

DEVELOPMENT OF NEW ANTITHYROID COMPOUNDS WITH REDUCED ANTIOXIDANT PROPERTY

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استنادًا على ما نشر من أبحاث عن المركبات المستخدمة في علاج مرض الدرق الجحوظي (داء غريف) وضعنا فرضية مفادها أننا لكي نسيطر على هذا المرض وما تسببه العقاقير المستخدمة في علاجه من فقدان للكريات البيض المحببة، فإنه يجب تحويل هذه العقاقير لتشمل مركبات الثيورورا غير الحلقية ذات الخاصية قليلة التضاد للأكسدة. لهذا تم استحداث علاقة خطية بين معاملات الحالات الكهروطوبولوجية لشطر الثيوريلين لأدوية الدرق وبين النسبة المثوبة لتثبيتها للتألق الكيميائي المحفز بالليومينول (%cl) للكريات البيض المفصصة النوى (PMNLs) المنشطة والتي اخذت بياناتها من مقال إمامورا ورفاقه المنشور عام ١٩٧٦م. ولقد استخدمت العلاقة الخطية الناتجة للاسترشاد بها في الاختيار المنطقي للمركبات من نوع ثيوريلين قليلة التضاد للأكسدة ممن تزيد معاملات حالاتها الكهروطوبولوجية عن ٨. وقد تم تقدير خاصية التضاد للأكسدة للمركبات التي وقع الاختيار عليها باستخدام تأثيرها على التألق الكيميائي المحفز بالليومينول للكريات البيض المفصصة النوى المحفزة بإداة خلايا مريستات الفوربول، وقد وجد أنها تتفق مع الدلائل المشتقة من بيانات إمامورا ورفاقه. وتم تحديد ثابت التكوين للشحنة المنقولة المركبة مع اليود للمركبات المختارة بواسطة التحليل الطيفي واستخدمت كمعيار مبدئي لنشاطها المحتمل ضد الدرق. بالإضافة إلى ذلك، تم تقصي النشاط المضاد للدرق لهذه المركبات على الجرذان باستخدام طريقة تفرغ شحنة اليود - ١٢٥ ثيوسيانات. ولقد تم تقدير الفاعلية النسبية (مقارنة بجرعة مكافئة من البروبايل ثيوراسيل) لهذه المركبات فيما يتعلق بمعدل الاستحواذ على اليود - ١٢٥ ومعدل تصريفه، وأثبت ذلك أن واحدًا على الأقل من المركبات الثلاث التي تم اختبارها وهو أبو ثيولين له نشاط مضاد للدرقية أكبر من البروبايل ثيوراسيل بالنسبة لمعدل تصريف اليود - ١٢٥. إن الفاعلية النسبية لجرعة مكافئة من الأبو ثيولين كانت ١٣، ١٣١، ٧٢، ٥١% من فاعلية بروبايل ثيوراسيل فيما يتعلق بتصريف اليود - ١٢٥ والاستحواذ عليه، على التوالي. كما تتم مناقشة أهمية تصريف اليود - ١٢٥ الدرقي في العلاجات المضادة لنشاط الغدة الدرقية. وحيث أن نسبة معدل تصريف اليود - ١٢٥ على معدل الاستحواذ عليه تبلغ ٢، ٥٤، فإنه يُستشف من ذلك أن لأبو ثيولين قيمة محتملة كدواء مضاد للدرقية بعد تعديل جرعته بالنسبة للجرعة العلاجية للبروبايل ثيوراسيل. وفي المحصلة، فإن مركب أبو ثيولين أقل سُمية ($LD_{50} > 800 \text{mg/kg}$)، وله خاصية قليلة التضاد للأكسدة، وفاعلية مضادة للدرق أكبر من فاعلية بروبايل ثيوراسيل فيما يتعلق بتصريف اليود - ١٢٥. وعلى ضوء الآثار السامة لخاصية التضاد للأكسدة التي تتمتع بها الأدوية الحالية المضادة للدرق، فإن أبو ثيولين سيكون مفيدًا في تخفيف حدة التفاعل المناعي لكل من داء غريف ومرض فقدان الكريات البيض المحببة الناتج عن الأدوية. وهناك المزيد من الدراسات المؤيدة لذلك مازالت جارية.

