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Antithyroid Drugs and Their Analogues: Synthesis, Structure, and Mechanism of Action

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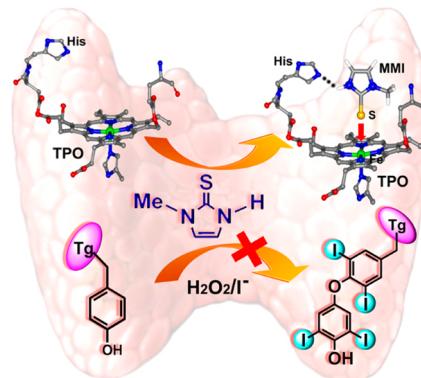
CONSPECTUS

Thyroid hormones are essential for the development and differentiation of all cells of the human body. They regulate protein, fat, and carbohydrate metabolism. In this Account, we discuss the synthesis, structure, and mechanism of action of thyroid hormones and their analogues.

The prohormone thyroxine (T4) is synthesized on thyroglobulin by thyroid peroxidase (TPO), a heme enzyme that uses iodide and hydrogen peroxide to perform iodination and phenolic coupling reactions. The monodeiodination of T4 to 3,3',5-triiodothyronine (T3) by selenium-containing deiodinases (ID-1, ID-2) is a key step in the activation of thyroid hormones. The type 3 deiodinase (ID-3) catalyzes the deactivation of thyroid hormone in a process that removes iodine selectively from the tyrosyl ring of T4 to produce 3,3',5'-triiodothyronine (rT3). Several physiological and pathological stimuli influence thyroid hormone synthesis. The overproduction of thyroid hormones leads to hyperthyroidism, which is treated by antithyroid drugs that either inhibit the thyroid hormone biosynthesis and/or decrease the conversion of T4 to T3.

Antithyroid drugs are thiourea-based compounds, which include propylthiouracil (PTU), methimazole (MMI), and carbimazole (CBZ). The thyroid gland actively concentrates these heterocyclic compounds against a concentration gradient. Recently, the selenium analogues of PTU, MMI, and CBZ attracted significant attention because the selenium moiety in these compounds has a higher nucleophilicity than that of the sulfur moiety. Researchers have developed new methods for the synthesis of the selenium compounds. Several experimental and theoretical investigations revealed that the selone ($C=Se$) in the selenium analogues is more polarized than the thione ($C=S$) in the sulfur compounds, and the selones exist predominantly in their zwitterionic forms.

Although the thionamide-based antithyroid drugs have been used for almost 70 years, the mechanism of their action is not completely understood. Most investigations have revealed that MMI and PTU irreversibly inhibit TPO. PTU, MTU, and their selenium analogues also inhibit ID-1, most likely by reacting with the selenenyl iodide intermediate. The good ID-1 inhibitory activity of PTU and its analogues can be ascribed to the presence of the $-N(H)-C(=O)-$ functionality that can form hydrogen bonds with nearby amino acid residues in the selenenyl sulfide state. In addition to the TPO and ID-1 inhibition, the selenium analogues are very good antioxidants. In the presence of cellular reducing agents such as GSH, these compounds catalytically reduce hydrogen peroxide. They can also efficiently scavenge peroxynitrite, a potent biological oxidant and nitrating agent.



Introduction

Thyroid hormones, produced by the thyroid gland, are iodine-containing compounds that regulate gene expression in every vertebrate tissue and control the metabolism in the body. The iodide ions are transported into the follicular cells of the thyroid gland by the sodium/iodide symporter (NIS), a transmembrane glycoprotein with a molecular weight of 87 kDa. For each iodide anion (I^-),

NIS transports two sodium cations (Na^+) into the cell, and the active NIS-mediated transport increases the concentration of iodide inside the cells by 20–50 times with respect to the iodide concentration in plasma.¹ The synthesis of the prohormone thyroxine (T4) on thyroglobulin is catalyzed by thyroid peroxidase (TPO), a heme enzyme that utilizes iodide and hydrogen peroxide to perform two distinct reactions: iodination of tyrosyl residues and phenolic

