Synthesis and Antithyroid Activity of Pyridine, Pyrimidine and Pyrazine Derivatives of Thiazole-2-thiol and 2-Thiazoline-2-thiol

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A series of compounds was synthesized by linking various derivatives of pyridine, pyrimidine or pyrazine to thiazole-2-thiol or to its partially hydrogenated derivative 2-thiazoline-2-thiol. The reactions of the compounds with molecular iodine and lactoperoxidase were examined *in vitro*. Their antithyroid activity was also examined *in vivo* in the rat. T_4 and TSH levels were determined, and the thyroid gland was examined histologically. 2-(3-Hydroxy-2-pyridyl)-2-thiothiazoline had the highest antithyroid activity of the compounds tested ($K_c = 14931 \cdot mol^{-1}$, IC_{50} 0.65×10^{-4} M, activity of thryoid gland + + +).

Keywords thiazole-2-thiol; 2-thiazoline-2-thiol; pyridyl derivative; pyrimidyl derivative; pyrazyl derivative

Drugs currently employed as antithyroid agents are either based on imidazole (*e.g.* mercapto-1-methylimidazole or methimazole) or thiouracil (*e.g.* benzylthiouracil). Methimazole, which possesses a free SH group, is a particularly strong antithyroid agent, but it may give rise to adverse reactions as severe as hypothyroiditis and agranulocytosis. Thiouracils, especially the methyl and propyl derivatives, are also toxic, and only benzylthiouracil is still in clinical use. Thioureas are too toxic to be employed therapeutically. Other molecules based on other structures therefore need to be sought.

In previous studies, we found a relationship between electron-donating power and antithyroid activity.[1] The electron-donating power is determined by the capacity of a compound to transfer an available pair of electrons to an acceptor molecule. Strong electron donors may interfere with thyroid peroxidase (TPO) or molecular iodine[2] or both.

We describe here the synthesis of derivatives of thiazole-2-thiol, the thiazole ring replacing that of imidazole. We also synthesized derivatives of a partially hydrogenated thiazole, 2-thiazoline-2-thiol, which has been employed as an antithyroid agent,[3] although it has been withdrawn due to toxicity. We blocked the free SH group with derivatives of pyridine, pyrimidine or pyrazine in an attempt to circumvent side effects while preserving electron-donating activity.

Experimental

The derivatives were obtained by condensation of a chloropyridine, chloropyrimidine or chloropyrazine with an alkaline thiolate of thiazole or thiazoline (Chart 1). Thiazole-2-thiol, 2-thiazoline-2-thiol, chloropyridines and chlorodiazines were purchased from Fluka (Buchs, Switzerland) and were purified by HPLC (Waters, France). Melting points were measured on a Koffler block. Elemental analyses were carried out at the CNRS microanalysis center (Vernaison, France). The results were within ±0.4% of the expected values. ^{1}H-NMR (DMSO-d_6) spectra were recorded at 200 MHz on a Bruker instrument by the Inter University NMR center at the Faculty of Pharmacy in Marseille (France). Chemical shifts (δ) are expressed in ppm with respect to tetramethylsilane (TMS) used as an internal reference. The IR spectra were recorded on a Beckman 4250 spectrometer, and the UV spectra on a Perkin-Elmer 554 UV/vis instrument.

Synthesis of Compounds The same methods were used to synthesize derivatives of the two basic structures.

Method A. 2-(2-Pyridyl)thiothiazole (Compound **1**): Equimolecular quantities of chloropyridine and thiazole-2-thiol were heated to melting point, and the temperature was allowed to rise spontaneously. The mixture solidified, and after cooling the residue was taken up in aqueous-ethanol (50:50, v/v). The solution was filtered and neutralized by addition of a 5% aqueous solution of $NaHCO_3$. The precipitate was recrystallized from water–ethanol (30:10, v/v). mp 185 °C, yield 52%. IR ν_{max}^{KBr} cm^{-1}: 3080 (CH arom.), 1558—1584 (pyridine nucleus), 1525—1470 (C=C), 1500 (C=N). ^{1}H-NMR (DMSO-d_6): 8.4 (s, 1H, H_3 pyridine), 7.2 (m, 1H, H_4 pyridine), 7.1 (m, 1H, H_6 pyridine), 8.8 (s, 1H, H_4 thiazole). This method was used to obtain compounds **1**, **2**, **8** and **9**.