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Based on literature evaluation, we hypothesize that "in order to control Graves' disease and the onset of drug-induced agranulocytosis the trends in antithyroid therapy should be modified to include acyclic thiourea with reduced antioxidant property". A linear relationship was developed between percentage of inhibition of luminol-enhanced chemiluminescence (%CL) of stimulated polymorphonuclear leucocytes (PMNLs) obtained from Imamura *et al.* (Acta Endocrinologica, 1986; 112:210-216.) data of currently used antithyroids and related compounds and their atom level Electrotological-state (E-state) indexes of their thiourylene moiety ($S_{N\&S}$). The resulted relationship [%CL-Inhibition = $349.254 (\pm 8.52) - 37.692 (\pm 1.13) (S_{N\&S})$; $n = 3$; $r = 0.999$; SEM = 0.47; F = 1108.1; P = 0.019] was utilized as a guideline for the rational selection of thiourylene-type compounds (with $S_{N\&S} > 8$) with reduced antioxidant property. The compounds selected were evaluated for their antioxidant property utilizing their influence on luminol-enhanced chemiluminescence (CL) of phorbol myristate acetate (PMA)-induced human PMNLs and was found to agree with the guideline extracted from Imamura *et al.* data. The formation constant of the selected compounds of their charge transfer complex with iodine was determined spectroscopically and utilized as a primary criteria for their potential antithyroid activity. In addition, these agents were screened for antithyroid activity in rats using ^{125}I -thiocyanate discharge technique. The relative efficacy (in relation to equimolar dose of propylthiouracil) of the tested agents with respect to both ^{125}I -uptake and the rate of ^{125}I -discharge were determined and proved that at least one of the three investigated compounds (abouthiouline) has higher antithyroidal activity than propylthiouracil with respect to the rate of ^{125}I -discharge. The relative efficacy of abouthiouline [1-cyclohexyl-3(3-quinolyl)-2-thiourea], after equimolar dose, was 131.13% and 51.72% of that of propylthiouracil with respect to the rate of ^{125}I -discharge and ^{125}I -uptake respectively. The importance of the thyroid ^{125}I -discharge in antithyroid therapy in reducing the dose and the duration of treatment is discussed. Since the ratio of the percent efficacy with respect to the rate of ^{125}I -discharge to ^{125}I -uptake of abouthiouline is 2.54, it is perceived that abouthiouline is of potential value as antithyroid agent, after adjusting the dose with respect to the therapeutic dose of propylthiouracil. In conclusion, abouthiouline has lower toxicity ($\text{LD}_{50} > 800 \text{ mg/kg}$), reduced antioxidant property as well as higher antithyroidal efficacy than propylthiouracil with respect to the rate of ^{125}I -discharge. In view of the toxic implications of the antioxidant property of the currently used antithyroid agents, it is suggested that abouthiouline will be beneficial in reducing the severity of the immune reaction of both the Graves' disease and the drug-induced agranulocytosis. Further supportive investigations are in progress.

Key words: antithyroids; agranulocytosis; electrotopological-state indexes; objective-drug design; antioxidants; charge transfer complex; abouthiouline; drug development; QSAR.

