Atom Level Electrotopological State Indexes in QSAR: Designing and Testing Antithyroid Agents

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Purpose. To design new antithyroid agents with less side effects, the electrotopological-state (E-state) indexes of thiourylene moiety ($S_{N\&S}$) was utilized as a guideline to develop five acyclic thiourylene-type compounds with reduced antioxidant property.

Methods. These agents were synthesized and screened for antithyroid activity in rats using ^{125}I-thiocyanate discharge technique. In addition, chemiluminescence studies on the activated polymorphonuclear leukocytes (PMNLs) were also conducted to reveal antioxidant properties of the tested compounds.

Results. A linear relationship between the *in vitro* literature value of the formation constants of thiourylene-type compounds with iodine (K_c) and the $S_{N\&S}$ was observed and utilized in designing those agents. At least one of the compounds (abouthiouzine) was found to have a potential value as an antithyroid agent. The relative efficacy of abouthiouzine [1-n-butyl-3(isonicotinamido)-2-thiourea], after equimolar dose, was 102% and 51.5% of that of propylthiouracil with respect to the rate of ^{125}I-discharge and ^{125}I-uptake, respectively. In addition, Chemiluminescence studies on PMNLs revealed that abouthiouzine has slight oxidant property. Such properties may provide advantages in avoiding the iatrogenic hypothyroidism and antithyroid-induced immunological reactions.

Conclusions. The E-state approach provides guidelines to economize efforts and cost of designing new antithyroid drugs.

KEY WORDS: antithyroid activity; electrotopological-state index; QSAR; abouthiouzine; chemiluminescence.

INTRODUCTION

The currently used antithyroid drugs, propylthiouracil and methimazole, act primarily to control the thyrotoxicosis of Graves' disease by blocking iodine organification. These compounds are structurally related thioamides and are known to have serious side effects such as agranulocytosis, immunological reactions and aplastic anemia (1,2). The exact mechanism of the antithyroid drug induced-agranulocytosis is not known. However, Wall *et al.*, (3) proposed the involvement of cyclic thioamide group in the stimulation of antineutrophil-antibodies that mediate agranulocytosis. Whereas, Wilson *et al.* (4) reported the involvement of the antioxidant property of both propylthiouracil and methimazole in the production of antibodies. Therefore, we suggested (2) the development of compounds with acyclic thiourea and reduced antioxidant property to control Graves' disease and drug-induced agranulocytosis. For this purpose, data of currently used antithyroids, obtained from Imamura *et al.*, (5) were re-evaluated with respect to $S_{N\&S}$. A linear relationship between % inhibition of luminol-enhanced chemiluminescence (% CL-inhibition) of stimulated PMNLs and their $S_{N\&S}$ was obtained (Equation 1).

$$\%\ \text{CL-Inhibition} = 349.254\ (\pm 8.52) - 37.692\ (\pm 1.13)\ (S_{N\&S}) \quad (1)$$
$$[n = 3;\ r = 0.999;\ SEM = 0.47;\ F = 1108.1;\ P = 0.019]$$

This relationship was utilized as a guideline for the rational selection of thiourylene-type compounds with reduced antioxidant property (with $S_{N\&S} > 8$) (2).

Although Raby and Buxeraud (6) have suggested the use of K_c with iodine as a preliminary criterion for antithyroid activity ($K_c \geq 100\ M^{-1}$), however, no attempt was made to quantify the $S_{N\&S}$ in relation to K_c to use it for the rational selection of thiourylene-type compounds with potential antithyroid activity. Such guideline may lead to economization of efforts and cost spent in synthesis and pharmacological investigations.

The aim of this study is to develop a relationship between $S_{N\&S}$ of thiourylene-type compounds with their K_c with iodine to utilize it as a guideline in the development of new antithyroid drugs with acyclic thiourea moiety and reduced antioxidant property to alleviate the current antithyroid drug side effects.

MATERIALS AND METHODS

Materials

Propylthiouracil (Aldrich, Milwaukee, Wisconsin), phorbol 12-myristate 13-acetate (PMA) and luminol (Sigma, St. Louis, Missouri), nycodenz (Nygaard & Co., Torshov, Norway), Na^{125}I (Radiochemical Center, Amersham, England), potassium thiocyanate (BDH, Poole, England), other reagents and chemicals (Aldrich, Milwaukee, Wisconsin, USA). The compounds I-V were synthesized in our laboratory.

Computation of Skeletal Atoms E-state Indexes

The calculations were performed as described (2,7,8). Table I shows the diagonal matrix of the field influence between skeletal atoms within the molecule of propylthiouracil. All computations were performed using ABOUFAC-ET program (2). Scheme I shows the E-state indexes of the currently used antithyroid drugs.

