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# SYNTHESIS AND ANTITHYROID ACTIVITY OF FLUORINATED 2-THIOURACIL ANALOGUES

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The synthesis of four fluorinated-2-thiouracil derivatives, namely, 6-trifluoromethyl*-2-thiouracil, 1-methyl-6-trifluoromethyl-2-thiouracil, 1-acetyl-6-trifluoromethyl-2-thiouracil and 6-n-heptafluoropropyl-2-thiouracil was achieved. 6-n-Heptafluoropropyl-2-thiouracil displayed higher antithyroid activity followed by 6-trifluoromethyl derivative and 1-methyl-6-trifluoromethyl analogue when compared to 6-n-propyl-2-thiouracil. 1-Acetyl 6-trifluoromethyl-2-thiouracil exhibited no antithyroid activity. The structure-activity relationships (SAR) of this class of compounds are discussed.

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

## INTRODUCTION

6-n-Propyl-2-thiouracil (PTU, **1**) is currently one of the drugs of choice in the treatment of hyperthyroidism. The primary action of propylthiouracil is on the thyroid gland. It inhibits thyroid peroxidase, resulting in a blockade of iodide utilization for thyroid hormone synthesis[1]. Furthermore, propylthiouracil also inhibits the peripheral action of thyroxine[2] and apparently crosses the placenta, producing adverse effects on the developing foetus[3]. Lindsay *et al.*[4] reported the antiperoxidase activity and the structure-activity relationships (SAR) of several propylthiouracil derivatives and metabolites. Also, Aboul-Enein *et al.*[5] studied the SAR of position 5 in PTU and suggested the requirements for better binding to a proposed receptor site.

Furthermore, Aboul-Enein *et al.*[6] recently reported the synthesis of 6-n-propyl-2-selenouracil and its 6-methyl analogue replacing the sulfur atom at $C_2$ by selenium. The antiperoxidase activity of these selenouracil analogs were five fold when compared to PTU. However, these selenouracil analogues were light sensitive and susceptible to degradation on exposure to air and light.

In search for new thyroid inhibitors, four fluorinated 2-thiouracil analogs were synthesized (**2–5**), in order to, (a) study the bioisosteric effect of replacement of

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TABLE I
Physical properties of Fluorinated-2-Thiouracils.

| Compound | $R_1$ | $R_2$ | Yield, % | M.p., °C |
|---|---|---|---|---|
| PTU* **1** | H | H | – | 218–220 |
| **2** | H | $CF_3$ | 86 | 241–2 |
| **3** | $CH_3$ | $CF_3$ | 89 | 236–9 |
| **4** | $CH_3CO$ | $CF_3$ | 88 | 240–2 |
| **5** | H | n-$C_3F_7$ | 78 | 230–2 |

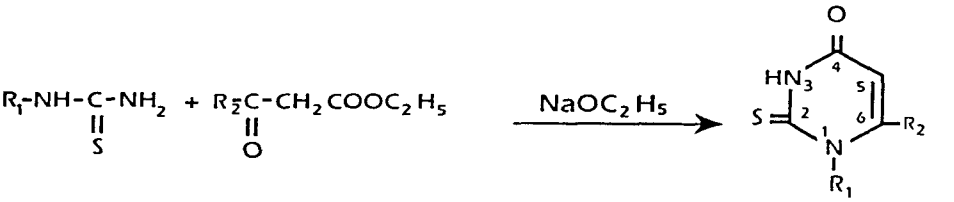
*Obtained from Sigma Chemical Co. St. Louis, MO, U.S.A.

hydrogen by fluorine at the alkyl group at $C_6$; (b) to substantiate the role of the alkyl group at $C_6$ in reinforcing the antithyroid activity of thioamide derivatives as suggested by Raby and Buxfraud[7]. These new fluorinated 2-thiouracil derivatives include: 6-trifluoromethyl-2-thiouracil (**2**), 1-methyl-6-trifluoromethyl-2-thriouracil (**3**), 1-acetyl-6-trifluoromethyl-2-thiouracil (**4**) and 6-n-heptafluoropropyl-2-thiouracil (**5**) (see Table 1).

## MATERIALS & METHODS

### *Chemistry*

The synthesis of compounds **2, 3, 4** and **5** was achieved by condensation of ethyl 4,4,4-trifluoroacetoacetate, ethyl 4,4,5,5,6,6,6,-heptafluoro-3-oxo-hexanoate and the appropriate thiourea derivatives in the presence of sodium ethoxide. Ethyl 4,4,5,5,6,6,6-heptafluoro-3-oxo-hexanoate was prepared by condensation of ethylacetate and ethyl heptafluorobutyrate in presence of sodium hydride.




