2O): δ = 50.4, 58.7, 120.2, 148.6 ppm (^1J(Se,C) = 216 Hz; C=Se of the imidazole ring); ^{77}Se NMR (D_2O): δ = −45 ppm; HRMS (TOF): *m/z* calcd for $C_7H_{12}N_2O_2Se$: 258.9962 $[M+Na]^+$; found: 258.9952.

Method B: A mixture of imidazole (1.00 g, 14.68 mmol) and chloroethanol (2.96 g, 36.72 mmol) was placed in a 250 mL two-necked round-bottomed flask that was fitted with a reflux condenser and septum and the mixture was warmed to 80°C for 96 h to produce a brown solid. The solid was placed in a 250 mL two-neck round bottom flask that was fitted with a reflux condenser and treated with dry MeOH (25 mL), selenium powder (1.15 g, 14.68 mmol), and anhydrous potassium carbonate (2.02 g, 14.68 mmol). Then, the reaction mixture was heated at reflux for 24 h before being hot filtered through Celite. The desired compound was obtained from the filtrate as a white solid and it was crystallized by the slow evaporation of its solution in MeOH. Yield: 1.89 g (55%).

*Synthesis of Compound **27**[12]*

Step 1: To a solution of 1,2-dibromoethane (5.72 g, 30.44 mmol) in THF (35 mL) was added 1-methylimidazole (5.00 g, 60.89 mmol). Then, the reaction mixture was heated at reflux for 3 h to afford a white crystalline product, which was filtered, washed with ether, and dried under vacuum for 1 h. Step 2: The solid compound as obtained in the first step was placed in a 250 mL two-necked round-bottom flask that was fitted with a reflux condenser and treated with dry MeOH (40 mL), anhydrous potassium carbonate (8.41 g, 60.89 mmol), and selenium powder (4.8 g, 60.89 mmol). The reaction mixture was heated at reflux for 24 h and then cooled. MeOH was evaporated under reduced pressure and the residue was extracted with $CHCl_3$. The combined organic extracts were filtered through Celite and the desired compound was obtained from the filtrate as a white solid, which was purified by column chromatography on silica gel (EtOAc/petroleum ether). Yield: 12.0 g (57%); M.p. 190–193°C; ^{1}H NMR ($CDCl_3$): δ = 3.67 (s, 6H), 4.58 (s, 4H), 6.76 (d, J(H,H) = 2.4 Hz, 2H), 6.80 ppm (d, J(H,H) = 2.4 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ = 37.1, 47.3, 119.8, 119.9, 155.7 ppm; ^{77}Se NMR (D_2O): δ = 4 ppm; HRMS (TOF): *m/z* calcd for $C_{10}H_{14}N_4Se_2$: 350.9627 $[M+H]^+$; found: 350.9634.

*Synthesis of Compound **28***

A mixture of 1-benzyl imidazole (0.75 g, 4.74 mmol) and 1,2-dibromoethane (0.21, 2.37 mmol) was heated at reflux in dry THF (20 mL)for 2 days. The white solid that formed was filtered, washed with dry THF, and dried under vacuum. The white powder was placed in a 100 mL two-necked round-bottomed flask that was fitted with a reflux condenser, dry MeOH (30 mL) was added, and the mixture was stirred at RT for 5 min. To the stirring solution were added sulfur powder (0.15 g, 4.74 mmol) and anhydrous potassium carbonate (0.6 g, 4.30 mmol) and the mixture was heated at reflux for 24 h. The unreacted sulfur powder was removed by passing through a pad of Celite and washed with dry MeOH. Small impurities could be removed by column chromatography on silica gel (petroleum ether/EtOAc, 3:1). The desired product was obtained as a white crystalline solid. Yield: 0.73 g (38%); M.p. 157–159°C; ^{1}H NMR ($CDCl_3$): δ = 4.56 (s; 4H of NCH_2CH_2N), 5.22 (s, 4H; NCH_2Ph), 6.45 (d, J(H,H) = 2.4 Hz, 2H; CH of the imidazole ring), 6.54 (d, J(H,H) = 2.4 Hz, 2H; CH of the imidazole ring), 7.33 ppm (m, 10H; CH of the benzene ring); ^{13}C NMR ($CDCl_3$): δ = 45.23 (NCH_2CH_2N), 51.10 (CH_2 of the benzyl group), 116.34, 118.27, (CH of the imidazole ring), 128.20, 128.23, 128.86, 135.63 (CH of the benzene ring), 162.37 ppm (C=S of the imidazole ring); elemental analysis calcd for $C_{22}H_{22}N_4S$: C 64.99, H 5.45, N 13.78; found: C 64.04, H 5.32, N 13.69.