Relationship Between the E-state of Skeletal Atoms of N',N'-Alkylthiourea Derivatives and Their K_c with Iodine

Twelve compounds of thiourylene- and thioamide- type

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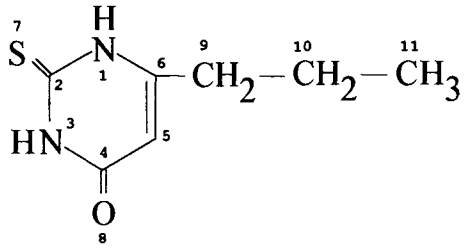
Table I. The Electrotopological-state of Propylthiouracil



PTU (Arbitrary numbered)

Atom no. (I_i)	Intrinsic values[d]	i	j 1	2	3	4	5	6	7	8	9	10	11	ΔI_i[b]	S_i[c]
I(1)	2.50	1	0.00	0.21	0.00	0.05[a]	0.06	0.21	−0.13	−0.18	0.11	0.06	0.02	0.41	2.91
I(2)	1.67	2	−0.21	0.00	−0.21	0.00	−0.02	0.00	−0.50	−0.33	0.01	0.01	−0.01	−1.26	0.40
I(3)	2.50	3	0.00	0.21	0.00	0.21	0.06	0.05	−0.13	−0.50	0.04	0.03	0.01	−0.03	2.47
I(4)	1.67	4	−0.05	0.00	−0.21	0.00	−0.08	0.00	−0.13	−1.33	0.01	0.01	−0.01	−1.79	−0.13
I(5)	2.00	5	−0.06	0.02	−0.06	0.08	0.00	0.08	−0.07	−0.56	0.06	0.03	0.00	−0.46	1.54
I(6)	1.67	6	−0.21	0.00	−0.05	0.00	−0.08	0.00	−0.13	−0.33	0.04	0.02	−0.02	−0.76	0.90
I(7)	3.67	7	0.13	0.50	0.13	0.13	0.07	0.13	0.00	−0.13	0.09	0.06	0.03	1.12	4.79
I(8)	7.00	8	0.18	0.33	0.50	1.33	0.56	0.33	0.13	0.00	0.22	0.15	0.10	3.84	10.84
I(9)	1.50	9	−0.11	−0.01	−0.04	−0.01	−0.06	−0.04	−0.09	−0.22	0.00	0.00	−0.06	−0.63	0.87
I(10)	1.50	10	−0.06	−0.01	−0.03	−0.01	−0.03	−0.02	−0.06	−0.15	0.00	0.00	−0.13	−0.49	1.01
I(11)	2.00	11	−0.02	0.01	−0.01	0.01	0.00	0.02	−0.03	−0.10	0.06	0.13	0.00	0.05	2.05
														0.00	

[a] Perturbation on atom i $(=(I_i - I_j)/(r_{ij})^2$.
[b] Total perturbation of I_i.
[c] $S_i = I_i + \Delta I_i$.
[d] Value in column $(I_i) = [(2/N)^2 \delta^v + 1]/\delta$. ($\delta^v$ and δ are the count of valence and sigma electrons; r_{iu} is the graph separation between atoms i and j; N is the principal quantum number).

were selected and their atom level E-state indexes calculated to quantitate the influence of different substituents on the E-state value of various skeletal atoms in the molecule, especially those of sulfur and nitrogen. The available literature values of K_c of these compounds with iodine (6,9) were collected in relation to that of methimazole. Regression analyses of the E-state indexes of all selected atoms of these agents with respect to K_c were performed to seek the *in vitro* QSAR model with the best statistical fit. Since complexation of antithyroid agents with iodine represents the major step in their mechanism of action (10), the selected agents (I-V) (Scheme II) were based on the developed *in vitro* QSAR model. Additional consideration was given to structural modifications and related physicochemical properties that influence the physical accessibility at the biological site.

Chemistry

The thiourea derivatives (I-IV) were prepared by refluxing 0.0134 mole of isothiocyanate derivative with equimolar quantity of amine derivatives in dry benzene. After cooling, the solid crude products were collected by filtration, washed with diethyl ether and crystallized from ethyl acetate/petroleum ether. Compound V was obtained in good yield, when reflux was done in absolute ethanol.

Animals

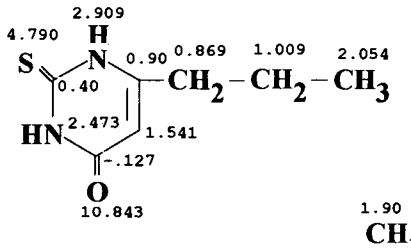
Wistar albino rats (220-250 gm) and Swiss albino mice (25-30 gm), obtained from the Experimental Animal Care Center, King Saud University, were randomly assigned to different experimental groups and maintained as per universal standards. The principles of laboratory care (NIH #85-23) were followed in undertaking the different experiments.

Antithyroid Testing

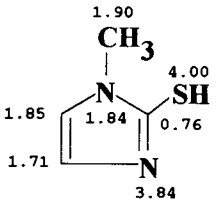
The antithyroid activity of these compounds was tested using ^{125}I-thiocyanate discharge technique (2,11) and compared with propylthiouracil after equimolar dose in dimethylsulfoxide (DMSO). Wistar albino rats, used in the treatment, were divided into nine groups (30 rats each). The test compounds were administered intraperitoneally (0.1 ml/day = 5.716 μmole kg^{-1}) for 30 days prior to the administration (i.p.) of carrier-free $Na^{125}I$ (~353460 cpm) per rat. The % efficacies of compounds I-V with respect to idoine uptake and release, relative to rats treated with propylthiouracil and DMSO control, were calculated according to Eq. (2)

$$Percentage\ Efficacy = \frac{(PAR)_t - (PAR)_c}{(PAR)_{ptu} - (PAR)_c} \times 100 \quad (2)$$

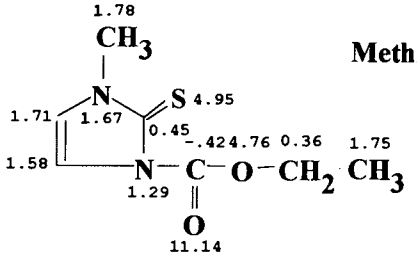
where PAR is the parameter used in determining % efficacy (either AUC or rate of ^{125}I-release) and the subscripts "t", "ptu" and "c" are for rats treated with tested agent, propylthiouracil and DMSO-control, respectively.