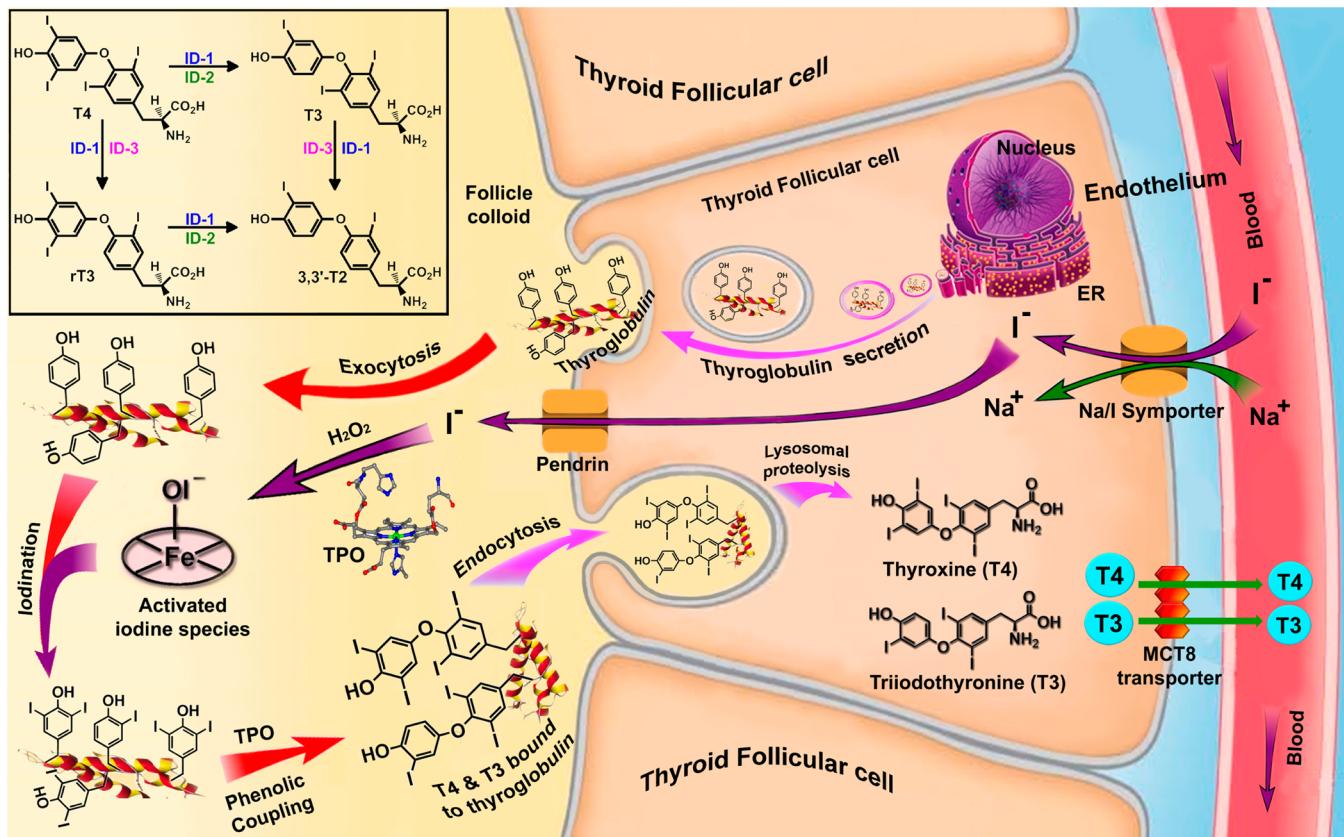


FIGURE 1. Biosynthesis of thyroid hormones from thyroglobulin by thyroid peroxidase and regioselective deiodination of thyroxine (inset).

coupling of the resulting iodinated tyrosyl residues of thyroglobulin. Subsequently, the tyroxyl residues are cleaved by proteolysis to produce free T4 (Figure 1).² Hydrogen peroxide, which is essential for the synthesis, is produced by the NADPH-dependent flavoproteins thyroid oxidases DUOX (dual oxidases) 1 and 2.³ Although a heme-linked iodine species [TPO-Fe(III)-OI⁻] has been proposed as the “iodinating intermediate”, there is no conclusive evidence for the formation of such an intermediate. While a small amount of T3 (3,3'-5-triiodothyronine), the active thyroid hormone, is also produced during synthesis, most of the circulating T3 is generated via 5'-deiodination of T4 and catalyzed by the selenoenzymes, type 1 and 2 iodothyronine deiodinases (ID-1 and ID-2).⁴⁻⁷ The deactivation of the thyroid hormone is catalyzed by the type 3 deiodinase (ID-3), which removes iodine exclusively from the tyrosyl ring of T4 to produce 3,3',5'-triiodothyronine (rT3). The triiodo derivatives T3 and rT3 are further metabolized by the three selenoenzymes to produce 3,3'-diiodothyronine (3,3'-T2) (Figure 1, inset).

The activation of thyroid stimulating hormone (TSH) receptor by autoantibodies leads to an overproduction of

thyroid hormones.⁸ These antibodies also stimulate the deiodinases to produce a larger amount of T3. As the activity of these antibodies is not controlled by the hypothalamic–pituitary–thyroid axis, the feedback system does not exert any negative influence on the thyroid activity. Therefore, the uncontrolled production of active thyroid hormones leads to “hyperthyroidism”. Autonomous adenoma, caused either by constitutive activation of the TSH receptor or by the Gs protein (gsp), is another cause of hyperthyroidism. The overproduction of T4 and T3 can be controlled by specific inhibitors (antithyroid agents), which either block the thyroid hormone biosynthesis or decrease the conversion of T4 to T3. Antithyroid drugs are thiourea-based compounds having a thione moiety within the heterocyclic structure (Figure 2). Propylthiouracil (**1**, PTU, 6-n-propyl-2-thiouracil) and methimazole (**2**, MMI, 1-methyl-2-mercaptopimidazole) are the antithyroid drugs used in the United States and MMI and its analogue, carbimazole (**3**, CBZ), are used in most European and Asian countries.^{9,10} A closely related methyl analogue, 6-methyl-2-thiouracil (**4**, MTU), also exhibits antithyroid activity, but it is not used clinically. These compounds are actively concentrated by the thyroid gland against a concentration gradient.

