Effect of 2,4-Dihydro-3*H*-1,2,4-triazole-3-thiones and Thiosemicarbazones on Iodide Uptake by the Mouse Thyroid: the Relationship between Their Structure and Anti-thyroid Activity

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Antithyroid activity of 2,4-dihydro-3*H*-1,2,4-triazole-3-thiones and thiosemicarbazones was tested by measuring the uptake ratio of thyroid : serum (*T*/*S*) of ^{125}I through the mouse thyroid. Substitution with an alkyl group at the 5-position of the triazole nucleus remarkably increased the activity but substitution at the N-2 and/or N-4 positions caused a significant decrease in the activity, indicating the necessity of unsubstituted thioureylene moiety for the antithyroid activity. Thiosemicarbazone derivatives which are an open ring structure of triazoles showed comparable antithyroid activities to those in a ring form, but one thiosemicarbazone showed a much higher toxicity than the corresponding ring form compound. This suggests that the ring structure is not essential for the activity but is necessary to reduce toxic effect. Of fourteen compounds tested, 5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione was the most potent antithyroid compound with low toxicity, with a potency tenfold that of propylthiouracil, a drug currently used.

Keywords 2,4-dihydro-3*H*-1,2,4-triazole-3-thione; thiosemicarbazone; triazole; antithyroid drug; iodide uptake; thyroid; mouse

Introduction

Thiocarbamides have been the most commonly used antithyroid drug. There are two major prototype structures: the first group is a thiouracil derivative and the second is a thioimidazoline derivative. Both groups of compounds have in common in their structures a functional thioureylene moiety.[1] Although currently used antithyroid drugs have excellent efficacy, a recent survey has revealed various side effects of those compounds carrying the thioureylenyl moiety.[2] Therefore, continuous efforts to reduce undesired effects and to develop a new type of antithyroid drug are necessary.

It has been reported that 1*H*-1,2,4-triazole derivatives and 5-aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones exhibit diuretic[3] and antidepressant activities[4] respectively, while, 3-amino-1*H*-1,2,4-triazole exhibits antithyroid activity.[5] These findings led us to investigate the antithyroid activity of various 2,4-dihydro-3*H*-1,2,4-triazole-3-thione derivatives.

This paper deals with the relationship between the blocking activity against a radioiodide uptake in the mouse thyroid and structures of 2,4-dihydro-3*H*-1,2,4-triazole-3-thione derivatives. To determine the relevant role of the triazole nucleus in antithyroid activity, thiosemicarbazones, an open chain type of triazole, were also investigated.

Materials and Methods

Chemicals Polyethyleneglycol (PEG), sodium carboxymethyl cellulose (CMC), 1*H*-1,2,4-triazole, 3-amino-1*H*-1,2,4-triazole and glycerol were purchased from Wako Pure Chemical Industries, Ltd. 6-*n*-Propyl-2-thiouracil (PTU) was purchased from Sigma Chemical Company (St. Louis, Mo., U.S.A.). A carrier free radioiodide in NaI form (^{125}I) was obtained from Daiichi Isotope Institute (Tokyo, Japan). The compounds used are listed in Tables I and II. 2,4-Dihydro-3*H*-1,2,4-triazole-3-thiones **1**,[6] **2**,[6], **3**,[6] **4**,[6] **5**,[7] **6**,[6] **7**,[8] **8**,[8] **10**[3] and **11**[9] were prepared by the methods described in the literature. Thiosemicarbazones **12**—**14** were prepared by condensation of proper aldehydes with thiosemicarbazides.

Synthesis Melting points were determined by the capillary method and were uncorrected. Infrared (IR) spectra were recorded on a Hitachi 215 spectrometer. ^1H-Nuclear magnetic resonance (^1H-NMR) spectra were recorded on a JEOL PS-200 spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were measured with a JEOL D-300 instrument.