Method B. 2-(3-Nitro-2-pyridyl)thiothiazole (Compound **3**): 2-Chloro-3-nitropyridine (0.01 mol) and the sodium salt of 2-thiazoline-2-thiol (0.01 mol) dissolved in 30 ml of ethanol were refluxed for 2 h. The product was precipitated by addition of excess water, and recrystallized from water–ethanol (30:70, v/v), mp 107 °C, yield 58%. IR ν_{max}^{KBr} cm^{-1}: 3139—3070 (CH arom.), 1558 (s pyridine nucleus), 1507 (NO_2), 1340 (s NO_2), 1480 (C=N). ^{1}H-NMR (DMSO-d_6): 8.4 (m, 1H, H_4 pyridine), 7.6 (m, 1H, H_5 pyridine), 8.0 (m, 1H, H_5 pyridine), 8.5 (s, 1H, H_4 thiazole). This method was used to obtain compound **3**, **4**, **10** and **11**.

Method C. 2-(3,5-Dinitro-2-pyridyl)thiothiazole (Compound **5**): Equimolar quantities of 10% solutions of 2-chloro-3,5-dinitropyridine and thiazole-2-thiol in chloroform were mixed. The reaction took place immediately, and after 15 min, the chloroform was evaporated. The residue was taken up in ethanol–water (70:30, v/v) and the product was precipitated on neutralizing the solution with $NaHCO_3$. mp 144 °C, yield 75%. IR ν_{max}^{KBr} cm^{-1}: 3102 (CH arom.), 1512 (NO_2), 1585—1516 (CH pyridine), 1500 (C=N), 1339 (s, NO_2). ^{1}H-NMR (DMSO-d_6): 9.0 (d, 1H, H_4 pyridine); 8.0 (d, 1H, H_6 pyridine); 8.2 (s, 1H, H_4 thiazole). This

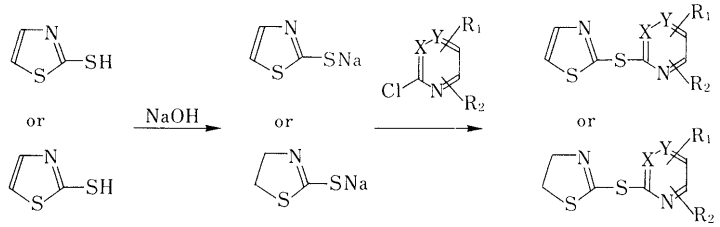


Chart 1

method was also used to obtain compound **12** starting from 2-thiazoline-2-thiol.

Method D. 2-(2-Pyrimidyl)thiothiazole (Compound **6**): The sodium salt of thiazole-2-thiol was prepared by mixing 0.02 mol of the thiol with 0.02 mol of sodium hydroxide solution (22 ml of 1 N NaOH). The mixture was stirred for 10 min, and evaporated. Then 0.02 mol of chloropyrimidine was added to a suspension of 0.02 mol of the sodium thiolate in 30 ml of octanol in a round-bottomed flask. The mixture was refluxed for 2 h. After rapid cooling, the product was precipitated by addition of excess water, and recrystallized from water–methanol (30 : 70, v/v). mp 175 °C, yield 40%. IR ν_{max}^{KBr} cm^{-1}: 3138 (CH arom.), 1500 (C=N). ^{1}H-NMR (DMSO-d_6): 8.4 (d, 2H, H_3, H_4 pyrimidine), 7.4 (s, 1H, H_6 pyrimidine), 8.5 (s, 1H, H_4 thiazole).

This method was used for compounds **13** and **14** starting from 2-thiazoline-2-thiol and the corresponding diazines, chloropyrimidine and chloropyrazine, respectively.