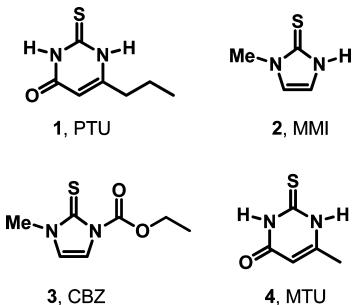
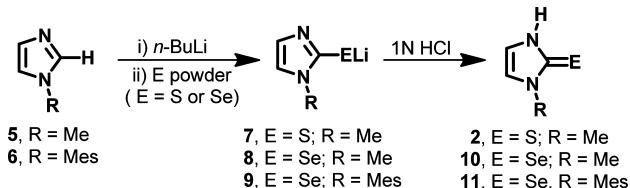


FIGURE 2. Chemical structures of commonly used antithyroid drugs.

SCHEME 1. Synthesis of MMI and its Selenium Analogue by Lithiation Route^{12–14}



Selenium Analogues of Antithyroid Drugs

The role of selenium in the thyroid gland is now well-established. In fact, the thyroid contains more selenium per gram of tissue than any other organ, and therefore, selenium is considered an essential trace element for normal thyroid function and thyroid hormone homeostasis. In recent years, the selenium analogues of MMI, PTU, and MTU attracted considerable attention due to the higher nucleophilic character of the selenium moiety in selones as compared to that of the sulfur in thiones, and the selenium analogues may inhibit the TPO activity by a different mechanism.¹¹ In general, selones can be synthesized in good yields by using electrophilic selenium, nucleophilic selenium, and carbon diselenide. Guziec et al. were the first to report the synthesis of the MMI and its selenium analogue (**10**) by using an electrophilic selenium route.¹² The low-temperature metalation of 1-methylimidazole (**5**) by *n*-BuLi followed by treatment with elemental sulfur/selenium produced the lithium thiolate (**7**) and selenolate (**8**). The addition of a 1 N HCl solution followed by an aqueous workup afforded compound **2** and **10**, respectively. This method has also been used to synthesize **11** having a mesityl group (Scheme 1).

Although the lithiation method can be used to synthesize MMI and its selenium analogue (**10**), the stability of **10** is significantly different from that of MMI. The selenium analogue (**10**) is very unstable and undergoes a facile oxidation to produce the diselenide (**13**) quantitatively.¹³ When the workup procedure was carried out under aerobic conditions,

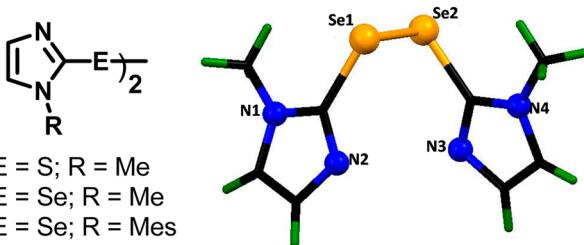


FIGURE 3. (a) Oxidized form of MMI, MSe1, and **14**. (b) Single crystal X-ray structure of MSe1 (**13**).¹³

only the diselenide could be obtained. The structure of **13** was confirmed by several techniques, including ⁷⁷Se NMR studies and single-crystal X-ray diffraction analysis (Figure 3).¹³ The introduction of sterically bulky groups does not block the diselenide formation, as compound **11** having a mesityl group also undergoes oxidation in air to produce the diselenide **14**.¹⁴ The expected selones **10** and **11** can be isolated and characterized when the workup procedure is carried out strictly under anaerobic conditions. The single crystal X-ray structures of compounds **10** and **11** confirmed the selone nature of the selenium moiety in its reduced form.^{14,15} However, a spontaneous oxidation of **10** to the corresponding diselenide (**13**) was observed during the crystallization under aerobic conditions. In contrast, MMI was found to be very stable and could not be converted to the disulfide (**12**), even by using oxidizing agents such as O₂ and H₂O₂.¹⁶

Studies on the keto–enol type tautomerism in the antithyroid drugs and their selenium analogues are important to understand the antithyroid activity of these compounds. It is clear that MMI (**2**) exists almost exclusively as the thione tautomer, which is important for the inhibition of thyroid hormone synthesis.¹⁷ The other sulfur-containing drugs, PTU (**1**), CBZ (**3**), and MTU (**4**), also exist as thione tautomers.^{18,19} The stability of the thione tautomer may prevent these compounds from the spontaneous oxidation to their corresponding disulfides, which may account for their high antithyroidal activity. As mentioned earlier, the selenium analogue of MMI (**10**) is not stable under aerobic conditions, leading to an assumption that **10** does not have a strong C–Se double bond, as the pi-overlap between carbon and selenium is expected to be weaker as compared to a pi-bond between carbon and sulfur atoms. Although the C–Se coupling constant (220 Hz) in the ¹³C NMR spectrum of **10** indicated the presence of a double bond,^{12,15} the recent experimental and theoretical studies confirmed that the compound exists as a zwitterion in which the selenium atom carries a negative charge (Figure 4).¹⁶

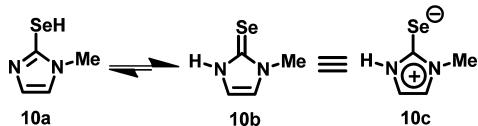
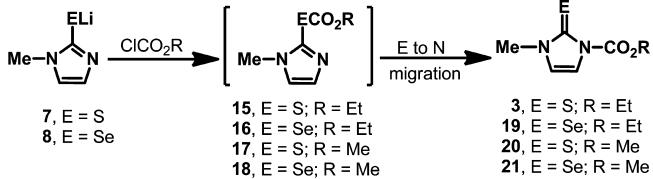


FIGURE 4. Selenol–selone tautomerism in compound **10**. The compound exists predominantly in its zwitterionic form **10c**, which may only have a partial C–Se double-bond character.¹⁶

