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# SYNTHESIS AND THE ANTIPEROXIDASE ACTIVITY OF SELENO ANALOGUES OF THE ANTITHYROID DRUG PROPYLTHIOURACIL

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The synthesis of 6-n-propyl-2-selenouracil (PSeU, **Ib**) and its methyl derivative (MSeU, **Ic**) are described. Replacement of the sulfur atom at $C_2$ by selenium increased with antiperoxidase activity of these analogues five fold when compared to the clinically used antithyroid drug propylthiouracil (PTU).

The structure-activity relationships of these agents are discussed.

KEY WORDS: Seleno analogues of propylthiouracil, antithyroid activity, antiperoxidase activity, structure-activity relationships.

## INTRODUCTION

Information relating to the pharmacology of antithyroid drugs was obtained primarily in the 1940's and 1950's with the compounds of interest at that time, thiourea and thiouracil. Because of toxicity, these drugs have been superceded by propylthiouracil (6-n-propyl-2-thiouracil; PTU, Figure **Ia**), which is currently the drug of choice in the treatment of hyperthyroidism.

The primary action of propylthiouracil is on the thyroid gland where it inhibits thyroid peroxidase, resulting in a blockade of iodide utilization for thyroid hormone synthesis.[1] Propylthiouracil also inhibits the peripheral action of thyroxine and apparently crosses the placenta, producing adverse effects in the developing fetus.[2]

In the present study, two selenium analogues of propylthiouracil, namely, 6-n-propyl-2-selenouracil (PSeU, Figure **Ib**) and 6-methyl-2-selenouracil (MSeU, Figure **Ic**) have been synthesized and evaluated to determine if they inhibit thyroid peroxidase activity against the sulfur congener, namely PTU. This study is aimed at developing a potent antithyroid drug which does not cross the placenta or have inhibitory action on peripheral thyroxine effects. Furthermore, it will add information to the structure-activity relationships (SAR) of this class of compounds.

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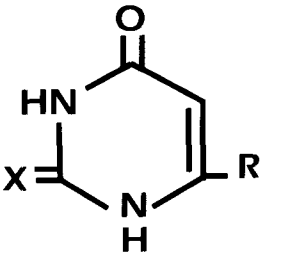


| | X | R | |
|---|---|---|---|
| Ia | S | n- $C_3H_7$ | PTU |
| Ib | Se | n-$C_3H_7$ | PSeU |
| Ic | Se | $CH_3$ | MSeU |

**Figure 1** Chemical structures of propylthiouracil and selenouracil analogues.

## MATERIALS AND METHODS

### *Chemistry*

PTU was obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). The synthesis of PSeU and MSeU are shown below in Figure 2. Ethyl acetoacetate, ethyl butyrylacetate and selenourea were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin, USA).

All IR and NMR spectra were in agreement with the assigned structures. Elemental analyses (C, H & N) were performed by Galbraith Laboratories, Inc. Knoxville, TN, USA, and by PRC, Inc. Gainesville, Fl., USA and were in agreement with theoretical calculations within $\pm 0.4\%$ experimental error. All mass spectra were obtained with a Finnigan Model 1015 SL quadrupole instrument operating at 700 $\mu$°A, an ionization energy of 70 eV, an ion source temperature of 150°C and at a vacuum of $1-2\times10^{-7}$

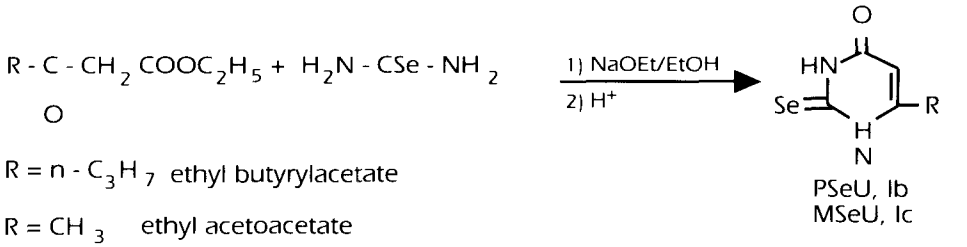


**Figure 2** The synthesis for the selenouracil analogues Ib and Ic.

Torr. Samples were introduced into the ion source directly. The detailed fragmentation patterns of these compounds has been previously published by Aboul-Enein.[3]