5-(4-Dimethylaminophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (9) A mixture of 4-dimethylaminobenzoic acid 2-(aminothioxomethyl) hydrazide (2.57 g) and 1 N aqueous sodium hydroxide (15 ml) was refluxed for 3 h. The reaction mixture was neutralized with 1 N aqueous hydrochloric acid and concentrated under reduced pressure. The residue was recrystallized from methanol to give yellowish crystals (1.51 g, 64%), mp 285—286 °C. IR ν cm^{-1}: 3405, 1610. ^1H-NMR (Me_2SO-d_6) δ: 2.97 (6H, S, $(CH_3)_2N$), 6.77 (2H, d, J=9 Hz, ArH), 7.72 (2H, d, J=9 Hz, ArH), 13.40 (1H, br s, NH), 13.49 (1H, br s, NH). MS *m/z*: 220 (M^+). *Anal.* Calcd for $C_{10}H_{12}N_4S$: C, 54.52; H, 5.49; N, 25.43. Found: C, 54.22; H, 5.52; N, 25.33.

Animal Experiment ddY Male mice weighing 20—25 g were maintained on a 12 h light–dark cycle in an air-conditioned room at 23 °C. Animals were given a standard chow and tap water *ad libitum*. Chemicals were first dissolved in physiological saline solution at room temperature with mechanical stirring. Insoluble compounds were subjected to immersion in boiling water, then tested in a series of 10% ethanol, glycerol, 20% HCO-60, 50% PEG, and 50% PEG in 20% HCO-60. For animal experiments, the solvents that were inappropriate for injection due to high viscosity or their irritable nature were replaced by CMC. Toxic effects were examined by an intraperitoneal injection of each concentration of test compounds to at least three mice and an approximate median lethal dosage (LD_{50}) was determined by the up-and-down method. Approximately one tenth of the LD_{50} was used for the dose testing the antithyroid potency. For compounds for which LD_{50} values were not obtainable due to their poor solubility, the maximally available amount was given to an animal.

Radioiodide Uptake Test The radioiodide uptake by the thyroid was carried out by the method of Halmi *et al.*,[10] and the uptake ratio of 100 mg thyroid tissue to 100 μl serum (*T*/*S* ratio) in the presence and absence of compounds was obtained. The uptake blocking activity of chemicals was defined by the following equation as the specific inhibitory rate:

$$\text{specific inhibitory rate} = \left(1 - \frac{T/S \text{ of treated group}}{T/S \text{ of control group}}\right) \times \frac{1}{\text{dosage (mg)}}$$

Results and Discussion

According to the acute toxicity test, compound **12** was most toxic, showing an approximate LD_{50} of 20 mg/kg body weight. Compounds **1**, **6** and **7** were also highly toxic, showing LD_{50} values of 300, 210 and 320 mg/kg, respectively. The LD_{50} of compounds **2**, **3**, **4** and **10** was in a range of 1500—2000 mg/kg. However, toxic dosages of compounds **5**, **8**, **9**, **11**, **13** and **14** were not determinable due to their limited solubility (data not shown).

We examined the antithyroid nature of 2,4-dihydro-3*H*-

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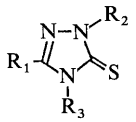
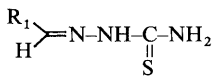
TABLE I. 2,4-Dihydro-3*H*-1,2,4-triazole-3-thiones and Their Antithyroid Potency



Compound	R_1	R_2	R_3	Specific inhibitory rate
1	H	H	H	4.95±0.28
2	H	CH_3	H	1.65±0.07
3	H	H	CH_3	1.37±0.12
4	H	CH_3	CH_3	6.47±0.17
5	CH_3	H	H	296.60±9.73
6	CH_3	H	CH_3	9.79±0.10
7	Ph	H	H	8.00±1.12
8	4-CH_3OPh	H	H	26.68±3.82
9	4-Me_2NPh	H	H	4.57±1.50
10	4-Py	H	H	24.00±2.75
11	$PhCH_2$	H	H	12.56±1.45
1*H*-1,2,4-Triazole				1.12±0.77
3-Amino-1*H*-1,2,4-triazole				0.51±0.02
PTU				30.13±2.74

Specific inhibitory rate was defined in the text. Values represent the mean±S.D. of at least 5 mice.