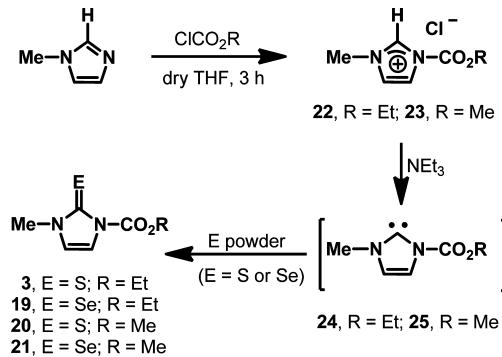
SCHEME 2. One-Pot Synthesis of CBZ and its Analogues by an Alkoxy-carbonyl Migration Route²¹



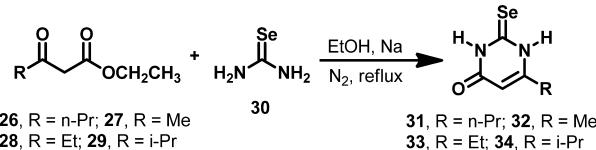
It is known that compound **3**, the ethoxycarbonyl derivative of MMI, acts as a prodrug, and the rapid enzymatic cleavage of the ethoxycarbonyl group in vivo leads to the formation of MMI.²⁰ As the spontaneous oxidation of selones to the corresponding diselenides may reduce the applicability of the selones in the drug development, the synthesis and biological evaluation of the selenium analogues of CBZ have been considered. The introduction of the $-\text{CO}_2\text{Et}$ group on the imidazole nitrogen of compound **8** should prevent the formation of a diselenide. Recently, a one-pot synthesis of **3** and **19** was achieved by an unusual ethoxycarbonyl migration route. The treatment of lithium thiolate **7** and selenolate **8**, generated by lithiation as shown in Scheme 1, with ethylchloroformate afforded **3** and **19**, respectively (Scheme 2).²¹ It should be noted that the reactions of **7** and **8** with ethylchloroformate generate the corresponding S- and Se-substituted derivatives (**15**, **16**) as intermediates. A rapid migration of the ethoxycarbonyl group from sulfur or selenium to the nitrogen leads to the formation of the expected compounds. Compounds **20** and **21** were synthesized by following a similar method using methylchloroformate.

Interestingly, a one-pot carbene route can also be used conveniently to synthesize carbimazole (**3**, CBZ) and its analogues **19–21** (Scheme 3).²¹ In this method, 1-methyl-imidazole was treated with ethyl- or methylchloroformate to produce the corresponding imidazolium salts (**22** and **23**). Treatment of these salts with triethylamine produced the *N*-heterocyclic carbenes (NHC) **24** and **25** as reactive intermediates, which upon addition of sulfur or selenium powder afforded the desired compounds **3**, and **19–21**. As these thiones and selones are readily soluble in organic solvents

SCHEME 3. The Synthesis of CBZ and its Analogues by Using Heterocyclic Carbenes Generated in Situ from the Corresponding Imidazolium Salts²¹



SCHEME 4. Synthesis of the Selenium Analogue of PTU from Selenouracil^{23–25}



such as chloroform, they can easily be separated from other saltlike impurities, which helps in the purification. The use of NEt_3 was found to be important for the generation of **24** and **25**, although imidazole-based carbenes are normally generated by using Na_2CO_3 or K_2CO_3 as a base.²² It should be noted that none of the reactions afforded the expected compounds when Na_2CO_3 or K_2CO_3 was used as the base, indicating the instability of the N–C bond in the presence of metal carbonates.

In addition to the selenium analogues of MMI, some selenouracil derivatives were studied as potent inhibitors of ID-1 and TPO.²³ The development of selenium-based compounds as inhibitors is based on the assumption that the selones, due to their greater nucleophilicity as compared to that of thiones, may react faster with the selenenyl iodide intermediate to form a stable diselenide bond with ID-1 (vide infra). Visser et al. and Guziec et al. reported the synthesis of the selenium analogues of PTU and MTU (Scheme 4).^{23–25} The reaction of ethyl 3-oxohexanoate (**26**) with selenourea (**30**) affords the selenium analogue of PTU (**31**). Compounds **32–34** having a methyl, ethyl, or *i*-propyl substituent can be prepared by following a similar procedure. The X-ray crystal structures of compounds **31–34** indicate that the compounds exist in their selone ($\text{C}=\text{Se}$) tautomeric forms and the N–H, C=O, and C=Se moieties are involved in extensive hydrogen bonding.²⁶