Method E. 2-(3-Methoxy-2-pyridyl)thiothiazole (Compound **7**): The procedure for method D was employed, with the exception that the mixture was stirred for 12 h before refluxing for 3 h. mp 142 °C, yield 10%. IR ν_{max}^{KBr} cm^{-1}: 3137 (CH arom.), 1480 (C=N). ^{1}H-NMR (DMSO-d_6): 4.0 (s, 3H, OCH_3), 8.1 (d, 1H, H_3 pyridine), 8.0 (d, 1H, H_4 pyridine), 7.4 (s, 1H, H_5 pyridine), 8.5 (s, 1H, H_4 thiazole).

This method was also used for compound **15** starting from 2-thiazoline-2-thiol, and compound **16** (2-thiazoline-2-thiol and 3-hydroxypyridine).

Compound **15**: IR ν_{max}^{KBr} cm^{-1}: 3137 (CH arom.), 1480 (C=N). ^{1}H-NMR (DMSO-d_6): 4.0 (s, 3H, CH_3), 8.0 (d, 1H, H_4 pyridine), 7.4 (s, 1H, H_5 pyridine), 3.4 (s, 2H, H_4 thiazoline), 6.0 (d, 1H, NH).

Compound **16**: IR δ_{max}^{KBr} cm^{-1}: 3135 (CH arom.), 1480 (C=N), 1200 (OH). ^{1}H-NMR (DMSO-d_6): 7.2 (d, 1H, H_3 pyridine), 7.4 (m, 1H, H_4 pyridine), 7.0 (d, 1H, H_6 pyridine), 8.0 (s, 1H, OH), 6.8 (d, 1H, H_5 thiazoline), 6.0 (s, 1H, NH).

Determination of Formation Constant of Complex The method has been described in detail elsewhere.[4] Briefly, solutions of compounds were made up by dilution of stock solutions which had been prepared by accurate weighing. The reactions were carried out directly in the spectrophotometer cuvettes by mixing 1.5 ml of a solution of iodine and 1.5 ml of a solution of the compound. Spectra were recorded immediately. Formation constants K_c were calculated by a modification of Lang's method.

The values of K_c could not be determined for some compounds, especially the derivatives of thiazole-2-thiol, as they were either insoluble in conventional solvents, or the complex formed was unstable, liberating I_3^- ions. Furthermore, in some cases, complexes of higher stochiometry were produced for which we do not yet have a suitable method for calculation of the value of K_c.

Peroxidase Assay We employed lactoperoxidase (LPO) to test the activity of the compounds. This enzyme is available in standardized batches, and so results could be readily compared between the different compounds. LPO closely resembles thyroid peroxidase (TPO).[5] TPO is hard extract and purify, and only small amounts can be obtained, which effectively precludes extensive testing. The method used has been described elsewhere[2] using LPO obtained from Sigma. The measurements were carried out in a Kontron 860 spectrophotometer (UVIKON-France).

Experiments *in Vivo* Male Wistar rats weighing 140—150 g were obtained from Iffa-Credo (Lyon, France). They were divided into groups of 10 animals. Before the start of the experiments, the rats were fed on a diet with normal iodine content (10.75 μg iodine per day) with free access to water. The rats received a 5% suspension of the compound in gum arabic at a dose of 50 mg/kg/d by gastric intubation. A control group received the same volume of a 5% solution of gum arabic. After three weeks' treatment, the animals were anesthetized with diethyl ether, and blood samples were taken by cardiac puncture. T_4 was assayed by an immunological method based on measurement of fluorescence polarization (Abbott). TSH was meaured immunoenzymologically (Abbott). The animals were then killed, and the thyroid glands were removed. Sections of gland fixed in Bouin's solution and stained with hematein–eosin–safran were examined under the microscope.

Statistical Analysis The results were compared using Student's *t*-test.

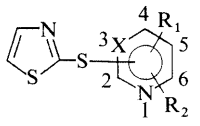
Results and Discussion

Chemistry Condensation of chloropyridine and the chlorodiazines (pyrimidine or pyrazine) with the alkaline thiolate of thiazole or 2-thiazoline gave good yields of product, apart from the hydroxylated (**16**) or methoxylated derivatives (**7** and **15**). Products were isolated either as the free base or the hydrochloride (Tables I and II).