Propylthiouracil



Methimazole



Carbimazole

Scheme I. The Electrotopological-state of Currently Used Antithyroid Drugs of the Thioureylene-Type.

Acute Toxicity

The acute lethality (LD_{50}) of the compounds was determined using male mice as described (2,12).

Chemiluminescence (CL) Activity and Viability Testing

PMNLs were isolated from the blood collected from healthy human volunteers (2). The effect of the tested agents on luminol-dependent CL of PMA-stimulated PMNLs was studied as described by Tono-oka *et al.* (13) using an LKB-Wallac ^{125}I Luminator. The percentage CL response was calculated from the integrated area under the CL curve of tested compound [$(AUC\text{-}CL)_t$] and from that of the control [$(AUC - CL)_c$] according to Eq.(3):

Percentage CL Response =

$$\frac{(AUC - CL)_t - (AUC - CL)_c}{(AUC - CL)_c} \times 100 \qquad (3)$$

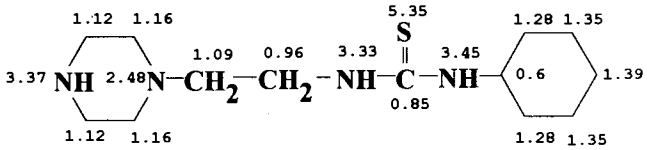
The resulting positive or negative value indicates stimulation or inhibition of oxidant and antioxidant property, respectively. The results were evaluated by using a two-tailed Student's t-test. The effect of the compounds I-V on the viability of PMNLs was tested at 10 and 30 minutes following incubation at 37°C. The percentage of viable cells was estimated by trypan blue exclusion test (2).

RESULTS AND DISCUSSION

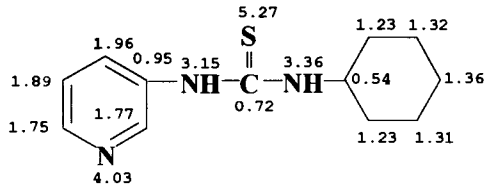
Quantitative Modeling Approach to Drug Design of New Antithyroid Agents

In general, antithyroid drugs inhibit the iodide organification after iodide was trapped in the thyroid and thus inhibit the formation of thyroid hormones (1,10). Lagorce *et al.* (10) indirectly proved the existence of molecular iodine intermediate through the formation of the charge transfer complexation and found a good correlation between the value of K_c with iodine and the *in vivo* antithyroid activity of the drug.

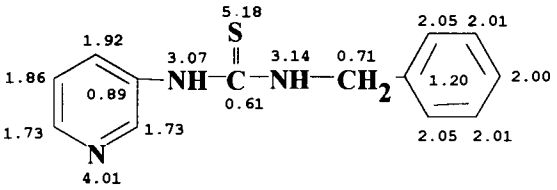
Table II shows the calculated E-state values of sulfur and nitrogen skeletal atoms of five N',N'-alkylthiourea and six thioamide derivatives and the available literature values of K_c of these compounds with iodine (6,9) in relation to that of methimazole. Attempts to delineate the skeletal atoms responsible for the *in vitro* charge transfer complexation with iodine showed that the K_c is independent on the E-state of each of both the skeletal atoms of sulfur (S_S) and the average E-state of nitrogen atoms (S_N) in the thiourylene moiety.



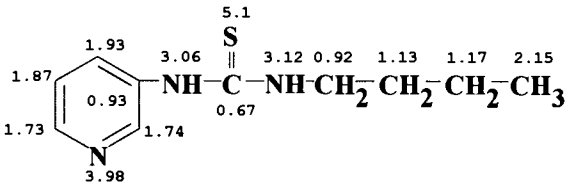
1-Cyclohexyl-3(2-piperazinoethyl)-2-thiourea (I).



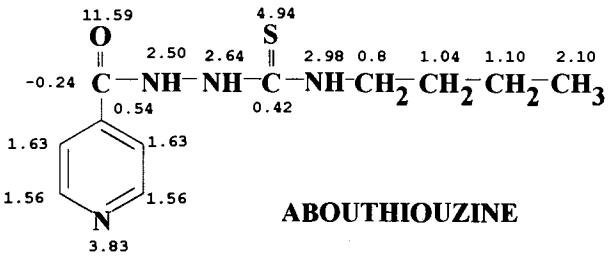
1-Cyclohexyl-3(3-pyridyl)-2-thiourea (II).



1-Benzyl-3(3-pyridyl)-2-thiourea (III).



1-n-Butyl-3(3-pyridyl)-2-thiourea (IV).



ABOUTHIOUZINE

1-n-Butyl-3(isonicotinamido)-2-thiourea (V).