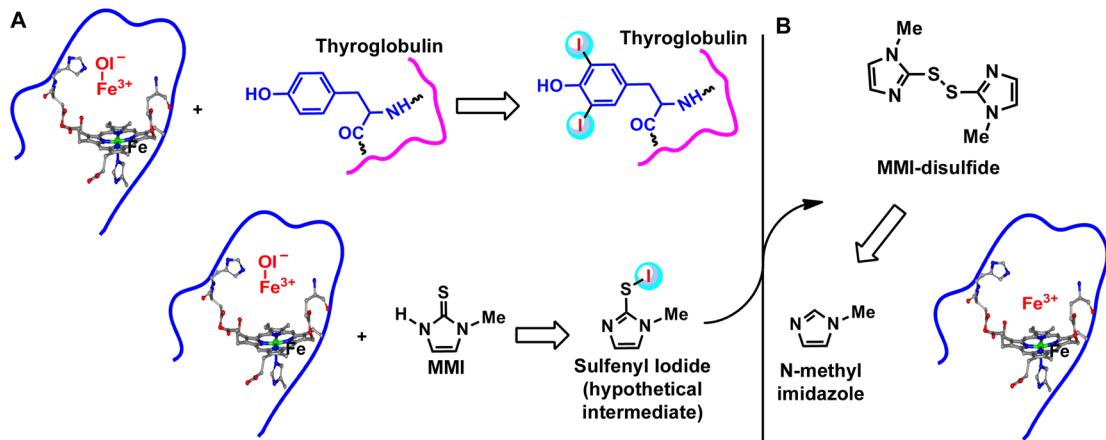


FIGURE 5. Proposed mechanism for the inhibition of TPO-catalyzed iodination of tyrosyl residues of thyroglobulin. (A) MMI is preferentially iodinated by TPO in the presence of thyroglobulin. (B) Formation of inactive MMI oxidation products (modified figure from ref 10).

Mechanism of Antithyroid Action

Although thionamide-based antithyroid drugs have been in use for almost seven decades, the mechanism of their action is not completely understood.¹⁰ MMI and PTU inhibit the thyroid hormone synthesis by blocking the TPO-mediated iodination of tyrosine residues in thyroglobulin, a key step in the synthesis of T4 (Figure 1). In an initially proposed mechanism, the antithyroid drugs have been shown to inhibit the iodination reaction by diverting the oxidized iodide ($\text{TPO}-\text{I}_{\text{ox}}$) away from thyroglobulin, although the nature of the “iodinating intermediate” ($\text{TPO}-\text{I}_{\text{ox}}$) is not known. While the heme-linked hypoidote ($\text{TPO}-\text{OI}^-$) can iodinate tyrosyl residues,²⁷ most of the earlier studies suggest the involvement of hypoidous acid (HOI) as the iodinating species.²⁸ This assumption is based on the mechanism of other haloperoxidases, such as chloroperoxidase or bromoperoxidase, which involves HOCl or HOBr, respectively, as halogenating species.²⁸ However, in the presence of MMI, the iodination occurs preferably at the sulfur center of MMI instead of the tyrosyl residues of thyroglobulin, leading to the formation of a sulfenyl iodide derivative (Figure 5).¹⁰ In this case, MMI serves as an alternative substrate for the iodinating intermediate, competing with thyroglobulin-linked tyrosine residues. The unstable sulfenyl iodide may undergo a disproportionation reaction to produce the corresponding disulfide, which spontaneously degrades to an inactive desulfurated molecule, *N*-methylimidazole (Figure 5).^{10,29}

As most of the investigations confirmed that MMI and PTU irreversibly inhibit TPO even in the presence of a relatively high concentration of iodide, the mechanism shown in Figure 5 does not explain the irreversibility of the

inhibition. Another mechanism proposed for the inhibition of TPO involving competitive coordination of the sulfur atom of MMI to the Fe(III) center³⁰ also does not correlate well with the in vivo inhibitory effect of MMI. However, it is quite possible that some of the intermediates derived from MMI (Figure 5) may bind to the heme center of TPO. Poulsom et al. reported that the sulfinic acid derivative of MMI is very labile and it undergoes a rapid desulfuration to produce *N*-methyl imidazole.³¹ The formation of *N*-methyl imidazole was also observed from the reaction of MMI with peroxy nitrite.^{32,33} On the other hand, Neal has proposed that the S-monoxide of MMI decomposes to imidazole carbene and sulfur monoxide.³⁴ As MMI can react with metal ions to form metal–carbene complexes,³⁵ a possible mechanism involving the formation a stable metal–carbene complex with the heme center cannot be ruled out.

Taurog et al. showed that the inhibitory effect of PSeU on TPO-catalyzed iodination and its mechanism are almost identical to that of PTU.^{24,25} In the TPO inhibition, compound **10** was found to be 4–5 times less potent than MMI in a guaiacol assay, but this compound was only two times less active than MMI in an iodination assay. Further in vivo experiments with rats indicated that **10** was 50 times less potent than MMI in inhibiting thyroidal organic iodine formation, suggesting that the selenium compound is poorly concentrated by the thyroid.²⁴ Recent studies suggest that the selenium analogues of MMI inhibit the peroxidase-mediated iodination and oxidation by a mechanism different from that of MMI.^{13,16} As selenium compounds are more susceptible to oxidation than their sulfur analogues, it is possible that the facile oxidation of the selenium moiety may lead to inhibition of peroxidase activity. When lactoperoxidase (LPO) was employed, compound **10** inhibited the

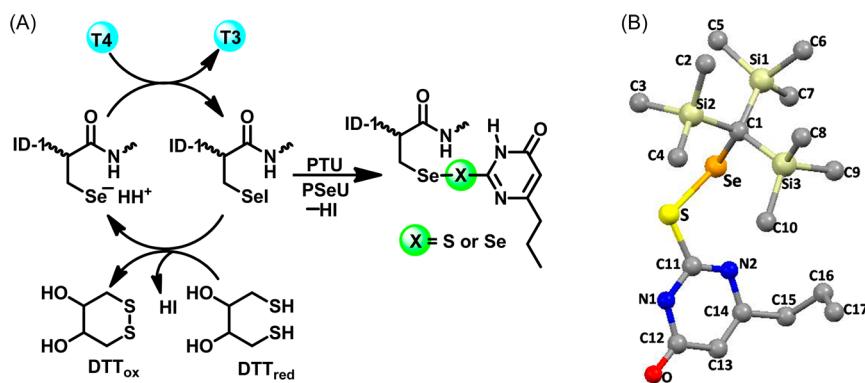


FIGURE 6. (A) Deiodination of T4 by ID-1 and inhibition deiodinase activity by PTU and PSeU. (B) X-ray structure of a model selenenyl sulfide obtained from the reaction of a stable selenenyl iodide and PTU.³⁹

