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Biomimetic Studies on Anti-Thyroid Drugs and Thyroid Hormone Synthesis

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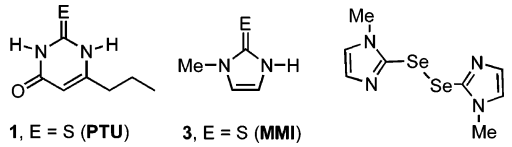
Thyroxine (**T4**) is the main secretory product of the thyroid gland, and the deiodination of this prohormone to the biologically active hormone, 3,5,3′-triiodothyronine (**T3**), is the first step in thyroid hormone action. It is well known that type I iodothyronine deiodinase (ID-1), a selenocysteine-containing enzyme, is responsible for most of this conversion.[1] The activation of thyroid stimulating hormone (TSH) receptor by autoantibodies leads to an overproduction of thyroid hormones, which can be controlled by specific inhibitors such as 6-*n*-propyl-2-thiouracil (**1**, PTU) and methimazole (**3**, MMI) that either block the thyroid hormone biosynthesis or reduce the conversion of **T4** to **T3**.[1]



1, E = S (**PTU**)
2, E = Se (**PSeU**)
3, E = S (**MMI**)
4, E = Se (**MSeI**)
5 (**MSeI$_{ox}$**)

Although these are the most commonly employed drugs for hyperthyroidism, the mechanism of their action is still not clear. It has been proposed that these drugs form stable electron donor−acceptor complexes with I_2 and divert oxidized iodides away from thyroglobulin, which effectively reduces the biosynthesis of thyroid hormones.[2] Another mechanism suggests that these drugs may coordinate to the thyroid peroxidase (TPO), a heme enzyme, which catalyzes the oxidation of iodides and the coupling of iodothyrosine residues of thyroglobulin.[3] It has also been reported that PTU can block the conversion of **T4** to **T3** by reacting with the selenenyl iodide intermediate (E-SeI) of ID-1 to form a selenenyl sulfide as a dead end product.[4]

Recently, the selenium analogues **2** (**PSeU**) and **4** (**MSeI**) attracted considerable attention because these are expected to be more nucleophilic than their sulfur analogues and the formation of an −Se−Se− bond with ID-1 may occur more readily than the formation of an −Se−S− bond.[5] However, the selenium compounds were found to be less potent than the sulfur analogues, and the reason for the relatively low potency is still not clear.[5] In view of this, we have undertaken a biomimetic study on **MSeI** to probe the mechanism by which the selenium derivatives exert their inhibitory action. In contrast to **MMI**, the selenium analogue exists in a diselenide form (**5**, **MSeI$_{ox}$**, Figure 1).[6]

In this study, we employed the Fe-containing lactoperoxidase (LPO) as a model for TPO, because both of the enzymes have been shown to have similar properties.[7] The IC_{50} values for the inhibition of LPO-catalyzed oxidation 2,2′-azino-bis-3-ethylbenzthiazoline sulfonic acid (ABTS) by the test compounds are summarized in Table 1, and the inhibition curves for **MMI** and **5** are shown in Figure 2.

As expected, **MMI** exhibited a strong inhibition with an IC_{50} value of 6.6 μM. The selenium analogue (**5**) also showed a strong inhibition, although its activity was found to be almost 2 times lower than that of **MMI**. This suggests that the selenium analogue

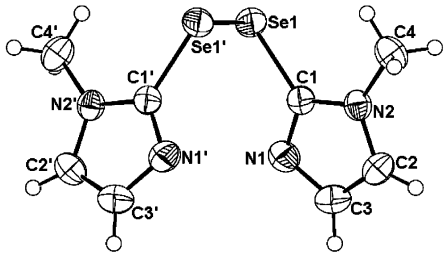


Figure 1. Molecular structure of **5**.

Table 1. Inhibition of LPO Activity by **MMI**, and **5**−**7**

no.	compound	IC_{50} (μM)[a]
1	**MMI**	6.6
2	**5**	16.7
3	**6**	15.9
4	**7**	20.1

[a] Concentration of the compound causing 50% inhibition.