Scheme II. Structures and chemical names of compounds I-V and their Electrotopological-state.

Table II. The Electrotopological-state of Some Skeletal Atoms of Thiourylene and Thioamide Derivatives and the Formation Constants of Their Charge Transfer Complexes with Iodine, with Respect to Methimazole

No.	Structure of thiourylene and thioamide derivatives	Electrotopological-state				K_c (L/mole)		
		$S_{>NH}$	S_S	$S_{>NH}$	$S_{N\&S}$	Value (±SD)	Ref	$Ln(K_c)$
1	H_2N-C(S)-NH_2	4.62[a]	4.09	4.62	8.71	8825 (±505)	6	−9.09
2	CH_3(p)ph-NH-C(S)-NH-ph(p)CH_3	3.15	5.26	3.15	8.41	11620 (±400)	9	−9.36
3	CH_3O(p)ph-NH-C(S)-NH-ph(p)OCH	3.10	5.26	3.10	8.36	14010 (±350)	9	−9.55
4	$(CH_3)_2$-CH-NH-C(S)NH-$CH(CH_3)_2$	3.09	4.98	3.09	8.07	16430	9	−9.71
5	CH_3-NH-C(S)-NH-$C(CH_3)_3$	2.84	4.88	3.09	7.84[a]	20820	9	−9.94
6	Methimazole	3.84[b]	4.0[c]	1.84	7.84[d]	23194 (±667)	6	−10.05
7	H_2N-C(S)-CH_2OH	4.77	4.22	—	8.99	NA[e]	—	
8	H_2N-C(S)-CH_2CH_3	5.02	4.47	—	9.49	NA	—	
9	H_2N-C(S)-CH_2Cl	4.89	4.34	—	9.23	NA	—	
10	H_2N-C(S)-CH_2F	4.62	4.10	—	8.72	NA	—	
11	H_2N-C(S)-CH_2SH	4.95	4.40	—	9.35	NA	—	
12	H_2N-C(S)-$CH(CH_3)_2$	5.17	4.60	—	9.77	NA	—	

[a] Average of the E-state value of two skeletal nitrogen atoms (>NH) plus E-state value of (S=).
[b] E-state value of (−N=).
[c] E-state value of (−SH).
[d] Sum of E-state values of (−N=) and (−SH).
[e] NA, data are not available in the literature.

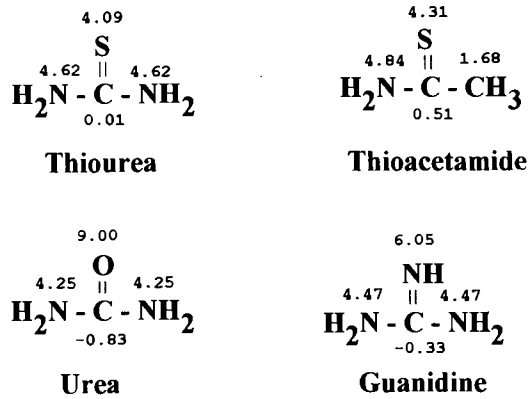
This indicates that complexation of thiourea derivatives with iodine is not due to the electrical donor properties of either sulfur or nitrogen atom alone, which agrees with an earlier report (14). Therefore, we attempted to relate the E-state of the sum of S_S and S_N ($S_{N\&S}$) of thiourylene moiety with their ln (K_c). The relationship between [-ln (K_c)] and the E-state of $S_{N\&S}$ was found to be linear as shown in Eq. (4):

$$-\ln(K_c) = -17.984\ (\pm 0.724) + 1.02\ (\pm 0.088)\ (S_{N\&S}) \quad (4)$$
$$[r = 0.985,\ SEM = 0.07,\ F = 133.7,\ n = 6,\ p = 0.0003]$$

where the figures in between parentheses are the standard errors of the mean.

Validity of the *in Vitro* QSAR Model

Thorough examination of Eq. (4) and the structural influences on the value of $S_{N\&S}$ of thiourylene- and thioamide-type compounds in Table II and Schemes I and III, explains that thiourylene and thioamide moieties are essential for antithyroid activity. However, thiourylene-type compounds are more potent than thioamide-type, which agrees with the reported findings (15,16). Eq. (4) also expects that the replacement of sulfur atom by either oxygen (O) or =NH, as in case of urea ($S_{N\&O}$ = 13.45) and guanidine ($S_{N\&N}$ = 10.52), will decrease K_c value and consequently, the antithyroid activity will be lost which agrees with literature findings (14,15).



Scheme III. Electrotopological-state of Thiourea, Thioacetamide, Urea and Guanidine.

Selection of Compounds for Antithyroid Testing

Equation (4) reveals that the E-state of thiourylene moiety ($S_{N\&S}$) represents the donor site for complexation, but the Equation is limited to *in vitro* complexation of iodine with N′,N′-alkylthiourea derivatives that have $S_{N\&S}$ value between 7.6 and 8.75 and to structural configurations similar to the examined agents. On this basis and on the selective accumulation of thiourylene-type agents in the thyroid gland (1,17), compounds I-V (Scheme II) were selected. The $S_{N\&S}$ value of compounds I-V are 8.74, 8.52, 8.285, 8.19, 7.75, respectively. The structure compactness and the general lipophilicity of the alkyl substituents of N′,N′-alkylthiourea compounds were also considered. Data in Table II and Schemes I and II could lead to the development of self-intuition and experience guide for the type of substituents, branching, cyclization needed to make *a priori* predictive knowledge utilizing the quantitative modeling approach to drug design.