Synthesis of Compound ***29***

A mixture of 1-benzyl imidazole (0.75 g, 4.74 mmol) and 1,2-dibromoethane (0.21, 2.37 mmol) was heated at reflux in dry THF (20 mL) for 2 days. The white solid that formed was filtered, washed with dry THF, and dried under vacuum. The white powder was placed in a 100 mL two-necked round-bottomed flask that was fitted with a reflux condenser, dry MeOH (30 mL) was added, and the mixture was stirred at RT for 5 min. To the stirring solution were added selenium powder (0.38 g, 4.74 mmol) and anhydrous potassium carbonate (0.6 g, 4.30 mmol) and the mixture was heated at reflux for 24 h. Unreacted selenium powder was removed by passing the mixture through a pad of Celite and washing with dry MeOH. Small impurities could be removed by column chromatography on silica gel (petroleum ether/EtOAc, 5:1). The desired product was obtained as a white crystalline solid. Yield: 0.83 g (35%); M.p. 162–164°C; ^{1}H NMR ($CDCl_3$): δ = 4.70 (s; 4H of NCH_2CH_2N), 5.32 (s, 4H; NCH_2Ph), 6.58 (d, J(H,H) = 2 Hz, 2H; CH of the imidazole ring), 6.80 (d, J(H,H) = 2 Hz, 2H; CH of the imidazole ring), 7.33 ppm (m, 10H; CH of the benzene ring); ^{13}C NMR ($CDCl_3$): δ = 47.03 (NCH_2CH_2N), 53.08 (CH_2 of the benzyl group), 118.46, 120.54, (CH of the imidazole ring), 128.37, 128.46, 128.97, 135.32 (CH of the benzene ring), 156.04 ppm (C=Se of the imidazole ring); ^{77}Se NMR ($CDCl_3$): δ = 7.5 ppm; elemental analysis calcd for $C_{22}H_{22}N_2Se$: C 52.81, H 4.43, N 11.20; found: C 52.68, H 3.93, N 11.26.

Synthesis of Compound ***32***

Step 1: To a solution of α,α′-dibromo-*m*-xylene (1.59 g, 6.1 mmol) in THF (25 mL) was added 1-methylimidazole (1.00 g mL, 12.17 mmol). The reaction mixture was heated at reflux for 3 h and the white crystalline product was filtered, washed with ether, and dried in vacuo for 1 h. Step 2: The solid compound as obtained in the previous step was placed in a 250 mL two-necked round-bottomed flask that was fitted with a reflux condenser and treated with dry MeOH (40 mL), anhydrous potassium carbonate (1.68 g, 12.2 mmol), and sulfur powder (0.39 g, 12.17 mmol). The reaction mixture was heated at reflux for 24 h and then cooled. MeOH was evaporated under reduced pressure and the residue was extracted with $CHCl_3$. The combined organic extracts were filtered through Celite and the desired compound was obtained from the filtrate as a white solid, which was purified by column chromatography on silica gel (EtOAc/petroleum ether). Yield: 1.9 g (46%); M.p. 175–178°C; ^{1}H NMR ($CDCl_3$): δ = 3.65 (s, 6H), 5.24 (s, 4H), 6.60 (d, J(H,H) = 2 Hz, 2H), 6.68 (d, J(H,H) = 2 Hz, 2H), 7.23–7.34 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): δ = 35.4, 51.13, 116.5, 118.2, 127.9, 129.5, 136.6, 163.0 ppm; HRMS (TOF): m/z calcd for $C_{16}H_{18}N_4S_2$: 331.1051 $[M+H]^+$; found: 331.1058.

Synthesis of Compound ***33***

This compound was synthesized according to the procedure described for the corresponding thione; because the first step is identical to that of thione **32**, only the second step is described here. The solid compound that was obtained from the first step was placed in a 250 mL two-necked round-bottomed flask that was fitted with a reflux condenser and treated with dry MeOH (40 mL), anhydrous potassium carbonate (1.68 g, 12.2 mmol), and selenium powder (0.96 g, 12.17 mmol). The reaction mixture was heated at reflux for 24 h and then cooled. MeOH was evaporated under reduced pressure and the residue was extracted with $CHCl_3$. The combined organic extracts were filtered through Celite and the desired compound was obtained as a white solid, which was purified by column chromatography on silica gel (EtOAc/petroleum ether). Yield: 2.4 g (48%); M.p. 185–188°C; ^{1}H NMR ($CDCl_3$): δ = 3.74 (s, 6H), 5.34 (s, 4H), 6.78 (d, J(H,H) = 1.6 Hz, 2H), 6.86 (d, J(H,H) = 1.6 Hz, 2H), 7.28–7.33 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): δ = 37.4, 53.0, 118.6, 120.3, 128.2, 129.6, 136.4, 156.7 ppm; ^{77}Se NMR ($CDCl_3$): δ = −0.8 ppm; HRMS (TOF): m/z calcd for $C_{16}H_{18}N_4Se_2$: 426.9940 $[M+H]^+$; found: 426.9951.