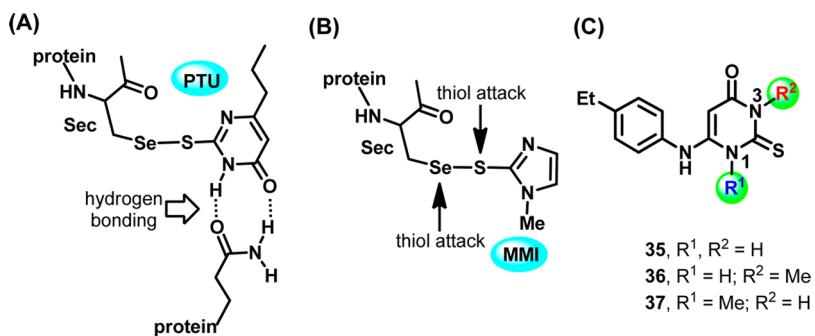


FIGURE 7. A hypothetical model showing (A) a possible hydrogen bond-assisted stabilization of an ID-1-PTU complex and (B) the absence of hydrogen bonding in a ID1-MMI complex (Sec = selenocysteine). (C) Aniline-based thiouracils as human placenta ID-1 inhibitors.⁴²

enzyme activity due to the facile oxidation of the reactive selenone moiety by H₂O₂ or by the oxidized enzyme.¹⁶ Compound **10** was found to be two times less efficient than MMI, but it was almost three times more potent than PTU and MTU. While the LPO activity was completely inhibited by MMI, the enzyme's activity could not be recovered by increasing the H₂O₂ concentration, suggesting that MMI does not act on H₂O₂. In contrast, in the case of compound **10**, the enzyme's activity could be completely recovered by increasing the H₂O₂ concentration.^{16,36} These observations strongly support the assumption that MSel (**10**), in contrast to MMI, does not interfere with the native enzyme directly but inhibits the LPO activity by reducing the H₂O₂, which is required for the oxidation of the iron center in LPO.

In contrast to MMI, which has an effect only on the TPO-catalyzed iodination, PTU and MTU can inhibit ID-1 possibly by reacting with one of the enzyme intermediates. PTU and related thiouracils do not inhibit all deiodinases in various species and the sequences of the active sites of some deiodinases are resistant to PTU inhibition. It has been proposed that PTU may react with the selenenyl iodide intermediate of ID-1 to form a stable selenenyl sulfide (Figure 6A).^{5–7,37} This led to the assumption that the

selenium analogue of PTU might be a more potent inhibitor of ID-1 than PTU. To test this hypothesis, Taurog et al. studied the inhibition of ID-1 by PTU and PSeU in a test system containing ¹²⁵I-rT3, rat liver microsomes, and DTT.²⁵ However, PTU and PSeU were found to be essentially equipotent as inhibitors of ID-1, indicating that the substitution of sulfur in PTU with selenium does not offer any significant advantage. The potent inhibitory effects of PTU toward ID-1 can be ascribed to the presence of the –N(H)–C(=O)– functionality that can form hydrogen bonding with nearby amino acid residues after the formation of a stable selenenyl sulfide, as shown in Figure 7A. Some amino acid residues such as Asn or Gln may play a crucial role in fixing the amino and carbonyl groups of the thiouracil moiety by strong hydrogen bonding. The existence of such hydrogen-bonding arrangements between the uracil moiety and Asn and His residues has been demonstrated for the uracil–DNA glycosylation.³⁸ The Se–S bond is, therefore, stabilized by the hydrogen-bond network.

Model studies on the reactivity of selenenyl iodides toward thiols/thiones supported the assumptions that PTU does not react with the native enzyme but only with an E-Sel intermediate containing a covalent Se–I bond.^{39–41}

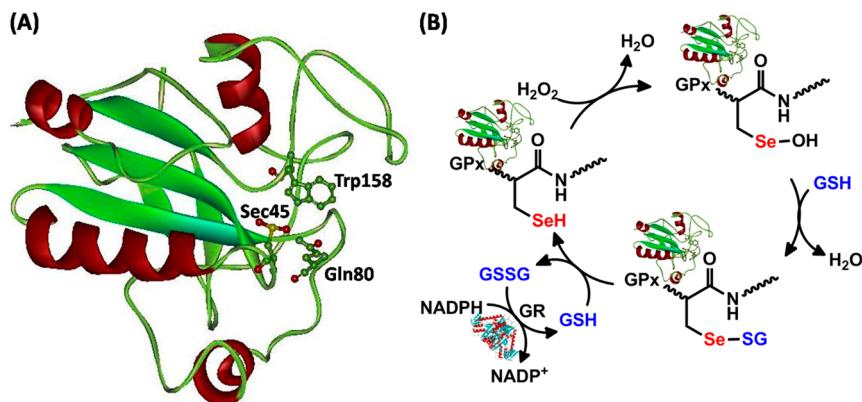


FIGURE 8. (A) X-ray crystal structures of GPx (PDB:1GP1).⁵⁰ (B) Proposed catalytic mechanism of GPx, involving selenol, selenenyl sulphide, and selenenic acid intermediates. GR = glutathione reductase.