Introduction

The antithyroid drugs currently used for the control of hyperthyroidism and goiter in Graves' disease include propylthiouracil and methimazole. These drugs are structurally related thioamides and are known to have serious side effects such as agranulocytosis and aplastic anemia (1-4). In the last five decades, efforts were concentrated on the investigation of the mechanism of action of antithyroid drugs and their side effects (5-10). Graves' disease has been shown to be a disease of disordered immunity in which circulating antibodies to the thyrotropin receptor stimulate thyroid func-

tion (7,8). The exact mode of action of the antithyroid drug induced agranulocytosis, decrease of natural killer cells and relapse of Graves' disease, after discontinuation of medication, is not known. However, different mechanisms of antithyroid drug-induced agranulocytosis were proposed in the literature (11,12). Wall *et al.*, (11) proposed the involvement of cyclic thioamide group, which is common to propylthiouracil and methimazole, in the stimulation of antineutrophil-antibodies that mediate agranulocytosis. Furthermore it was also explained that the cyclic thioamide group acts either as an antigen or become antigenic by forming a complex with neutrophil protein. Whereas,

Wilson *et al.* (12) reported the involvement of the free radical scavenging property of both propylthiouracil and methimazole in the production of antibodies. Heufelder *et al.* (13) reported that the relapse of Graves' disease after discontinuation of propylthiouracil and methimazole to be also related to their free radical scavenging activity.

The antioxidant property of these antithyroid agents has been described to enhance interleukin-2 production, alter the function of natural killer cells and modulate the thyrocyte HLA-DR expression and phagocytosis (14-17). Furthermore, the antioxidant property of a compound is also known to be related to the proliferative activity of cells and production of antibodies (5,18-21). Recent studies have demonstrated that the antioxidant property of antithyroid drugs increases the endogenous levels of interleukin-2 which reflects the activation of T and B lymphocytes and the production of antibodies (12,14-16,22). In addition to these effects, propylthiouracil and methimazole have also been shown to exert immunomodulatory effect on lymphocytes from both normal volunteers and patients with Graves' hyperthyroidism (12,14,15,22,23), and increase the serum soluble interleukin-2 receptors in normal human (24). Furthermore, Paschke *et al.*, (25) suggested that the intrathyroidal lymphocytes may be the major source of the thyroidal autoantibodies and hence the intensity of the intrathyroidal lymphocytic infiltration and microsomal antibodies are generally believed to reflect the activity of the autoimmune process in Graves' disease. Aguaya *et al.*, (17) and Wenzel *et al.*, (26) provided evidence against immunosuppressive effect of antithyroid drugs and indicated that they may cause *in vivo* immunomodulatory effects, secondary to their influence on thyroid hormone formation and/or inhibition of thyroid antigen. Therefore, in order to control the disease and the onset of agranulocytosis the trends in antithyroid therapy should be modified to include acyclic thiourea with reduced antioxidant property that might cause suppression of immune system by reducing lymphocytic infiltration and production of antibodies. Since hyperthyroidism is a common endocrine problem and no alternative safe drug therapy is available, there is an imperative need to develop less toxic antithyroid drugs.

Minimal efforts were made to synthesize new

antithyroid agents with low toxicity. However, Raby and Buxeraud (27) studied the structure-activity relationship of antithyroid drug molecules, possessing thiourylene moiety (NCS), based on their formation of charge transfer complexes with iodine. The value of formation constant (K_c) of the complexation with iodine has been used as a criterion for evaluation of potential antithyroid activity of such compounds (27-31). It has been suggested that compounds with a value of $K_c \geq 100 \text{ M}^{-1}$ have potential antithyroid activity (10). However, no attempt has been made to relate the antioxidant/oxidant property to skeletal atomic informations of thiourylene-type compounds as depicted by E-state indexes (32,33). The responsiveness of E-state indexes to structural changes in the molecule and their correlation with molecular orbital parameters have been proven to be useful in correlation with biological data (34-37). Thus, the application of E-state approach of Kier and Hall as a measure of atom electronic accessibility might provide a guideline for rational selection of the designed compounds.