Synthesis of Compound ***34***

Step 1: *Preparation of 2-(1H-Imidazol-1-yl)ethanol (**22**)*. As mentioned during the synthesis of compound **24**, a solution of sodium hydride (2.98 g, 1.28 mol) in dry DMF (150 mL) was placed in a 500 mL two-necked round-bottomed flask that was fitted with a sidearm. To this solution was slowly added a solution of imidazole (7.67 g, 1.13 mol) in dry DMF (25 mL) and the mixture was warmed at 90°C for 1 h and then cooled. A solution of chloroethanol (9.02 g, 1.13 mol) in DMF (25 mL) was slowly added to the reaction mixture, which was stirred for 24 h at 90°C. After cooling to RT, the mixture was filtered and the filtrate was evaporated under reduced pressure to yield crude compound **22**, which was purified by column chromatography on neutral aluminum oxide ($CHCl_3$/MeOH, 5:1) and then further used in the next step. Step 2: To a solution of α,α′-dibromo-*m*-xylene (1.17 g, 4.46 mmol) in THF (25 mL) was dropwise added 2-(1*H*-imidazol-1-yl)ethanol (**22**, 1.00 g mL, 8.92 mmol) in THF (10 mL). The reaction mixture was heated at reflux for 3 h and the white crystalline product was filtered, washed with ether, and dried in vacuo for 1 h. Step 3: The solid compound as obtained from the previous step was placed in a 250 mL two-necked round-bottomed flask that was fitted with a reflux condenser and treated with dry MeOH (40 mL), anhydrous potassium carbonate (1.23 g, 8.92 mmol), and sulfur powder (0.71 g, 8.92 mmol). The reaction mixture was heated at reflux for 24 h and then cooled. MeOH was evaporated under reduced pressure and the residue was extracted with $CHCl_3$. The combined organic extracts were filtered through Celite and the desired compound was obtained from the filtrate as a white solid, which was purified by column chromatography on silica gel (EtOAc/petroleum ether). Yield: 2.5 g (58%); M.p. 140–142°Cl ^{1}H NMR ([D$_6$]DMSO): δ = 3.68 (q, 4H), 4.11 (t, 4H), 4.94 (t, 2H), 5.30 (s, 4H), 7.20 (d, 2H), 7.29 (m, 5H), 7.38 ppm (s, 1H); ^{13}C NMR ([D$_6$]DMSO): δ = 48.3, 50.9, 51.1, 58.4, 118.7, 120.9, 126.7, 127.1, 128.5, 136.7, 154.5 ppm; ^{77}Se NMR ([D$_6$]DMSO): δ = −0.8 ppm; HRMS (TOF): m/z calcd for $C_{18}H_{22}N_4O_2Se_2$: 508.9971 $[M+Na]^+$; found: 509.0016.

Synthesis of Compound ***36***

Step 1: To a solution of 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene (1.60 g, 4.05 mmol) in THF (25 mL) was added 1-methylimidazole (1.00 g mL, 12.17 mmol). Then, the reaction mixture was heated at reflux for 3 h and the white crystalline product was filtered, washed with ether, and dried under vacuum for 1 h. Step 2: The solid compound as obtained from the first step was placed in a 250 mL two-necked round-bottomed flask that was fitted with a reflux condenser and treated with dry MeOH (40 mL), anhydrous potassium carbonate (1.68 g, 12.2 mmol), and sulfur powder (0.39 g, 12.17 mmol). The reaction mixture was heated at reflux for 24 h and then cooled. MeOH was evaporated under reduced pressure and the residue was extracted with $CHCl_3$. The combined organic extracts were filtered through Celite and the desired compound was obtained from the filtrate as a white solid, which was purified by column chromatography on silica gel (EtOAc/petroleum ether). Yield: 3.0 g (51%); M.p. 250–255°C; ^{1}H NMR ($CDCl_3$): δ = 2.05 (s, 9H), 3.65 (s, 9H), 5.21 (s, 6H), 6.09 (d, J(H,H) = 2 Hz, 3H), 6.63 ppm (d, J(H,H) = 2 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ = 16.7, 35.15, 47.0, 114.4, 118.1, 131.3, 139.8, 162.3 ppm; HRMS (TOF): m/z calcd for $C_{24}H_{30}N_6S_3$: 499.1772 $[M+H]^+$; found: 499.1769.

Synthesis of Compound ***37***

In the second step, the solid compound as obtained from the first step was placed in a 250 mL two-necked round-bottomed flask that was fitted with a reflux condenser and treated with dry MeOH (40 mL), anhydrous potassium carbonate (1.68 g, 12.2 mmol), and selenium powder (0.96 g, 12.17 mmol). The reaction mixture was heated at reflux for 24 h and then cooled. MeOH was evaporated under reduced pressure and the residue was extracted with $CHCl_3$. The combined organic extracts were filtered through Celite and the desired compound was obtained from the filtrate as a white solid, which was purified by column chromatography on silica gel (EtOAc/petroleum ether). Yield: 4.2 g (54%); M.p. 285°C; ^{1}H NMR ($CDCl_3$): δ = 2.19 (s, 9H), 3.72 (s, 9H), 5.27 (s, 6H), 6.24 (d, J(H,H) = 1.6 Hz, 3H), 6.80 ppm (d, J(H,H) = 2 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ = 17.0, 37.2, 49.2, 116.6, 120.1, 131.3, 140.2, 156.2 ppm; ^{77}Se NMR ($CDCl_3$): δ = 8 ppm; HRMS (TOF) m/z calcd for $C_{24}H_{30}N_6Se_3$: 664.9926 $[M+Na]^+$; found: 664.9916.