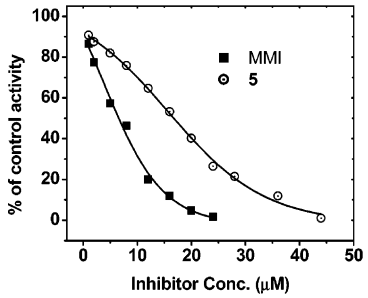
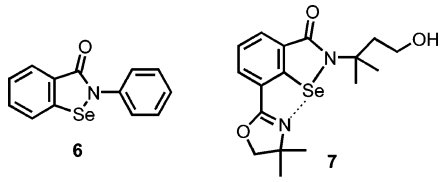


Figure 2. Inhibition curves for the LPO-catalyzed oxidation of ABTS.

may inhibit the LPO by a different mechanism. To verify this hypothesis, we have carried out the inhibition experiments with ebselen **6** and selenazole **7**,[6] which do not have any thione or selenone moiety that has been proposed to be important for TPO or LPO inhibition. Interestingly, the activities of **6** and **7** were found to be comparable to that of **5**, indicating that thione or selenone moieties are not required for the inhibition of LPO. To the best of our knowledge, compounds **5**−**7** represent the first examples of selenium compounds that inhibit the LPO activity.



Recent studies on thyroid hormone metabolism suggest that glutathione peroxidase (GPx),[8] another selenoenzyme present in the thyroid gland, degrades intracellular H_2O_2 and thereby inhibits the iodination reactions.[9] These observations indicate the possibility

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Table 2. Initial Rates (v_0) for the Reduction of H_2O_2 in the Presence of Selenium Catalysts[a]

no.	compound	v_0 (μM min^{-1})
1	**GPx**[b]	30.55 ± 0.87
2	**MSeI$_{ox}$ (5)**	81.05 ± 0.40
3	**Ebselen (6)**	78.46 ± 0.63
4	**7**	132.70 ± 2.84
5	**GPx + MSeI$_{ox}$**	106.69 ± 3.97
6	**ebselen + MSeI$_{ox}$**	150.56 ± 3.53
7	**7 + MSeI$_{ox}$**	195.52 ± 2.03

[a] Conditions: GSH, 1 mM; EDTA, 1 mM; GSSG reductase, 0.6 unit/mL; NADPH, 0.2 mM; H_2O_2, 1 mM; selenium catalysts, 0.025 mM; in 0.1 M potassium phosphate buffer, pH 7.3. [b] GPx: 5 nM.

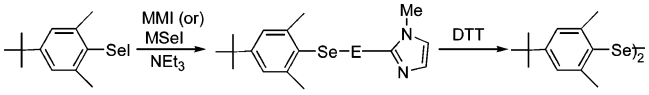
that some of the anti-thyroid drugs may inhibit the thyroid hormone biosynthesis by reducing H_2O_2 (GPx-like activity), because the oxidation of iron center in TPO by H_2O_2 is the first step in thyroid hormone synthesis. Therefore, we focused our attention on the GPx activity of **MSeI$_{ox}$** and some related compounds. Interestingly, **MSeI$_{ox}$** exhibited high GPx activity, providing a novel mechanism for its inhibitory action. The activity of **MSeI$_{ox}$** was found to be comparable to that of ebselen (**6**),[10] a well-known GPx mimic (Table 2). On the other hand, the sulfur analogue, **MMI**, did not show any noticeable activity under identical experimental conditions.

The high GPx activity of **5**−**7** suggests that the selenium analogues of the anti-thyroid drugs and other GPx mimics inhibit the LPO activity by reducing H_2O_2 and these compounds may also act as antioxidants and protect cells from oxidative damage. It has been suggested that an increase in the H_2O_2 concentration in the thyroid could damage the gland and be an important factor in the pathophysiology of myxedematous cretinism.[11]

We also studied the effect of **3** and **5** on the GPx activity of the natural enzyme and some GPx mimics because the electrophilic reactivity of selenium in the selenenic acid (E-SeOH) intermediate in the GPx cycle is similar to that of the E-SeI intermediate in the deiodinase cycle. Therefore, the anti-thyroid drugs may interfere with GPx by reacting with the E-SeOH to form stable −Se−S− or −Se−Se− bonds. As it can be seen from Table 2, the activities of GPx, ebselen (**6**), and compound **7** in the presence of **MSeI$_{ox}$** are almost equal to the sum of the initial reduction rates observed in the individual cases. The sulfur compound, **MMI**, also did not inhibit the GPx or the model compounds, suggesting that the anti-thyroid drugs do not interfere with other selenoenzymes such as GPx in the thyroid gland.