TABLE II. Thiosemicarbazones and Their Antithyroid Potency



Compound	R_1	Specific inhibitory rate
12	CH_3	285.90±20.5
13	4-Me_2Ph	126.70±4.77
14	4-HOPh	11.92±1.71

The explanations are identical to those for Table I.

1,2,4-triazole-3-thione derivatives **1**—**11** and a few aldehyde thiosemicarbazone derivatives **12**—**14** by measuring the *T*/*S* ratios. The results are shown in Tables I and II. Since the blocking activity was expressed by a specific inhibitory rate, the higher values represent greater potency in blocking the iodide uptake. Compound **5** was the most potent and other 2,4-dihydro-3*H*-1,2,4-triazole-3-thione derivatives were in the following order of potency: **8**>**10**>**11**>**6**>**7**>**4**>**9**>**1**>**2**>**3**. Neither 3-amino-1,2,4-triazole nor 1*H*-1,2,4-triazole showed any inhibition against iodide uptake under the conditions applied. For thiosemicarbazone derivatives, the order of the potency was **12**>**13**>**14**. Of these fourteen compounds, **5**, **12** and **13** showed much greater blocking activity than PTU, which is currently widely used as an antithyroid drug. On the basis of these results, it becomes clear that substitution at the C-5 position of the triazole nucleus is required for increasing the activity; the 5-methyl derivative **5** showed the strongest activity, about sixty times as high as that of unsubstituted 1,2,4-triazole-3-thione **1**, and ten times higher than that of PTU. The 5-phenyl derivative **7** was slightly more active than **1**. Introduction of the methoxy group (**8**) into the 4-position of the phenyl group of compound **7** increased the activity three times more than **7**, while the introduction of the dimethylamino group (**9**) was without effect. Replacement of the phenyl group of **7** by 4-pyridyl (**10**) or benzyl (**11**) resulted in no appreciable increase in activity.

Substitution of methyl group at the position of N-2 and/or N-4 of the triazole nucleus rather tended to reduce the activity (**2**—**4**, **6**). From these results, it is obvious that unsubstituted thyoureylene moiety is definitely required.

Compounds **12**, **13** and **14** are the thiosemicarbazone derivatives with an open triazole ring structure. Compound **12** was as highly effective as the ring form compound **5**. Compound **13** sustained a substantially high activity against the *T*/*S*, which was quite a contrast to compound **9**. Therefore, a ring structure may not be essential for the *T*/*S* blocking activity, although, as stated earlier, compound **5** in a ring form is much less toxic than **12** in a chain form, indicating the necessity of a triazole nucleus for reduction of toxicity.

References

1) J. D. Wilson and D. W. Foster (eds.), "Textbook of Endocrinology," 7th ed., W. B. Saunders, Philadelphia, 1985, p. 704.
2) S. S. Wing and I. G. Fantus, *Canad. Med. Assoc. J.*, **136**, 121 (1987).
3) M. H. Shah, M. Y. Mhasalkar, V. M. Patki, C. V. Deliwala and U. K. Sheth, *J. Pharm. Sci.*, **58**, 1398 (1969).
4) J. M. Kane, M. W. Dudley, S. M. Sorensen and F. P. Miller, *J. Med. Chem.*, **31**, 1253 (1988).
5) N. M. Alexander, *J. Biol. Chem.*, **234**, 148 (1959).
6) C. F. Kroger, W. Sattler and H. Beyer, *Ann. Chem.*, **643**, 128 (1961).
7) J. F. Willems and A. Vandenberghe, *Bull. Soc. Chim. Belgs.*, **75**, 358 (1966).
8) F. Kurzer, *J. Chem. Soc.*, (*C*), **1970**, 1813.
9) F. Malbee, R. Milcent and G. Barbier, *J. Heterocycl. Chem.*, **21**, 1689 (1984).
10) N. S. Halmi, B. N. Spirtos, E. M. Bogdanove and H. J. Lipner, *Endocrinol.*, **52**, 19 (1953).

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