MMI reacts with selenenyl iodide with a rate comparable to that of PTU,³⁹ indicating that it is not the difference in the reactivity toward the E-Sel intermediate, but it is the stability of the resulting selenenyl sulfides that controls the different degrees of inhibition.^{39–41} This is clearly connected with the fact that the MMI-derived selenenyl sulfide does not possess any N–H and/or $-N(H)C(=O)-$ groups for hydrogen bonding within the active site (Figure 7B). In agreement with this, compound **35** having two free N–H moieties showed strong inhibition of human placenta ID-1.⁴² On the other hand, compound **36** having a methyl group at the N-3 position or compound **37** that lacks N-1 hydrogen did not show any noticeable inhibition.⁴² These observations strongly suggest that not only the presence of N1–H for the reaction with Se–I intermediate but also the presence of N3–H for stabilization are essential for the efficient inhibition by PTU and related derivatives. It should be noted that PTU and related compounds do not inhibit the activity of ID-2 or ID-3, and the reason for this difference is still not clear.^{5–7} Recent model studies on ID-3 mimics indicate that the stability of the selenenyl iodide intermediate may play a crucial role in the inhibition.^{43–45}

Antioxidant Activity

Oxidative stress caused by reactive oxygen species (ROS) has been implicated in numerous autoimmune disorders, including Graves' disease.⁴⁶ The treatment of patients with MMI resulted in normalization of the ROS and antioxidant activity indices,⁴⁷ indicating that antithyroid drugs having antioxidant activity may have beneficial effects in the treatment for hyperthyroidism. The antioxidant and immunomodulatory effects of MMI on thyrocytes and immune cells have been studied.⁴⁷ The thyroid gland has remarkably high amounts of selenium and the large number of selenoproteins that are

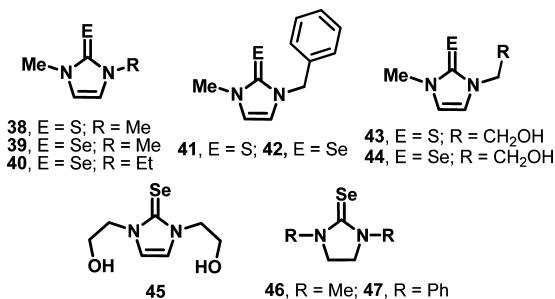
present may be involved in the protection of the gland against the ROS.⁴⁸ Particularly, the antioxidant selenoenzyme, GPx, may control the H_2O_2 level in the thyroid by catalytically reducing it with the help of glutathione (GSH).⁴⁹ The selenocysteine residue, which acts as a single redox center (Figure 8A), reacts with the peroxide to form a selenenic acid. A nucleophilic attack of GSH at the selenium center generates the selenenyl sulfide, which upon reaction with another GSH molecule generates the selenol, releasing GSSG as the byproduct. The concentration of GSH is maintained by glutathione reductase (GR), which catalytically reduces GSSG to GSH by using NADPH as a cofactor (Figure 8B).^{49,50}

As selenium compounds generally exhibit a much better antioxidant activity than their sulfur analogues,^{51,52} recent studies were focused on the GPx-like activity of thiones and selones. In the presence of GSH, the GPx activity of **10** was found to be much higher than that of MMI.^{13,16} A structure–activity correlation study by using H_2O_2 as a substrate and PhSH as a cofactor indicated that selones that are potent inhibitors of LPO-catalyzed reactions exhibit high GPx-like activity.⁵³ Interestingly, compound **10** with a free N–H group in the imidazole ring exhibited almost 80 times higher activity than ebselen, a well-known GPx mimic (Table 1). In contrast, MMI did not show any noticeable activity under identical conditions. The activity of **38–45** was found to be 4–12 times higher than that of ebselen (Table 1). Among the selones, compound **40** with an ethyl substituent exhibited the highest GPx activity. The GPx activity of compound **44** ($20.8 \mu\text{M}/\text{min}$) is almost 7 times higher than that of ebselen ($2.9 \mu\text{M}/\text{min}$). The substituents at the nitrogen atom of the imidazole ring appear to play an important role in their antioxidant activities. While compounds **39** and **45** exhibited almost similar GPx-like activities (16.3 and $15.2 \mu\text{M}/\text{min}$,

TABLE 1. GPx-Like Activity of Thiones and Selones in the Presence of PhSH⁵³

compd	initial rate ($\mu\text{M min}^{-1}$) ^a	compd	initial rate ($\mu\text{M min}^{-1}$) ^a
Ebselen	2.9 ± 0.4	42	11.2 ± 1.7
MSel (10)	231.7 ± 8.8	43	inactive
38	inactive	44	20.8 ± 1.8
39	16.3 ± 1.1	45	15.2 ± 1.5
40	29.1 ± 1.5	46	12.7 ± 0.3
41	inactive	47	13.9 ± 1.7

^aThe concentration was at 100 μM of the test compounds. The amount of diphenyl disulfide (PhSSPh) formed during the initial period of reaction was monitored by HPLC.