Our objective in this study is to develop new antithyroid drugs with acyclic thiourea moiety and reduced antioxidant property to alleviate immune side effects. These objectives were undertaken in the following sequence: (i) To utilize atom level E-state indexes of thiourylene moiety as a guideline for the rational selection of compounds with reduced antioxidant property, synthesize these compounds and evaluate their antioxidant/oxidant property, utilizing their influence on luminol-enhanced chemiluminescence (CL) of stimulated polymorphonuclear leukocytes (ii) To determine the formation constant (K_c) of the synthesized agents' charge transfer complex with iodine to utilize it as a primary criteria for their potential antithyroid activity. (iii) To subject the synthesized compounds to an *in vivo* antithyroid testing, utilizing ^{125}I -thiocyanate discharge technique in rats, in order to compare their efficacy with propylthiouracil.

Materials and Methods

Materials

Propylthiouracil (Aldrich Chemical Company, Milwaukee, WI), phorbol 12-myristate 13-acetate (PMA), luminol (Sigma Chemical Company,

St. Louis, MO), nycodenz (Nygaard & co., Torshov, Norway), phosphate buffer saline (PBS) (Electronucleonics Inc., Columbia, MD), Na¹²⁵I (Radiochemical Center, Amersham, England), potassium thiocyanate and chloroform for HPLC (BDH, Poole, England), and an ultra pure iodine (Merck, Darmstadt). Compounds I-III were prepared in our laboratory. All other reagents and chemicals were of general grade and purchased from Aldrich Chemical Company (Milwaukee, WI, USA).

Instruments

Beckman Gama 4000 scintillation counting system, LKB-Wallac 125I Luminator (Wallac OY, 20101 Turku 10, Finland), DuPont TA9900 computer thermal analyzer (Wilmington, DE, USA), Varian NMR spectrometer XL200 (Sunnyvale, CA, USA), Perkin-Elmer 580B infrared spectrometer, and Perkin-Elmer 240B elemental analyzer (Norwalk, Conn., USA).

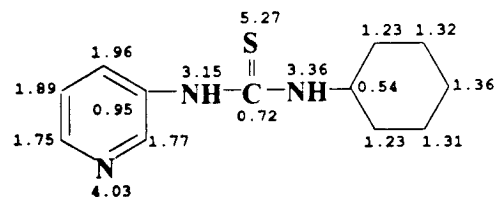
Chemistry

Compounds I, II and III (Scheme 1) were prepared by refluxing 0.0134 mole of isothiocyanate derivative with equimolar quantity of amine derivatives in dry benzene (50 ml) for 6-8 hours. After cooling, the crude product was separated as a solid powder by filtration. Finally, the crude products were washed with diethyl ether and crystallized from ethyl acetate/petroleum ether.

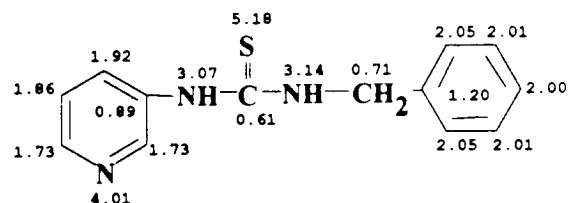
Computation of E-state indexes

The E-state index is a nonempirical description of an atom in a molecule in which both electronic and topological attributes are unified into a single index for each skeletal atom (or hydride group). The E-state value of a skeletal atom is unique and encodes electron distribution information modified by both the local and global topological environment within the molecule; thus the E-state value was considered by Kier and Hall as a measure of atom electronic accessibility (32-37). Furthermore, E-state indexes give the chance to compare the nature and/or the effect of one atom with another within a given molecule or among molecules.

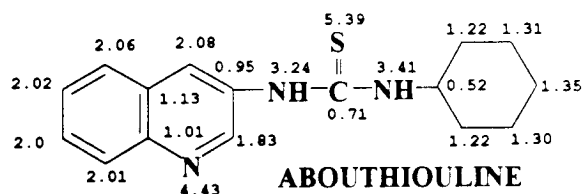
The method is based upon the hydrogen-suppressed chemical graph which represents the molecular structure. The calculations are per-



1-Cyclohexyl-3(3-pyridyl)-2-thiourea (I)



1-Benzyl-3(3-pyridyl)-2-thiourea (II)



1-Cyclohexyl-3(3-quinoly)-2-thiourea (III).