*Synthesis of Compounds **41–43***

General Procedure

A mixture of the amine (50.0 mmol for monoamines, 25.0 mmol for diamines) triethylorthoformate (50 mmol, 100% excess), and selenium (25.0 mmol) was sealed in a Teflon-lined bomb and heated at 190°C for 8 h under autogenous pressure, followed by slow cooling to RT. The cold reaction mixture was dried under an oil vacuum for 3 h, dissolved in the minimum amount of ether/EtOH (3:1), decolorized with charcoal, and filtered. Slow evaporation gave the selenoureas as colorless crystalline solids.[13]

*1,3-Dimethyl-imidazolidine-2-selone (**41**)*

^{1}H NMR ($CDCl_3$): $\delta = 3.19$(s, 6H), 3.55 ppm (s, 4H); ^{13}C NMR ($CDCl_3$): $\delta = 36.9$ (CH_3), 49.3 (CH_2 of the imidazolidine ring), 181.4 ppm (C=Se); ^{77}Se NMR ($CDCl_3$): $\delta = 62.8$ ppm.

*1,3-Diphenyl-imidazolidine-2-selone (**42**)*

^{1}H NMR ($CDCl_3$): $\delta = 4.19$(s, 4H), 7.31–7.58 ppm (m, 10H); ^{13}C NMR ($CDCl_3$): $\delta = 51.1$ (CH_2 of the imidazolidine ring), 126.5, 127.3, 129.0, 141.4 (C of the benzene ring), 180.5 ppm (C=Se); ^{77}Se NMR ($CDCl_3$): $\delta = 161.1$ ppm.

*1,3-Di-tert-butyl-imidazolidine-2-selone (**43**)*

^{1}H NMR ($CDCl_3$): $\delta = 1.69$ (s, 18H), 3.48 ppm (s, 4H); ^{13}C NMR ($CDCl_3$): $\delta = 29.0$ (CH_3), 45.6 (CH_2 of the imidazolidine ring), 58.0 ($NC(CH_3)_3$), 178.9 ppm (C=Se); ^{77}Se NMR ($CDCl_3$): $\delta = 266.8$ ppm.

*Synthesis of Complex **44**[19]*

To a solution of compound **19** (100 mg, 0.39 mmol) in CH_2Cl_2 (10 mL) was added a solution of I_2 (99 mg, 0.39 mmol) in CH_2Cl_2 (25 mL) dropwise under a nitrogen atmosphere at 0°C. The red–brown solution was stirred at RT for 3 h. The resulting solution was concentrated to obtain a red–brown solid product. The product was recrystallized from CH_2Cl_2 to afford black crystals. Yield: 90 g (80%).

*Synthesis of Complex **45***

To a solution LPO/H_2O_2/KI in phosphate buffer (50 mM, pH 7.4),[22] a solution of compound **19** in MeOH was added dropwise with stirring. The final mixture was allowed to stand at RT for slow evaporation of the solvent. Upon evaporation of the solvent, bright-orange crystals were obtained in quantitative yield.

Inhibition of the LPO-Catalyzed Oxidation of ABTS

The LPO-inhibition experiments were performed in phosphate buffer (pH 7) at 25°C. The spectroscopic measurements were performed on a UV/Vis spectrophotometer and the assay of LPO enzyme activity was followed by catalysis of the oxidation of ABTS. The initial rate of the oxidation reaction was calculated by following the increase in UV absorption at 411 nm. The enzyme activity after the addition of various inhibitors was expressed as a percentage of that observed in the absence of inhibitors. The concentration of peroxide was always present in excess with respect to the enzyme. The inhibition plots were obtained by using Origin 6.1 software and these plots were used for the calculation of the IC_{50} values. In a typical experiment, 100 mM ABTS (from its diammonium salt) and 30 mM hydrogen peroxide solutions (from a 30% w/w solution) were freshly prepared in deionized water. A solution of the lactoperoxidase enzyme (0.15–0.25 unit mL^{-1}) was prepared in cold deionized water and used immediately for the assay. In a 1 mL reaction mixture, the final concentrations were 12.9 nM LPO, 28.7 μM H_2O_2, 1.4 mM ABTS, and 1–200 mM of the inhibitor.

Effect of H_2O_2 Concentration on the Inhibition of the LPO-Catalyzed Oxidation of ABTS

The effect of hydrogen-peroxide concentration on the inhibition of the LPO-catalyzed oxidation of ABTS was performed on a UV/Vis spectrophotometer in phosphate buffer (pH 7) at 25°C. The initial rate of the oxidation reaction was calculated by following the increase in UV absorption at 411 nm. All of the solutions were freshly prepared as mentioned above. In a typical experiment, for a 1 mL incubation mixture, 12.9 nM LPO, 1.4 mM ABTS, and a fixed concentration of inhibitor were incubated for 1 min at 25°C. The reaction was initiated with different concentrations of hydrogen peroxide. The rates were calculated at different concentrations of hydrogen peroxide and were plotted against various concentrations of hydrogen peroxide.