*6-n-Propyl-2-selenouracil* **(Ib)**
Selenourea (3.08 g, 25 mmoles) and ethyl butyrylacetate (3.96 g, 25 mmoles) were added simultaneously to a solution of sodium ethoxide (1.15 g of sodium in 70 ml of absolute ethanol). The mixture was refluxed for 8 h and ethanol was removed under reduced pressure. The residue was dissolved in 50 ml of water and acidified to pH 3 and 1 M hydrochloric acid. The crude product was filtered, washed with water and recrystallized from aqueous ethanol to yield 1.1 g (20%) of pure **Ib**, m.p. 233–234°C; NMR ($CDCl_3$): $\delta$0.88 (t, 3, $CH_3$), 1.51 (m, 2, $CH_2$), 2.28 (t, 2, $CH_2$) and 5.82 (s, 1, NH). Found: C, 31.76; H, 3.17; N, 14.77; $C_7H_{10}N_2OSe$ requires C, 31.76; H, 3.20; N, 14.82%.

*6-methyl-2-selenouracil* **(Ic)**
This was prepared by a similar method to that described for PSeU (**Ib**) using selenourea (3.08 g, 25 mmoles) and ethyl acetoacetate (3.3 g, 25 mmoles). The yield was 1.36 g (29%), m.p. 253–255°C; NMR ($CDCl_3$): 2.09 (s, 3, $CH_3$) and 5.82 (s, 1, NH). Found: C, 38.69; H, 4.54; N, 12.88. $C_5H_6N_2OSe$ requires: C, 38.71; H, 4.64; N, 12.90%.

*Pharmacology*

*Peroxidase assay*
Porcine thyroid peroxidase was prepared as described[4] and assayed by a modification of the guaiacol oxidation test.[5] Assay mixtures contained 190 $\mu$mol Tris/HCl (pH 7.4), 1 mg enzyme protein, and varying amounts of thio- or selenouracil in a final volume of 3 ml. Reactions were initiated by addition of $\mu$mol $H_2O_2$, and the increase in absorbance at 470 nm was measured during 15 s. Protein was determined by the method of Lowry *et al.*[6] Table 1 shows the thyroid peroxidase inhibitory activity of PTU as compared to its respective selenouracil derivatives **Ib** and **Ic**.

## RESULTS AND DISCUSSION

The data presented in Table 1 clearly suggest that the introduction of the less electronegative selenium atom in the $C_2$-position of the uracil ring system replacing the sulfur atom, improved the antiperoxidase activity by about five fold. Thus, the following observations for SAR can be considered: (1) As the atomic size at the 2-position of the ring increases and, electronegativity decreases, the activity increases, i.e., O > S > Se. This may be due to the presence and use of *d* orbitals in the sulfur and selenium atoms for the binding of PTU and PSeU to the peroxidase enzyme. (2) Lipophilicity of the alkyl group substitution at $C_6$ of selenouracil improves the antithyroid activity i.e., PSeU possesses better activity than MSeU. This observation is substantiated by the findings of Anderson *et al.*[7] on the thiouracil series where maximal antithyroid activity was reported for PTU having an n-propyl group at $C_6$.

However, these selenouracil compounds (**Ib** & **Ic**) are light sensitive and susceptible to degradation on exposure to air and light.

**Table 1** Inhibition of thyroid peroxidase by seleno analogues of 6-n-propyl-2-thiouracil

| Compound | Concentration $\times 10^{-5}$ M | Specific activity* of peroxidase | Inhibition % |
|---|---|---|---|
| None | — | 1.93 ± 0.11 | — |
| Propylthiouracil | 50 | 0.19 ± 0.02 | 90.2 |
| (PTU, **Ia**) | 10 | 1.11 ± 0.07 | 42.5 |
| | 5 | 1.47 ± 0.07 | 24.0 |
| 6-n-Propyl-2-selenouracil | 10 | 0.14 ± 0.01 | 92.8 |
| (PSeU, **Ib**) | 5 | 0.24 ± 0.05 | 37.5 |
| | 1 | 1.11 ± 0.06 | 42.5 |
| 6-Methyl-2-selenouracil | 50 | 0.24 ± 03 | 87.0 |
| (MSeU, **Ic**) | 5 | 0.21 ± 0.05 | 80.0 |
| | 1 | 1.11 ± 0.07 | 42.5 |

*Specific activity of peroxidase values were the mean of six determinations ± SD.

Studies are recently published to examine the effect of these analogues on Type I iodothyronine deiodinase (ID-I), a selenoenzyme, which is important for the conversion of thyroxine ($T_4$) to 3,3′,5-tri iodothyronine ($T_3$).[8]

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