Further experiments on the reduction of diselenide bonds show that the −Se−Se− bond in **MSeI$_{ox}$** can also be cleaved by reducing agents such as dithiothreitol (DTT). It should be mentioned that the identity of the physiological second substrate is uncertain for ID-1 and DTT is the major cosubstrate used in in vitro deiodination experiments. Therefore, DTT may reduce the diselenide to produce the corresponding selenone species, which can react with the E-SeI intermediate to form an enzyme−drug diselenide bond. To test this hypothesis, we synthesized selenenyl iodide **8** as a model for the E-SeI intermediate, and the reactivity of this compound toward **MMI** and **MSeI** was followed by ^{77}Se NMR spectroscopy (Scheme 1). The reactions of selenenyl iodides with selenourea derivatives have not been studied previously, although the reactivity of selenenyl iodides toward thiourea drugs has been reported.[4b] Reactions of **MMI** and **MSeI** with **8** in the presence of NEt_3 afforded the selenenyl sulfide (**9**) and diselenide (**10**), respectively. Interestingly, the Se−S bond in **9** and the Se−Se bond in **10** were

Scheme 1. Reaction of **8** with **MMI** and **MSeI**



8 **9**, E = S; **10**, E = Se **11**

readily cleaved by DTT to produce **11**. This suggests that, although **MMI** and **MSeI** can form the corresponding selenenyl sulfides and diselenides with stable selenenyl iodides, the resulting compounds are not stable under reducing conditions. This is in agreement with the recent observations that **MSeI** exhibits very weak inhibition on ID-1 even at higher concentrations.[5c]

In summary, we have shown that the selenium analogues of anti-thyroid drugs exhibit their anti-thyroid activity by a mechanism different from that of MMI. The inhibition of TPO by selenium compounds is mainly due to their ability to act as GPx mimics, and the insensitivity of ID-1 toward **MSeI** is due to the inability of the drug to form a stable −Se−Se− bond. This study suggests that suitably designed selenium compounds may solve the problems associated with **PTU** and **MMI**, which are irreversible inhibitors of TPO. Alternatively, the anti-thyroid drugs, together with GPx, may constitute a defense system against reactive oxygen species in the thyroid gland.

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Supporting Information Available: Experimental procedures (PDF) and X-ray data for **5** and **7** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) Crystal data for **5** ($C_8H_{10}N_4Se_2$): Mr = 320.12, monoclinic, space group $C2/c$, a = 12.618(7), b = 7.536(4), c = 11.418(6) Å, β = 97.446(9)°, V = 1076.5(10) Å^3, Z = 4, ρ_{calcd} = 1.975 Mg/m^3, Mo Kα radiation (λ = 0.71073 Å), T = 293(2) K; R_1 = 0.0349, wR_2 = 0.0995 ($I > 2\sigma(I)$); R_1 = 0.0387, wR_2 = 0.1021 (all data). Crystal data for **7** ($C_{16}H_{20}N_2O_3Se$): Mr = 367.30, triclinic, space group P, a = 9.486(6), b = 9.965(6), c = 10.953(6) Å, α = 91.403(9); β = 111.942(8); γ = 118.051(8)°, V = 821.8(8) Å^3, Z = 2, ρ_{calcd} = 1.484 Mg/m^3, Mo Kα radiation (λ = 0.71073 Å), T = 293(2) K; R_1 = 0.0264, wR_2 = 0.0674 ($I > 2\sigma(I)$); R_1 = 0.0297, wR_2 = 0.0693 (all data).
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