Glutathione Peroxidase-Like Activity

In this assay, we employed a mixture of PhSH and H_2O_2 (1:2 molar ratio) in MeOH/CH_2Cl_2 (1:1) at RT as a model system. Experiments were performed with and without different catalysts under the same conditions. Periodically, aliquots were removed and the concentrations of the diphenyl disulfide (PhSSPh) product were determined by reversed-phase HPLC, by using pure PhSSPh as an external standard. The amount of disulfide that formed during the course of the reaction was calculated from the calibration plot for each standard.

Computational Methods

All of the calculations were performed by using the Gaussian 03 suite of quantum chemical programs.[23] The hybrid Becke's three-parameter functional with the Lee–Yang–Parr correlation functional (B3LYP) was applied for the DFT calculations. Geometries were fully optimized at the B3LYP level of theory by using the 6-311++G(d,p) basis sets.[15] All stationary points were characterized as minima by their corresponding Hessian indices. The NMR calculations were performed at B3LYP/6-311++G(2d,p) level on B3LYP/6-311++G(d,p)-optimized geometries by using the GIAO method.[24] Orbital interactions were analyzed by using the natural bond orbital (NBO) method at the B3LYP/6-311++G(2d,p) level of theory and charges were calculated by natural population analysis (NPA).[25]

X-ray Crystallography

X-ray crystallographic studies were performed on a Bruker CCD diffractometer (graphite-monochromated $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å) that was controlled by a Pentium-based PC with the SMART software package.[26] Single crystals were mounted at RT onto the ends of glass fibers and the data were collected at RT. The structures were solved by using direct methods and refined by using the SHELXTL software package. All non-hydrogen atoms were refined anisotropically and hydrogen atoms were assigned at idealized locations. Empirical absorption corrections were applied to all structures by using SADABS.[27–31]

*Crystal Data for Compound **21***

$C_6H_{10}N_2OSe$; $M_w = 205.12$; orthorhombic; space group *Pbca*; $a = 14.74(2)$, $b = 7.487(10)$, $c = 15.08(2)$ Å; $\alpha = \beta = \gamma = 90.00°$; $V = 1665(4)$ Å^3; $\rho_{calcd} = 1.637$ mg m^{-3}; $Z = 8$; $\mu(Mo_{K\alpha}) = 4.449$ mm^{-1}; reflns collected/unique reflns: 13348/1995; parameters: 90; $R_{int} = 0.0901$; R(observed data)[a]: $R_1 = 0.0444$, $wR_2 = 0.0800$; R(all data)[b]: $R_1 = 0.0901$, $wR_2 = 0.0930$; GOF on F^2: 1.036; $\Delta\rho_{min/max}$: −0.312/0.610 e Å^{-3}.[32]

*Crystal Data for Compound **24***

$C_7H_{12}N_2O_2Se$; $M_w = 235.15$; orthorhombic; space group *Pbc*21; $a = 5.9400(17)$, $b = 16.674(5)$, $c = 9.506(3)$ Å; $\alpha = \beta = \gamma = 90.00°$; $V = 941.6(5)$ Å^3; $\rho_{calcd} = 1.659$ mg m^{-3}; $Z = 4$; $\mu(Mo_{K\alpha}) = 3.952$ mm^{-1}; reflns collected/unique reflns: 7580/2120; parameters: 109; $R_{int} = 0.0338$; R(observed data)[a]: $R_1 = 0.0281$, $wR_2 = 0.0837$; R(all data)[b]: $R_1 = 0.0338$, $wR_2 = 0.0914$; GOF on F^2: 0.707; $\Delta\rho_{min/max}$: −0.307/0.394 e Å^{-3}.[32]

*Crystal Data for Compound **27***

$C_{10}H_{14}N_4Se_2$; $M_w = 348.17$; monoclinic; space group *P*21/*c*; $a = 4.8188(10)$, $b = 19.537(5)$, $c = 7.1988(16)$ Å; $\beta = 90.00°$; $V = 647.8(2)$ Å^3; $\rho_{calcd} = 1.785$ mg m^{-3}; $Z = 2$; $\mu(Mo_{K\alpha}) = 5.687$ mm^{-1}; reflns collected/unique reflns: 5045/1315; parameters: 74; $R_{int} = 0.0412$; R(observed data)[a]: $R_1 = 0.0285$, $wR_2 = 0.0929$; R(all data)[b]: $R_1 = 0.0412$, $wR_2 = 0.1063$; GOF on F^2: 0.795; $\Delta\rho_{min/max}$: −0.465/0.298 e Å^{-3}.[32]

Crystal Data for Compound ***28***

$C_{22}H_{22}N_4S_2$; M_w=406.56; monoclinic; space group $P21/c$; a=8.8133(9), b=9.9281(10), c=24.024(2) Å; β=90.071(2)°; V=2102.1(4) Å^3; ρ_{calcd}=1.285 mg m^{-3}; Z=4; $\mu(Mo_{K\alpha})$=0.268 mm^{-1}; reflns collected/unique reflns: 17660/4943; parameters: 253; R_{int}=0.028; R(observed data)[a]: R_1=0.0567, wR_2=0.1420; R(all data)[b]: R_1=0.0955, wR_2=0.1686; GOF on F^2: 0.888; $\Delta\rho_{min/max}$: −0.174/0.294 e Å^{-3}.[32]