Scheme 1. The Electrotopological-state indexes. Structure and Chemical names of the investigated compounds.

formed as described by Kier and Hall (32). Each skeletal atom or group is assigned an intrinsic state value, I_i , as shown in Eq. (1):

$$I_i = [(2/N)^2 \delta^v + 1]/\delta \quad (1)$$

where δ^v and δ are the counts of valence and sigma electrons of atoms associated with the molecular skeleton. The intrinsic state value encodes the valence state of electronegativity of the atom as well as its local topology through the use of the molecular connectivity simple and valence delta value, δ and δ^v . The ratio of the squares of the principal quantum number relative to the second ($N = 2$) was used as a modifier of the δ^v value in Eq 1.

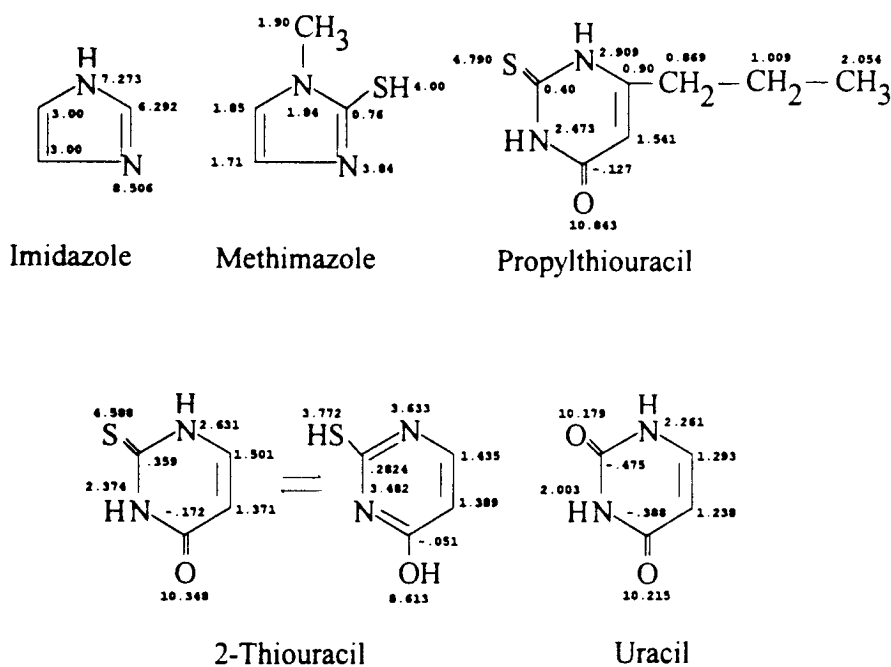
The E-state index of skeletal atom i , S_i , is defined as the sum of the intrinsic value and the perturbations due to the global topological environment within the molecule as described by Eq. (2):

$$S_i = I_i + \Delta I_i \quad (2)$$

where ΔI_i is the total perturbation of I_i . The perturbation on skeletal atom i , arising from the presence of all other atoms j , was estimated as the difference between the intrinsic atoms; $I_i - I_j$. Since the perturbation is diminishing over graph separation; the functional dependence of the total perturbation was quantified using the inverse-square law of the count of atoms in the shortest path (graph separation) between atoms i and j (r_{ij}). The total perturbations are summed over the whole molecule as shown in Eq. (3):

$$\Delta I_i = \sum_{j=1}^N (I_i - I_j) / r_{ij}^2 \quad (3)$$

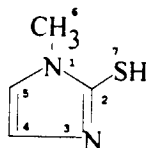
A computation example is illustrated in Table 1. Table 1 shows the diagonal matrix of the field influence between all skeletal atoms within the molecule of methimazole. All computations were performed using ABOUFAC-ET program.* Scheme 2 shows the E-state indexes of the currently used antithyroid drugs and related agents. E-state indexes of thiourylene moiety ($S_{N\&S}$) of the compounds were calculated from the average of the E-state value of the two skeletal nitrogen atoms ($>NH$ or $-N=$) plus the E-state value of ($S=$ or $-SH$) depending on the presence of the tautomeric structures. In case of tautomeric equilibrium, the average value of $S_{N\&S}$ of the two tautomeric forms (as in 2-thiouracil) was taken.