Melting points were determined on a Mettler FP5-51 instrument and are uncorrected. I.R. spectra were determined with a Perkin-Elmer 580 B infrared spectrophotometer in KBr discs or as liquid films. U.V. absorption spectra were measured with a Pye Unicam SP8-100 ultraviolet spectrophotometer in neutral methanol. Mass spectra were obtained with a Ribermag R-10-10 quadrupole spectrometer by direct inlet. All the spectral data are in agreement with the assigned structures. Elemental analyses were performed by Janssen Pharmaceutica, Beerse, Belgium and

were within ±0.4% of the theoretical values for C, H, and N. Ethyl 4,4,4-trifluoroacetoacetate, ethyl heptafluorobutyrate and the appropriate thiourea and thiourea derivatives were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.

*Ethyl 4,4,5,5,6,6,6-Heptafluoro-3-Oxo-Hexanoate*

This β-keto ester was prepared according to a general method described by Royals and Turpin[8]. A mixture of ethylheptafluorobutyrate (24 g, 0.1 mole) and ethyl acetate (8.8 g, 0.1 mole) was refluxed in dry benzene for 6 h in the presence of sodium hydride (2.6 g, 0.11 mole). The solvent was evaporated under vacuum and the residue was acidifed with 0.1 N hydrochloric acid and extracted with ether. The ethereal extract was dried ($Na_2SO_4$) and the solvent removed under vacuum when an oily yellow liquid (25 g, yield 88%) was obtained which gave a violet color with ferric chloride solution; i.r. (liquid film) 1720 $cm^{-1}$ (C = O aliphatic ester), 1750 $cm^{-1}$ (C = O β-keto ester).

*A General Method of the Synthesis of 6-Fluorinated 2-Thiouracil (**2–5**)*

An adaptation of Anderson *et al.*'s method[9] for the preparation of 6-n-propyl-2-thiouracil was used. A solution of sodium ethoxide was prepared by dissolving sodium metal (2.3 g, 0.1 g-atom) in absolute ethanol (70 ml), then thiourea or thiourea derivatives (0.05 mole) and the appropriate β-keto ester (0.05 mole) were added simultaneously. The mixture was refluxed for 8 h and the ethanol was removed by distillation under vacuum. The residue was dissolved in 50 ml of water and acidified to pH 3 with hydrochloric acid. The precipitate was filtered, washed with water and recrystallized from aqueous ethanol.

The physical properties of the fluorinated derivatives are shown in Table 1.

*Pharmacology*

*A. Antithyroid Activity by $I^{125}$ perchlorate discharge technique*

Compounds **2, 3, 4** and **5** were tested for their antithyroid activity compared to that of PTU using an adaptation of the $^{125}I$ perchlorate discharge technique[10]. The animals used were adult albino rats of Agoza strain 230–280 g. They were divided into six groups each comprising 10 rats. They had been maintained on a commercial diet (Grain and Silos, Saudi Arabia) and tap-water *ad lib*.

Propylthiouracil, as well as the compounds **2–5**, were dissolved in dimethyl sulfoxide to give a $50 \times 10^{-5}$ M concentration and 0.2 ml of the solution was daily injected intraperitoneally in each animal for 60 days. The control group was injected with 0.2 ml of dimethylsulfoxide.

At the end of 60 days the perchlorate discharge test was performed by oral administration of a tracer dose (400,000 c/m) of carrier free iodine-125 (Radiochemical Center, Amersham, England). After 3.5 h, 2.7 mg of potassium perchlorate (BDH, Poole, England) was orally given and the $^{125}I$-thyroid uptake was determined by direct counting of the isolated thyroid gland using a 4000 Beckman Gamma Counter at 6 and 6.5 h following the administration of radioiodine. The data was normalised to correct for differences in the administered $^{125}I$ dose.

TABLE II
Uptake space of iodine-125 by the Thyroid Gland of Rats Treated with Fluorinated 2-Thiouracil Derivatives.

| Compound | $^{125}$I-thyroid Uptake (c/min ± SD)* at 6 h | at 6.5 h |
|---|---|---|
| Control | 44428 ± 4086 | 43162 ± 3400 |
| Propylthiouracil (**1**) | 23462 ± 3824 | 29481 ± 3088 |
| 6-Trifluoromethyl-2-thiouracil (**2**) | 36232 ± 4825 | 23735 ± 4704 |
| 1-Methyl-6-trifluoromethyl-2-thiouracil (**3**) | 26586 ± 5158 | 34260 ± 4612 |
| 1-Acetyl-6-trifluoromethyl-2-thiouracil (**4**) | 50423 ± 6480 | 36934 ± 3804 |
| 6-n-Heptafluoropropyl-2-thiouracil (**5**) | 20133 ± 3146 | 22060 ± 4601 |