Crystal Data for Compound ***41***

$C_5H_{10}N_2Se$; M_w=177.11; monoclinic; space group $P21/n$; a=10.547(3), b=6.2838(16), c=11.672(3) Å; β=112.938(4)°; V=712.4(3) Å^3; ρ_{calcd}=1.651 mg m^{-3}; Z=4; $\mu(Mo_{K\alpha})$=5.173 mm^{-1}; reflns collected/unique reflns: 5851/1664; parameters: 75; R_{int}=0.0848; R(observed data)[a]: R_1=0.0434, wR_2=0.0875; R(all data)[b]: R_1=0.0848, wR_2=0.1019; GOF on F^2: 0.998; $\Delta\rho_{min/max}$: −0.348/0.486 e Å^{-3}.[32]

Crystal Data for Compound ***42***

$C_{15}H_{14}N_2Se$; M_w=301.24; monoclinic; space group $P21/n$; a=5.9711(7), b=21.688(3), c=20.463(3) Å; β=90.971(2)°; V=2649.5(5) Å^3; ρ_{calcd}=1.510 mg m^{-3}; Z=8; $\mu(Mo_{K\alpha})$=2.817 mm^{-1}; reflns collected/unique reflns: 22876/6220; parameters: 325; R_{int}=0.1227; R(observed data)[a]: R_1=0.0493, wR_2=0.0916; R(all data)[b]: R_1=0.1227, wR_2=0.1242; GOF on F^2: 0.986; $\Delta\rho_{min/max}$: −0.346/0.519 e Å^{-3}.[32]

Crystal Data for Compound ***44***

$C_{22}H_{24}I_2N_4OSe_2$; M_w=772.17; monoclinic; space group $P21/c$; a=10.2868(9), b=28.487(3), c=10.3754(9) Å; β=116.48°; V=2721.3(4) Å^3; ρ_{calcd}=1.885 mg m^{-3}; Z=4; $\mu(Mo_{K\alpha})$=5.006 mm^{-1}; reflns collected/unique reflns: 23718/6394; parameters: 282; R_{int}=0.1313; R(observed data)[a]: R_1=0.029, wR_2=0.093; R(all data)[b]: R_1=0.041, wR_2=0.106; GOF on F^2: 0.981; $\Delta\rho_{min/max}$: −0.593/0.883 e Å^{-3}.

[a] $R_1=\Sigma||F_o|-|F_c||/\Sigma|F_o|$; $wR_2=\{\Sigma[w(F_o^2-F_c^2)^2/\Sigma[w(F_o^2)^2]\}^{1/2}$. [b] $F_o>4\sigma(F_o)$.

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[1] a) B. Davidson, M. Soodak, J. T. Neary, H. V. Strout, J. D. Kieffer, H. Mover, F. Maloof, *Endocrinology* **1978**, *103*, 871–883; b) A. Taurog, M. L. Dorris, F. S. Guziec, Jr., *Endocrinology* **1989**, *124*, 30–39, and references therein; c) A. Taurog, *Endocrinology* **1976**, *98*, 1031–1046; d) T. Nakashima, A. Taurog, G. Riesco, *Endocrinology* **1978**, *103*, 2187–2197; e) H. Engler, A. Taurog, T. Nakashima, *Biochem. Pharmacol.* **1982**, *31*, 3801–3806; f) D. R. Doerge, *Arch. Biochem. Biophys.* **1986**, *244*, 678–685; g) A. Shiroozu, A. Taurog, H. Engler, M. L. Dorris, *Endocrinology* **1983**, *113*, 362–370; h) R. S. Magnusson, A. Taurog, *Biochem. Biophys. Res. Commun.* **1983**, *112*, 475–481; i) H. Engler, A. Taurog, C. Luthy, M. L. Dorris, *Endocrinology* **1983**, *112*, 86–95; j) R. P. Magnusson, A. Taurog, M. L. Dorris, *J. Biol. Chem.* **1984**, *259*, 13783–13790; k) R. P. Magnusson, A. Taurog, M. L. Dorris, *J. Biol. Chem.* **1984**, *259*, 197–205.

[2] a) T. Nogimori, L. E. Braverman, A. Taurog, S.-L. Fang, G. Wright, C. H. Emerson, *Endocrinology* **1986**, *118*, 1598–1605; b) A. Taurog, M. L. Dorris, *Endocrinology* **1988**, *122*, 592–601; c) A. Taurog, M. L. Dorris, F. S. Guziec, Jr., J. P. Uetrecht, *Endocrinology* **1989**, *124*, 3030–3037; d) A. Taurog, M. L. Dorris, *Endocrinology* **1989**, *124*, 3038–3042; e) A. Taurog in *Werner and Ingbar's The Thyroid* (Eds.: L. E. Braverman, R. D. Utiger), Lippincott-Raven, Philadelphia, **1991**, pp. 47–84.