Electron-Donating Effect Complexes of 1 : 1 stochiometry were formed between iodine and two derivatives of thiazole-2-thiol (**1** and **2**). They had values of K_c close to that of thiazole ($K_c = 10.27\ l \cdot mol^{-1}$). The K_c of thiazole-2-thiol could not be measured as the compound is insoluble in conventional solvents. We were unable to determine the K_c for the other derivatives of thiazole-2-thiol, or for some derivatives of 2-thiazoline-2-thiol (**9**, **13**, **15**), but they all formed complexes with iodine, as the iodine solution was bleached on contact with solutions of the compounds. High values of K_c were obtained for most of the derivatives of 2-thiazoline-2-thiol. Partial hydrogenation of the thiazole ring and the presence of an SH group increased the electron-donating effect (K_c of 2-thiazoline-2-thiol = 2527 $l \cdot mol^{-1}$). Derivatives **14** and **16** had particularly high values of K_c. We have found that compounds with a K_c above 100 $l \cdot mol^{-1}$ are liable to have antithyroid activity.[1] Thus most of the derivatives obtained were expected to have antithyroid activity. This was examined in the *in vivo* experiments (Table III).

Enzymology Synthetic antithyroid agents are thought to act on thyroid peroxidase.[6,7] Derivatives **1** and **2** of thiazole-2-thiol, in which the pyridine ring was unsub-

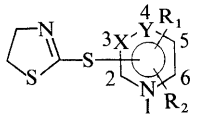
TABLE I. Derivatives of Thiazole-2-thiol



Compound No.	R_1	X	R_2	S-T[a]	mp (°C)	Yield (%)	Method	K_c ($l \cdot mol^{-1}$)
1	H	CH	H	2	185	52	A	21
2	H	CH	H	3	174	45	A	31
3	NO_2 (3)	CH	H	2	107	58	B	b)
4	NO_2 (5)	CH	H	2	197	60	B	b)
5	NO_2 (3)	CH	NO_2 (5)	2	144	75	C	b)
6	H	N	H	2	175	40	D	c)
7	OCH_3 (3)	CH	H	2	142	10	E	b)

a) Position of thiothiazole radical on pyridic or pyrimidi nucleus. b) Derivatives for which K_c could not be determined because the complex was unstable. c) Complex of stoichiometry 2 : 1.

TABLE II. Derivatives of 2-Thiazoline-2-thiol



Compound No.	R_1	X	Y	R_2	S-T[a]	mp (°C)	Yield	Method	K_c ($l \cdot mol^{-1}$)
8	H	CH	CH	H	2	105	48	A	356
9	H	CH	CH	H	3	104.5	52	A	[b]
10	NO_2 (3)	CH	CH	H	2	102	55	B	403
11	NO_2 (5)	CH	CH	H	2	142	42	B	433
12	NO_2 (3)	CH	CH	NO_2 (5)	2	176	52	C	187
13	H	N	CH	H	2	102	75	D	[b]
14	H	N	CH	H	2	128	68	D	795
15	OCH_3 (3)	CH	CH	H	2	108	9	E	[b]
16	OH (3)	CH	CH	H	2	103	5	E	1493

a) Position of thiothiazoline radical on the pyridic, pyrimidic, or pyrazic nucleus. *b*) Complex of stoichiometry 2:1.

TABLE III. Effect of Compounds on LPO, Thyroid Hormone Levels and Histology[a]