**FIGURE 9.** *N,N'*-disubstituted thiones and selones used for studying GPx-like activity.⁵³

respectively), introduction of a benzyl group appears to reduce the GPx activity, as the activity of compound **42** (11.2 $\mu\text{M}/\text{min}$) is much lower than that of the alkyl-based compounds **39**, **40**, and **44**. However, this compound was found to be almost 4 times more active than ebselen. Although **46** and **47** exhibited very weak LPO inhibition, the GPx activity was only slightly lower than that of the selones that have a C–C double bond in the imidazole ring (see Figure 9).⁵³

In addition to the GPx-like activity, the antithyroid drugs and their analogues effectively scavenge peroxynitrite (ONOO^- , PN), a potent biological oxidant and nitrating agent. PN, formed in vivo from the reaction between superoxide and nitric oxide radicals, can damage a wide array of molecules in cells, including DNA and proteins. PN is known to nitrate tyrosyl residues in peptides/proteins, and nitrotyrosine is detected in a large number of pathological conditions.⁵⁴ Therefore, nitrotyrosine is considered a biomarker of NO-dependent, reactive nitrogen species-induced nitrative stress. Recent evidence suggests that PN and/or nitrative stress may participate in the pathogenesis of thyroid diseases. MMI and its selenium analogues, PTU and MTU, significantly inhibit protein tyrosine nitration, although PTU and MTU are slightly less effective than MMI. As the S- and Se-methylated compounds exhibit a weak inhibitory effect in the nitration of tyrosine, it has been suggested that

the presence of a thione or selone moiety is important for the PN-scavenging activity.^{32,33} While the replacement of the N–H moiety in MMI by the *N*-methyl substituent significantly reduced the antioxidant activity, such substitution in the selenium analogue of MMI increased the activity of the parent compound. Theoretical studies indicated that the substitution of the N–H moiety by N–Me significantly increases the energy required for the PN-mediated oxidation of the thione. On the other hand, the selenium moieties in the selones undergo a facile oxidation by PN to produce the corresponding selenenic and seleninic acids. In contrast to the *N,N'*-disubstituted thiones, the selones predominantly exist in their zwitterionic forms, in which the selenium atom carries a negative charge which is responsible for their higher PN-scavenging activity.^{32,33} The PN-scavenging activity of antithyroid drugs raises the question of whether these compounds would also be inhibitors of NOX enzymes, which are shown to be expressed and are relevant to the thyroid structure and function.

Conclusions and Outlook

The surgical removal of the thyroid has been in practice for centuries for the treatment of thyrotoxicosis. In 1928, Chesney et al. reported that cabbage, cauliflowers, and turnips possess goitrogenic effects in animals.⁵⁵ The serendipitous discovery of compounds causing goiter in animals led to the introduction of thiourea and thiouracil as possible medical treatment of thyrotoxicosis in 1943.^{56,57} Since then, thionamide-based antithyroid drugs 1-methyl-2-mercaptoimidazole (methimazole, MMI), 6-*n*-propyl-2-thiouracil (PTU), and carbimazole (CBZ) are commonly used to treat hyperthyroidism caused by Graves' disease. These drugs block the synthesis of thyroid hormones by inhibiting the heme-containing thyroid peroxidase (TPO) that catalyzes the iodination and coupling of tyrosyl residues on thyroglobulin. In addition, PTU can inhibit the selenium-containing enzyme type I iodothyronine deiodinase (ID-1) and, thereby, block the conversion of thyroxine (T4) to 3,3',5-triiodothyronine (T3) within the thyroid and in peripheral tissues. However, the inhibition of ID-1 does not appear to have clinical importance in most instances, probably due to the fact that PTU does not inhibit ID-2, which also catalyzes the conversion of T4 to T3 by phenolic ring (5') deiodination.

For the last two decades, the selenium analogues of MMI, PTU, and MTU attracted considerable attention. As the hypermetabolic state due to an increase in the active thyroid hormone concentration is associated with tissue oxidative injury and hyperthyroid tissues exhibit an increased

production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), the unique redox properties of selenium may be beneficial in the treatment of hyperthyroidism. Several efficient methodologies have been developed for the synthesis of the selenium analogues of MMI, PTU, and MTU. Particularly, a novel, one-pot synthetic route involving carbene intermediates has been developed for the synthesis of carbimazole and its selenium analogue. In contrast to MMI, which irreversibly inhibits TPO, the inhibition by the selenium analogues is reversible. Furthermore, the antioxidant activity of the selenium compounds was found to be much higher than that of the sulfur compounds. In the presence of glutathione (GSH), the selenium analogue of MMI mimics the glutathione peroxidase (GPx) by catalytically reducing hydrogen peroxide to water. The selenium analogues also inhibit peroxynitrite-mediated oxidation and protein tyrosine nitration.

While the current antithyroid medication is found to be satisfactory in most cases, adverse effects have been reported, the most dangerous one being agranulocytosis, an acute condition involving a severe leukopenia. The future biomedical research on thyroid hormones and antithyroid drugs may focus on development of isoform-specific inhibitors for the deiodinases. At the moment, there are no reports of compounds that can selectively inhibit one of the deiodinases without affecting the thyroid hormone biosynthesis. As both excess and deficiency of T4/T3 can cause diseases, controlling the deiodinase activity by specific inhibitors may help in treating patients with neuropsychiatric disorders or cardiovascular diseases.

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FOOTNOTES

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