Chemistry

Characterization of the compounds was achieved

Table III. Physical and Analytical Data of the Prepared Thiourea Derivatives

Compound	Yield %	Melting point (°C)[a]	Chemical formula	Element	Analysis% Calc.	Analysis% Found	IR[b] ν(N–H)	IR[b] ν(C=S)
				C	5.77	57.4		
I	80	181.35 ± 0.15	$C_{13}H_{26}N_4S$	H	9.62	9.52	3250	1542
				N	20.7	20.3		
				C	61.28	61.53		
II	66	154.45 ± 0.05	$C_{12}H_{17}N_3S$	H	7.23	6.85	3345	1515
				N	17.87	18.15		
				C	64.2	63.8		
III	78	135.6 ± 3.2	$C_{13}H_{13}N_3S$	H	5.35	5.47	3230	1535
				N	17.28	16.89		
				C	54.79	54.41		
IV	67	98.8 ± 0.01	$C_{10}H_{15}N_3S$	H	6.85	6.52	3135	1550
				N	19.18	19.54		
				C	52.38	52.19		
Abouthiouzine[d]	82	215.2 ± 0.5	$C_{11}H_{16}N_4OS$	H	6.35	6.46	3300	1550
				N	22.22	22.49		

	MR characterization[c]			
	Cyclohexyl H (11 H,m)	– Butyl H	NH (1 H,s)	Other H
I	1.59–2.03	—	7.25 8.80	3.76–2.51 (12H,m,CH_2)
II	1.13–2.03	—	6.37 8.64	7.4–8.4(4H,m)
III	—	—	9.71	7.34–8.6(4H,m) 8.4(1H,s,CH_2)
IV	—	0.86(3H,t,CH_3) 1.44(4H,m,2CH_2) 3.58(2H,t,CH_2)	6.77, 9.05	7.26–8.4(4H,m)
Abouthiouzine[d]	—	0.87(3H,t,CH_3) 1.4(4H,m,2CH_2) 3.4(2H,t,CH_2)	8.17, 9.35	7.84(4H,m)

[a] Mean of 3–4 runs ± SD.
[b] Using KBr pellets.
[c] Solvent, DMSO-d_6.
[d] IR of (C=O) = 1675.

through IR and NMR spectrophotometry. The IR and ^1H NMR spectra of all compounds (Table III) were in agreement with the proposed structure. Further identification was obtained using elemental analysis (Table III).

Antithyroid Activity

The observed radioactivity of thyroid gland (% ^{125}I dose/g) is shown in Fig. 1. The resulting curves of tested agents are biphasic while the curves of both the normal control and the DMSO-control are not. In case of normal control, ^{125}I-thyroid uptake occurred at a constant rate for the entire two-hour period of the experiment (7.432×10^{-3} % Dose/min, r = 0.994, n = 4 [4 groups using Mean Value of 5 rats in each group]). On the other hand, the DMSO-control showed ^{125}I-uptake up to 60 min. (^{125}I-uptake phase) followed by a constant rate of 3.66×10^{-4} % Dose/min, r = 0.87, n = 3. The % efficacy of tested agents was calculated, according to Eq. (2), in relation to both the ^{125}I-uptake and the rate of its discharge from thyroid gland. A comparative antithyroid efficacy of the tested compounds in reference to DMSO-control and propylthiouracil-treated rats is given in Table IV. The % efficacy of compounds III and V (named abouthiouzine) with respect to ^{125}I-uptake are 23.2 and 51.5, respectively. Whereas, compounds I, II and IV have no efficacy. On the other hand, the % efficacy of compounds III and abouthouzine with respect to the rate of ^{125}I-discharge are 49.9 and 101.8, respectively. Therefore, increasing the dose of abouthiouzine to increase its ^{125}I-uptake from 51.5% to 100.0%, of that of propylthiouracil, will lead to an increase in the ^{125}I-discharge to a higher value than that of the therapeutic dose of propylthiouracil. Such an increase in the liberation of iodide during the reversible process tends to protect thyroid peroxidase from complete inactivation (Eq. (7) and Eq. (8)) and enhances the generation of molecular iodine (Eq. (8)) according to the following reactions (10,18).