[3] a) J. Buxeraud, A. C. Absil, J. Claude, C. Raby, G. Catanzano, C. Beck, *Eur. J. Med. Chem.* **1985**, *20*, 43–50; b) C. Raby, J. F. Lagorce, A. C. Jambut-Absil, J. Buxeraud, G. Catanzano, *Endocrinology* **1990**, *126*, 1683–1691; c) M. C. Aragoni, M. Arca, F. Demartin, F. A. Devillanova, A. Garau, F. Isaia, V. Lippolis, G. Verani, *J. Am. Chem. Soc.* **2002**, *124*, 4538–4539; d) F. Cristiani, F. A. Devillanova, F. Isaia, V. Lippolis, G. Verani, *Polyhedron* **1995**, *14*, 2937–2943; e) F. Cristiani, F. Demartin, F. A. Devillanova, F. Isaia, G. Saba, G. Verani, *J. Chem. Soc. Dalton Trans.* **1992**, 3553–3560; f) F. Cristiani, F. A. Devillanova, F. Demartin, F. Isaia, V. Lippolis, G. Verani, *Inorg. Chem.* **1994**, *33*, 6315–6324.

[4] Although there is no conclusive evidence for the coordination of the thione moiety of anti-thyroid drugs to iron, iron−sulfur coordinate interactions are frequently observed in metalloenzymes; see: a) C. Laurence, M. J. El Ghomari, M. Berthelot, *J. Chem. Soc. Perkin Trans. 2* **1998**, 1163–1166; b) C. Laurence, M. J. El Ghomari, J.-Y. Le Questel, M. Berthelot, R. Mokhlisse, *J. Chem. Soc. Perkin Trans. 2* **1998**, 1545–1551.

[5] a) C. Schmutzler, B. Mentrup, L. Schomburg, C. Hoang-Vu, V. Herzog, J. Köhrle, *Biol. Chem.* **2007**, *388*, 1053–1059; b) J. Aaseth, H. Frey, E. Glattre, G. Norheim, J. Ringstad, Y. Thomassen, *Biol. Trace Elem. Res.* **1990**, *24*, 147–152; c) J. Köhrle, F. Jakob, B. Contempré, J. E. Dumont, *Endocr. Rev.* **2005**, *26*, 944–984.

[6] a) U. Björkman, R. Ekholm, *Endocrinology* **1992**, *130*, 393–399; b) U. Björkman, R. Ekholm, *Mol. Cell. Endocrinol.* **1995**, *111*, 99–107; c) R. Ekholm, U. Björkman, *Endocrinology* **1997**, *138*, 2871–2878.

[7] A. C. F. Ferreira, L. D. Cardoso, D. Rosenthal, D. P. Carvalho, *Eur. J. Biochem.* **2003**, *270*, 2363–2368, and references therein.

[8] a) G. Roy, M. Nethaji, G. Mugesh, *J. Am. Chem. Soc.* **2004**, *126*, 2712–2713; b) G. Roy, G. Mugesh, *J. Am. Chem. Soc.* **2005**, *127*, 15207–15217.

[9] a) G. Roy, D. Das, G. Mugesh, *Inorg. Chim. Acta* **2007**, *360*, 303–316; b) P. N. Jayaram, G. Roy, G. Mugesh, *J. Chem. Sci.* **2008**, *120*, 143–154; c) D. J. Williams, M. R. Fawcett-Brown, R. R. Raye, D. VanDerveer, Y. T. Pang, R. L. Jones, K. L. Bergbauer, *Heteroat. Chem.* **1993**, *4*, 409–414.

[10] D. Das, G. Roy, G. Mugesh, *J. Med. Chem.* **2008**, *51*, 7313–7317.

[11] G. Roy, M. Nethaji, G. Mugesh, *Org. Biomol. Chem.* **2006**, *4*, 2883–2887.

[12] K. P. Bhabak, K. Satheeshkumar, S. Jayavelu, G. Mugesh, *Org. Biomol. Chem.* **2011**, *9*, 7343–7350.

[13] Y. Zhou, M. K. Denk, *Tetrahedron Lett.* **2003**, *44*, 1295–1299.

[14] a) H. Bock, S. Aygen, P. Rosmus, B. Solouki, E. Weissflog, *Chem. Ber.* **1984**, *117*, 187–202; b) S. Collins, T. G. Back, A. Rauk, *J. Am. Chem. Soc.* **1985**, *107*, 6589–6592; c) S. Dapprich, G. Frenking, *Chem. Phys. Lett.* **1993**, *205*, 337–342; d) J. S. Kwiatkowski, J. Leszczynski, *Mol. Phys.* **1994**, *81*, 119–131; e) T.-K. Ha, C. Puebla, *Chem. Phys.* **1994**, *181*, 47–55; f) E. D. Jemmis, K. T. Giju, J. Leszczynski, *J. Phys. Chem. A* **1997**, *101*, 7389–7389.