Compound No.	Inactivation of LPO IC_{50}	T4[b] μg/100 ml	TSH[b] μg/ml	Histology[e]
1	1.2×10^{-6}	1.86 ± 0.22[c]	0.38 ± 0.08[d]	++
2	3.7×10^{-6}	1.92 ± 0.16[c]	0.36 ± 0.06[d]	++
3	Activation	1.72 ± 0.10[c]	0.35 ± 0.02[d]	++
4	8.5×10^{-2}	1.66 ± 0.36[c]	0.42 ± 0.10[d]	+++
5	Activation	2.17 ± 0.34[c]	0.32 ± 0.06[c]	+++
6	6.5×10^{-5}	2.68 ± 0.21[c]	0.29 ± 0.04[c]	+
7	6.3×10^{-5}	2.04 ± 0.20[c]	0.38 ± 0.06[c]	++
8	4.7×10^{-3}	2.42 ± 0.26[d]	0.30 ± 0.08[d]	++
9	2.2×10^{-3}	2.48 ± 0.22[c]	0.31 ± 0.06[c]	++
10	4.4×10^{-4}	2.15 ± 0.46[d]	0.34 ± 0.04[c]	++
11	3.8×10^{-4}	2.28 ± 0.15[c]	0.28 ± 0.06[c]	++
12	7.0×10^{-3}	2.26 ± 0.51[d]	0.27 ± 0.08[c]	+
13	9.7×10^{-3}	2.44 ± 0.36[d]	0.28 ± 0.08[d]	+
14	6.5×10^{-3}	1.74 ± 0.22[c]	0.38 ± 0.06[c]	++
15	9.1×10^{-4}	1.82 ± 0.20[c]	0.38 ± 0.04[c]	+++
16	0.6×10^{-4}	1.42 ± 0.32[c]	0.44 ± 0.04[c]	+++
Control	—	3.35 ± 0.49	0.25 ± 0.02	−
Methimazole	2.4×10^{-5}	0.31 ± 0.04[c]	0.52 ± 0.04[c]	+++

a) The doses given *per os* to rats were 50 mg/kg per day during 3 weeks. Results are given as the mean ± S.E.M., $n = 10$. *b*) Significance of differences was determined by using Student's *t*-test. *c*) $p < 0.001$. *d*) $p < 0.01$. *e*) A ratio of the number of cylindrical cells to total cells of 25% was scored as +, a ratio of 50% as ++ and a ratio of 75% as +++. Cubic cells only, −.

stituted, were found to be the most active (around 10^{-6} M). On the other hand, the derivatives of 2-thiazoline-2-thiol were generally less active on LPO. It should be noted that the pyrimidine derivatives had less activity than the pyridine derivatives of both thiazole-2-thiol **6** and 2-thiazoline-2-thiol **13** (Table III).

Experiments *in Vivo* The *in vivo* experiments were designed to verify the *in vitro* findings, and test the combined action on molecular iodine and thyroid peroxidase (Table III). Thyroid gland weight is a good indicator of antithyroid activity, although if the action is of the excretor type, there will be no increase in colloid volume and the overall gland weight will not reflect the alteration in activity. Levels of T_4 also provide an indication of antithyroid activity. Total T_4 is increased in hyperthyroiditis and decreased in hypothyroiditis. T_4 levels were reduced by treatment with all compounds synthesized. Compound **16** displayed the most activity. Results of the TSH assay were in general agreement with the T_4 determinations, with the greatest increase observed for compound **16**, although levels (0.44 μg/ml) were below those observed with methimazole (0.52 μg/ml).

The histological appearance of the gland gives a good indication of its state of activity. Hyperactivity of the gland due to a decrease in thryroxine output is reflected by the number of cylindrical cells. The epithelium of thyreocytes of a normal gland has a cubic appearance, whereas a hyperfunctioning gland has a cylindrical epithelium. A ratio of the number of cylindrical cells to total cells of 25% was scored as +, a ratio of 50% as ++ and a ratio of 75% +++. Compounds **15** and **16** in the 2-thiazoline-2-thiol series, and compounds **4** and **5** of the thiazole-2-thiol series were found to lead to the greatest overactivity of the thyroid gland. Compound **16** (hydroxypyridine derivative of 2-thiazoline-2-thiol) with a high K_c (1493 $l \cdot mol^{-1}$) and a moderate action on peroxidase (IC_{50} 0.6×10^{-4}) was found to be the most active of the compounds synthesized, followed by the nitropyridine derivatives of thiazole-2-thiol (**4** and **5**). Compound **5**, like compound **3**, activated the peroxidase, but nevertheless had marked antithyroid activity *in vivo*, indicating that action on iodine may be involved in the antithyroid effect of these compounds.

Replacement of a diazole ring by a triazole, especially when partially hydrogenated, was found to confer antithyroid activity. Such compounds might thus be useful starting materials for preparation of novel antithyroid agents.

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