$$E + H_2O_2 \rightarrow EO \quad (5)$$

$$EO + I^- \rightarrow EOI \quad (6)$$

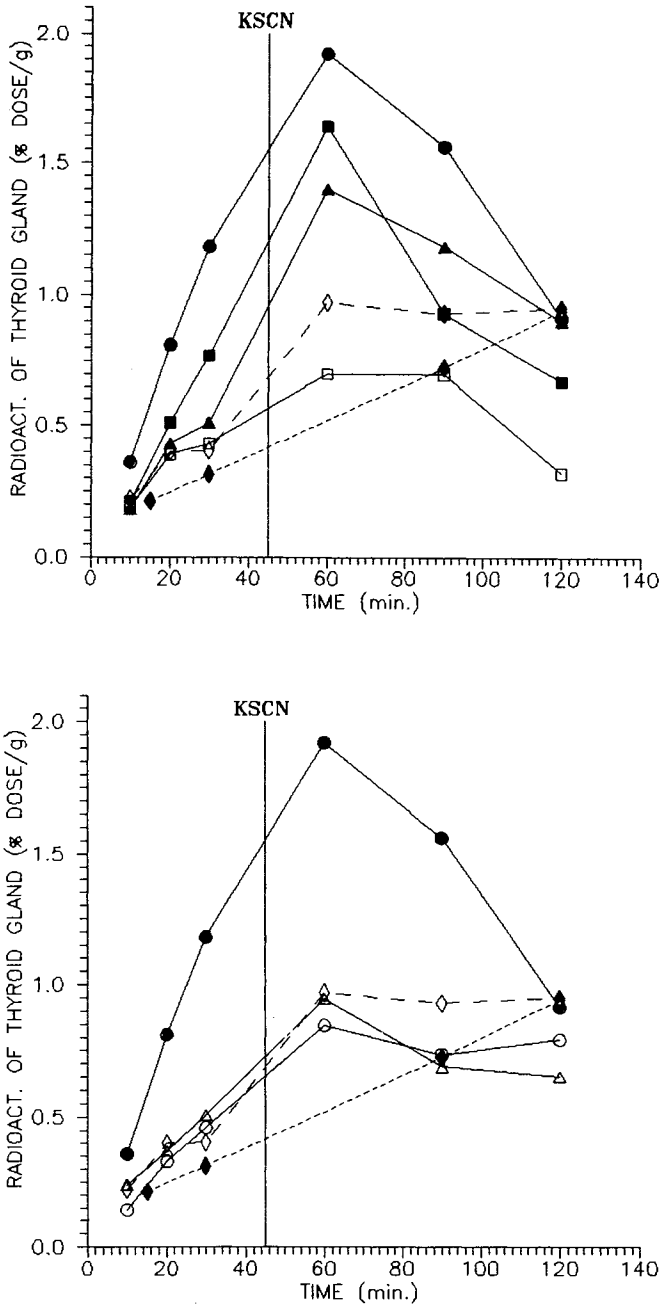


Fig. 1. Radioactivity of thyroid gland (%^{125}I Dose/g) in rats treated with compounds I-V. Equimolar doses of all the compounds were daily injected i.p. for 30 days before ^{125}I administration to rats. Each point was an average of 5 runs. At 45 minutes, all remaining rats were injected i.p. with KSCN [SE is included in the calculated area under the curve (Table IV)]. Key: (●) PTU; (◇) DMSO control; (◆) Normal control; (○) I; (△) II; (▲) III; (□) IV; (■) Abouthiouzine (V).

$$EOI^- + \text{tyrosyl} \rightarrow \text{iodotyrosyl} + E + H^+ \quad (7)$$

$$EOI^- + I^- \rightarrow I_2 + E \quad (8)$$

This property of abouthiouzine, might avoid the iatrogenic hypothyroidism and ensure the continuity of the formation of the charge transfer complex with antithyroid drugs.

Since the ratio of the % efficacy with respect to the rate of ^{125}I-discharge to ^{125}I-uptake (101.8% to 51.5%) of abouthiouzine is 1.98, it is perceived that abouthiouzine is of potential value as an antithyroid agent.

Correlation Between Antithyroid % Efficacy and $S_{N\&S}$

The quantitative relationship between $S_{N\&S}$ of the tested compounds and their % efficacy with respect to both ^{125}I-uptake [(% Efficacy)$_{IP}$] and ^{125}I-exchange [(% Efficacy)$_{IE}$] are shown in Eqs. (9) and (10).

$$(\%\ \text{Efficacy})_{IP} = 705.24\ (\pm 130.6) - 82.40\ (\pm 16.3) S_{N\&S} \quad (9)$$

[r = 0.963, SEM = 13.52, F = 25.62, n = 6, P = 0.037]

$$(\%\ \text{Efficacy})_{IE} = 713.02\ (\pm 91.2) - 80.78\ (\pm 11.2)\ S_{N\&S} \quad (10)$$

(r = 0.964, SEM = 11.79, F = 52.44, n = 6, P = 0.0019]

Using equations (9) and (10), only 92.7% (r^2 = 0.927) and 92.9% (r^2 = 0.929) of the variability in ^{125}I-uptake and ^{125}I-exchange data can be explained, respectively. The quality of these QSAR relationships may be related to the site of interaction rather than physical accessibility at the biological site of action.

Relating the % efficacy of the tested agents to their N-substitutions could lead to a misleading interpretation (Table IV and scheme II). However, the discrepancies in % efficacy of the tested compounds are inherited in the different influences of their N-substitutions on both the $S_{N\&S}$ and their physical accessibilities at the site of action. The comparative antithyroid % efficacy for both ^{125}I-uptake and discharge with $S_{N\&S}$ of the selected compounds demonstrates the applicability of the *in vitro* QSAR model in the drug design. Furthermore, the $S_{N\&S}$ index is confirmed as an important determinant of antithyroid activity (2).