*These values were the mean of six determinations ± SD

The least significant differences procedure was used to assess the difference between two means. The compounds were nontoxic in rats following daily intraperitoneal administration for 60 days within the standard dose levels. Abdullah *et al.*[11] published a report on the evaluation of these derivatives (**2–5**) as potential antithyroid inhibitors. The uptake of iodine-$^{125}$ radioactivity by the thyroid gland in the control animals and those treated with propylthiouracil and compounds (**2–5**) is shown in Table 2.

*B. Peroxidase Assay*

Compounds **2, 3, 4** and **5** were also tested for their thyroid peroxidase inhibitory activity compared to that of PTU (Table 3). Sheep thyroid peroxidase was prepared as described for the porcine thyroid peroxidase by Yu *et al.*[12] and assayed by a modification of the guaiacol test[13]. The assay medium contained 190 $\mu$ moles of guaiacol, 108 $\mu$ of Tris HCl (pH 7.4), 1.0 mg of enzyme protein and various concentrations of PTU and analogues (dissolved in 0.3 ml ethanol or water) in a final volume of 3.0 ml. The reaction was initiated by the addition of 2$\mu$ moles of hydrogen peroxide. The increase in absorbance of 470 nm was measured for 15 s during the initial linear reaction using a recording spectrophotometer. Protein was determined by the method of Lowry *et al.*[14]

## RESULTS AND DISCUSSION

The data from Table 2 indicate that **5**, the fluorobioisoster of PTU, displayed a significantly higher discharge (51.78%) than that of PTU (39.57%), this is followed by **2**, (37.47%) and **3** (30.46%), while **4** showed very low discharge (0.17%). These results

TABLE III
Inhibition of thyroid peroxidase by fluorinated 2-thiouracil analogues

| Compound | Concentration $\times 10^{-5}$M | Specific Activity of peroxidase | Inhibition % |
|---|---|---|---|
| None | – | 1.96 ± 0.12 | – |
| PTU (**1**) | 50 | 0.19 ± 0.02 | 90.3 |
| | 10 | 1.14 ± 0.07 | 41.8 |
| | 5 | 1.44 ± 0.07 | 26.5 |
| **2** | 50 | 0.25 ± 0.08 | 87.2 |
| | 10 | 1.20 ± 0.02 | 38.8 |
| | 5 | 1.53 ± 0.05 | 21.9 |
| **3** | 50 | 0.42 ± 0.02 | 78.6 |
| | 10 | 1.26 ± 0.05 | 35.7 |
| | 5 | 1.58 ± 0.01 | 19.4 |
| **4** | 50 | 1.65 ± 0.14 | 15.8 |
| | 10 | 1.81 ± 0.05 | 7.6 |
| | 5 | 1.92 ± 0.09 | 2.0 |
| **5** | 10 | 0.17 ± 0.03 | 91.3 |
| | 5 | 0.96 ± 0.02 | 51.0 |

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

show that **5** had a significantly higher antithyroid activity while **2** and **3** possessed slightly lower activity as compared to propylthiouracil. However, **4** is devoid of thyroid inhibitory effect. Furthermore, the data presented in Table 3 clearly indicate that **5** is about 5 times more potent as PTU as a thyroid peroxidase inhibitor followed by **2** and then **3**. Compound **4** was the least active among the analogues studied. It is evident that replacement of n-$C_3H_7$ group by n-$C_3F_7$ group at $C_6$ of 2-thiouracil has potentiated the biological activity and the thyroid peroxidase inhibitory activity. This could be due to the physicochemical properties conferred by the bioisoseric replacement of fluorine e.g. electronegativity, bond stability, atomic radius (van der Waals' radii) and increased lipophilicity. These might in turn cause high thyroid uptake and/or better binding to the enzymatic site responsible for iodide-iodine conversion.

The lower activity of **2** as compared to **5** was found to be in agreement with previous SAR studies of hydrogen analogs of 6-alkyl-2-thiouracil[9,15].

Methylation of the N-1 group as in **3** reduced the antithyroid activity slightly as compared to **1**. However, substitution at the same position with an acetyl group as in **4** abolishes the antithyroid activity. This may be due to the electron withdrawing effect of the acetyl group as compared to the electron-donating effect of the methyl group on the -N-CS-NH pharmacophoric group, which is essential for activity. Further studies

on the SAR of this class of compounds is required for a better understanding of the nature of the antithyroid activity.

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