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

[16] a) C. A. Bayse, *Inorg. Chem.* **2004**, *43*, 1208–1210; b) C. A. Bayse, *J. Chem. Theory Comput.* **2005**, *1*, 1119–1127; c) S. Hayashi, W. Nakanishi, *J. Org. Chem.* **1999**, *64*, 6688–6696; d) E. Block, M. Birringer, R. DeOrazio, J. Fabian, R. S. Glass, C. Guo, C. He, E. Lorance, Q. Qian, T. B. Schroeder, Z. Shan, M. Thiruvashi, G. S. Wilson, X. Zhang, *J. Am. Chem. Soc.* **2000**, *122*, 5052–5064; e) H. Fleischer, S. Glang, D. Schollmeyer, N. W. Mitzel, M. Bühl, *Dalton Trans.* **2004**, 3765–3771.

[17] a) R. Ditchfield, *Mol. Phys.* **1974**, *27*, 789–807; b) K. Wolinski, J. F. Hinton, P. Pulay, *J. Am. Chem. Soc.* **1990**, *112*, 8251–8260; c) T. Higashioji, M. Hada, M. Sugimoto, H. Nakatsuji, *Chem. Phys.* **1996**, *203*, 159–175; d) H. Nakatsuji, T. Higashioji, M. Sugimoto, *Bull. Chem. Soc. Jpn.* **1993**, *66*, 3235–3240; e) G. Magyarfalvi, P. Pulay, *Chem. Phys. Lett.* **1994**, *225*, 280–284; f) M. Bühl, W. Thiel, U. Fleischer, W. Kutzelnigg, *J. Phys. Chem.* **1995**, *99*, 4000–4007; g) G. Schreckenbach, Y. Ruiz-Morales, T. Ziegler, *J. Chem. Phys.* **1996**, *104*, 8605–8612.

[18] D. R. Doerge, R. S. Takazawa, *Chem. Res. Toxicol.* **1990**, *3*, 98–101.
[19] G. Roy, K. P. Bhabak, G. Mugesh, *Cryst. Growth Des.* **2011**, *11*, 2279–2286.
[20] a) L. Flohé, E. A. Günzler, H. H. Schock, *FEBS Lett.* **1973**, *32*, 132–134; b) J. T. Rotruck, A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, W. G. Hoekstra, *Science* **1973**, *179*, 588–590; c) Selenium Proteins Containing Selenocysteines. A. Bock in *Encyclopedia of Inorganic Chemistry*, *Vol. 8* (Ed.: R. B. King), Wiley, Chichester, England, **1994**, p. 3700; d) M. Birringer, S. Pilawa, L. Flohé, *Nat. Prod. Rep.* **2002**, *19*, 693–718; e) C. Jacob, G. I. Giles, N. M. Giles, H. Sies, *Angew. Chem.* **2003**, *115*, 4890–4907; *Angew. Chem. Int. Ed.* **2003**, *42*, 4742–4758.
[21] a) G. Mugesh, W.-W. du Mont, *Chem. Eur. J.* **2001**, *7*, 1365–1370; b) B. K. Sarma, G. Mugesh, *J. Am. Chem. Soc.* **2005**, *127*, 11477–11485; c) G. Roy, B. K. Sarma, P. P. Phadnis, G. Mugesh, *J. Chem. Sci.* **2005**, *117*, 287–303; d) K. P. Bhabak, G. Mugesh, *Chem. Eur. J.* **2007**, *13*, 4594–4601.
[22] A. Taurog, M. L. Dorris, W. X. Hu, F. S. Guziec, Jr., *Biochem. Pharmacol.* **1995**, *49*, 701.
[23] Gaussian 98, revision A.7, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Al-. Keith, M. A. Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. J. Replogle, A. J. Pople, Gaussian, Inc., Pittsburgh, PA, **1998**.
[24] W. Nakanishi, S. Hayashi, *J. Phys. Chem. A* **1999**, *103*, 6074–6081 and references therein.
[25] a) A. E. Reed, L. A. Curtiss, F. Weinhold, *Chem. Rev.* **1988**, *88*, 899–926; b) E. D. Glendening, J. E. Reed, J. E. Carpenter, F. Weinhold, *Natural Bond Orbital (NBO) Version 3.1*.
[26] Bruker. *SMART* (Version 6.028), *SAINT* (Version 6.02), *XPREP*. Bruker AXS Inc. Madison, Wisconsin, USA, **1998**.
[27] G. M. Sheldrick, *SADABS*. University of Göttingen, Germany, **1996**.
[28] SMART, Version 5.05, Bruker AXS, Madison, WI, **1998**.
[29] A. Altomare, G. Cascarano, C. Giacovazzo, A. Gualardi, *J. Appl. Crystallogr.* **1993**, *26*, 343–350.
[30] G. M. Sheldrick, *Acta Crystallogr. Sect. A* **1990**, *46*, 467–473.
[31] G. M. Sheldrick, SHELX-97, Program for the Refinement of Crystal Structures, University of Göttingen, Göttingen, Germany, **1997**.
[32] CCDC 926607 (**21**), CCDC 926608 (**24**), CCDC 926609 (**27**), CCDC 926610 (**41**), CCDC 926611 (**42**) and CCDC 926612 (**44**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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