Toxicity

The approximate LD_{50} for compounds I-V are >1000, 510, 570, 370 and >1000 mg/kg, respectively. Abouthiouzine has higher LD_{50} than propylthiouracil. Thus, higher doses of abouthiouzine can be administered safely to produce a comparable efficacy to propylthiouracil. It should be noted that the equimolar dose (1.429 μmole/day) in rat corresponds[4] to human dose of 11 and 16.1 mg/day of propylthiouracil and abouthiouzine, respectively.

Effect of the Tested Agents on CL-Response and PMNLs Viability

Table V summarizes the area under the curve (AUC), of luminol-dependent CL-response of PMA-stimulated PMNLs, induced by the tested agents at different concentrations (5-40 μg/ml). The % CL-stimulation (+) and % CL-inhibition (−), calculated according to Eq. (3), represent the

[4] Rat (200 gm) to man (70 kg) dose conversion was done according to the surface area ratio (= 56). Value stated = [(Dose in rat (mg/kg) / 5) × 56].

Table IV. Comparative ^{125}I-Uptake, Rate of ^{125}I-Release and Antithyroid Relative Efficacy of the Synthesized Agents with Reference to Equimolar Dose of Propylthiouracil in Rats

Compound	Total AUC[a] [AUC. ± SE; (%Dose/g) · min.]	^{125}I-Uptake [AUC. ± SE; (%Dose/g) · min.] up to maximum[b]	Rate of ^{125}I-release[c] (Dose%/min.) (×10^4)	% Efficacy[f] with respect to	
				^{125}I-uptake	^{125}I-release
Control—Normal[d]	61.28 ± 7.54	—	—	—	—
—DMSO[e]	85.45 ± 8.37	45.76 ± 3.95	3.66 (r = 0.870)	—	—
PTU	153.35 ± 11.63	105.58 ± 8.33	−159.43 (r = 0.988)	100	100
I	73.15 ± 7.38	43.32 ± 5.07	−9.167 (r = 0.500)	—	3.53
II	76.95 ± 9.39	49.49 ± 5.84	−49.17 (r = 0.919)	6.24	29.21
III	107.22 ± 9.80	59.62 ± 6.51	−81.33 (r = 0.998)	23.17	49.86
IV	61.2 ± 11.07	40.81 ± 6.59	−62.60 (r = 0.863)	—	37.8
Abouthiouzine	110.65 ± 7.45	76.56 ± 6.11	−162.25 (r = 0.967)	51.49	101.81

[a] Area under the curve from 0–120 min.
[b] From 0–60 min (uptake phase).
[c] From 60–120 min (release phase), n = 5 rats.
[d] Untreated rats.
[e] Rats were treated with 0.1 ml DMSO for 30 days before radioiodine administration.
[f] % efficacy = $100[(PAR_t - PAR_c)/(PAR_{ptu} - PAR_c)]$; where PAR is the parameter used in determining % efficacy (either AUC of ^{125}I-uptake or rate of ^{125}I-release), t and c are for tested compound and DMSO control, respectively. The regression coefficient (r) was calculated for the rate of ^{125}I-release from the decline phase of the curve (last 3 points).

magnitude of oxidant and antioxidant property, respectively. Propylthiouracil inhibited the PMNLs "respiratory burst" in a dose-dependent manner and varied between 41-75% for the tested concentration range, thus, it confirms the earlier findings on the antioxidant property of propylthiouracil (4,5). However, the maximum CL-inhibition observed at 40 μg/ml for compounds I-IV were 10%, 24%,40.6% and 35.4%, respectively. On the other hand, abouthiouzine stimulated CL for the concentration range tested. The PMNLs viability was not affected by any of the tested compounds. These results indicate that the effect of these drugs on the CL-response of the PMNLs was not due to direct lysis of the cell membrane but due to respiratory burst. In contrast to propylthiouracil, the stimulation of PMNLs "respiratory burst" by abouthiouzine demonstrates the production of reactive oxygen species (ROS). Since the ROS are known to inhibit cell proliferation by depleting the levels of glutathione (19), the oxidant property of abouthiouzine might inhibit proliferation of cells and decrease production of antibodies. In conclusion, compounds I-V (Scheme II) were designed to be acyclic thiourea derivatives with reduced antioxidant property, utilizing the developed *in vitro* QSAR models, to account for Wall *et al.*, and Wilson *et al.*, hypotheses related to the causes of agranulocytosis (3,4). At least one of the compounds (abouthiouzine) was found to have a potential value as an antithyroid agent. In addition, the Chemiluminescence studies on PMNLs revealed that abouthiouzine has slight oxidant property. Such properties are known to provide advantage in avoiding the iatrogenic hypothyroidism (10) and antithyroid-induced immunological reactions (3,4). Therefore, abouthiouzine may possess an immunosuppressive effect and reduce sensitization of antineutrophil-antibodies. This might cause suppression of agranulocytosis and prevention of the relapse of Graves' disease after discontinuation of medication (2). Hence, the E-state approach provides guidelines to economize efforts and cost of designing new antithyroid drugs.

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Table V. The Effect of the Investigated Agents on Chemiluminescence of Human Polymorphonuclear Leukocytes (1 × 10^5 Cells/ml), Stimulated with PMA, with Reference to Propylthiouracil

Treatment Dose (μg/ml)	Chemiluminescence area under the curve ± SD[a] (min. mV)					
	PTU	I	III	Abouthiouzine (V)	II	IV
Control	2590.5 (±131)	2590.5 (±131)	2590.5 (±131)	2590.5 (±131)	2590.5 (±131)	2590.5 (±131)
5 μg/ml	1510 ± 76	3618 ± 11	1896 ± 94	3874 ± 82	2401 ± 97	2163 ± 11
10 μg/ml	1321 ± 80	3383 ± 67	1663 ± 62	3516 ± 72	2382 ± 12	1856 ± 55
20 μg/ml	911 ± 46	2381 ± 58	1638 ± 48	3544 ± 106	2296 ± 68	1639 ± 49
40 μg/ml	648 ± 52	2335 ± 24	1563 ± 78	2980 ± 119	1967 ± 10	1674 ± 67

[a] Area under the curve from 0–14 min. (Mean ± Standard deviation); n = 2–6 runs.

REFERENCES

1. D. S. Cooper. Antithyroid drugs. *N. Engl. J. Med.* 311:1353-1362 (1984).
2. R. R. A. Abou-shaaban, H. A. Al-Khamees, H. S. Abou-Auda, and A.P. Simonelli. Development of New antithyroid compounds with reduced antioxidant property. *Saudi Pharm. J.* 3:156-175 (1995).
3. J. R. Wall, S. L. Fang, T. Kurocki, S. H. Lagbar, and L. E. Braverman. *In vitro* immunoactivity to propylthiouracil, methimazole, and carbimazole in patients with Graves' disease: a possible cause of antithyroid drug-induced agranulocytosis. *J. Clin. Endocrinol. Metab.* 58:868-872 (1984).
4. R. Wilson, J.H. McKillop, M. Travers, J. Smith, E. Smith, and J.A. Thomson. The effects of antithyroid drugs on intracellular mediators. *Acta. Endocrenol. Copenh.* 122: 605-609 (1990)
5. M. Imamura, N. Aoki, T. Saito, Y. Ohno, Y. Maruyama, J. Yamaguchi, T. Yamamoto. Inhibitory effects of antithyroid drugs on oxygen radical formation in human neutrophils. *Acta Endocrinological,* 112:210-216 (1986).
6. C. Raby and J. Buxeraud. Antithyroidiens: relation structure-activité. I. Formation de complexes moléculaires iodés. *Eur. J. Med. Chem.* 15:425-430 (1980).
7. L. B. Kier and L. H. Hall. An electropological state index for atoms in molecules. *Pharm. Res.* 7:801-807 (1990).
8. L. H. Hall, B. Mohney, and L. B. Kier. Comparison of electrotopological state indexes with molecular orbital parameters: Inhibition of MAO by hydrazides. *Quant. Struct. Act. Relat.* 12:44-48 (1993).
9. K. R. Bhaskar, R. K. Gosavi, and C. N. R. Rao. Spectroscopic studies on n-donor + o'-acceptor systems: Alkylthioureas and thiocarbanilides. *Trans. Faraday. Soc.* 62:29-38 (1966).
10. J. F. Lagorce, J. C. Thomas, G. Catanzano, J. Buxeraud, M. Raby, and C. Raby. Formation of molecular iodine during oxidation of iodide by the peroxidase/H_2O_2 system. *Biochem. Pharmacol.* 42:589-592 (1991).
11. H. Y. Abou-Enein, M. A. Loutfy, and M. E. Abdulla. Synthesis and antithyroid activity of 6-n-propyl-5-arylazo 2-thiouracils. *Toxicol. Environ. Chem.* 13:257-263 (1987).
12. M. N. Ghosh. *Fundamental of experimental pharmacology.* Scientific Book Agency, Calcutta, 1984, pp. 189-190.
13. T. Tono-oka, N. Ueno, T. Matsumoto, M. Ohkawa, and S. Matsumoto. Chemiluminescence of whole blood. I. A simple and rapid method for the estimation of phagocytic function of granulocytes and opsonic activity in whole blood. *Clin. Immunopathol.* 26:66-75 (1983).
14. R. P. Lang. Molecular complexes and their spectra. XIV. Iodine complexes with thiourea and thioacetamide. *J. Am. Chem. Soc.* 84:1185-1192 (1962).
15. E. B. Astwood, A. Bissell, and A. M. Hughes. Further studies on the chemical nature of compounds which inhibit the function of the thyroid gland. *Endocrinol.* 37:456-481 (1945).
16. D. R. Morris and L. P. Hager. Mechanism of the inhibition of enzymatic halogenation by antithyroid agents. *J. Biol. Chem.* 241:3582-3589 (1966).
17. B. Marchant, W. D. Alexander, J. H. Lazarus, Jean Lees, and D. H. Clark. The accumulation of ^{35}S-antithyroid drugs by the thyroid gland. *J. Clin. Endocr.* 34:847-851 (1972).
18. R. P. Magnusson, A. Taurog, and M.L. Dorris. Mechanism of thyroid peroxidase and lactoperoxidase-catalysed reaction involving iodide. *J. Biol. Chem.* 259:13783-13790 (1984).
19. R. R. A. Abou-Shaaban. Oxidative stress, HIV and AIDS: The basis for antioxidant-oriented antiretroviral nucleoside analogues. *Saudi Pharm. J.* 3:1-22 (1995).