Scheme 2. Structure of antithyroid drugs, related agents and their Electrotopological-state indexes.

* The program is associated with a database of several hundred structures where structure modification influence on S_i can be observed directly on the screen with minimum manipulations.

Table 1. The Electrotopological-State of Methimazole.



Atom No. (I _i)	Intrinsic values ^d	i	j							ΔI _i ^b	S _i ^c	
			1	2	3	4	5	6	7			
I(1)	2.00	1	0.000	0.083	-0.111	0.000	0.000	0.000	0.000	-0.136	-0.164	1.836
I(2)	1.67	2	-0.083	0.000	-0.333	-0.037	-0.037	-0.037	-0.037	-0.388	-0.913	0.757
I(3)	3.00	3	0.111	0.333	0.000	0.250	0.111	0.063	0.063	-0.025	0.843	3.843
I(4)	2.00	4	0.000	0.037	-0.250	0.000	0.000	0.000	0.000	-0.076	-0.290	1.710
I(5)	2.00	5	0.000	0.037	-0.111	0.000	0.000	0.000	0.000	-0.076	-0.151	1.849
I(6)	2.00	6	0.000	0.037	-0.063	0.000	0.000	0.000	0.000	-0.076	-0.102	1.898
I(7)	3.22	7	0.136	0.388	0.025	0.076	0.076	0.076	0.076	0.000	0.778	4.000
											0.00	

^a Values in matrix were calculated according to $(I_i - I_j) / r_{ij}^2$

^c Values in column were calculated according to eq. 2

^b Values in column were calculated according to eq. 3.

^d Values in column were calculated according to eq. 1.

Isolation of Polymorphonuclear Leucocytes (PMNLs)

PMNLs were isolated by using Nycodenz solution. Blood was collected by venopuncture from apparently normal human donors in sterile 5ml containers with heparin (10 IU). The heparinized blood was mixed with 1ml of 6% (w/v) Dextran T 500 in saline and incubated for 30 minutes at room temperature. The leukocyte-rich plasma layer was separated and mixed with 3 ml Nycodenz solution before centrifugation at 400 g for 15 minutes. The PMNL-rich portion at the bottom was then suspended in 10 ml of PBS. After centrifugation, the pellet was resuspended in PBS diluted with water (1:1) to lyse the erythrocytes. The cells were then centrifuged and the pellet was resuspended in normal PBS medium and the PMNLs were counted.

Effect of Teste Agents on Luminol-enhanced CL of Stimulated PMNLs:

An LKB-Wallac ¹²⁵I Luminator, was used to determine the CL of the stimulated PMNLs as described by Tono-oka *et al.* (38). This instrument was connected to an Apple IIe computer and was devised to have a constant temperature (37°C)

controller. The principle of oxidation of luminol, 5-amino-2,3-dehydrol-1,4-phthalazinedione, by reactive oxygen species, produced during phagocytosis in PMNLs, was applied to increase the amount of measurable light (39) in the Luminol-dependent CL measurements. To 10 μL of each of the compounds (in DMSO) of variable concentration; 0.9ml PBS medium containing 10⁻³ M luminol and 2 μg of PMA was added. After shaking, 0.1ml of isolated PMNLs suspension was added to the reaction mixture. A control was included in each experiment containing the solvent used in the experiment. The resultant light output in mV was continuously recorded on LKB (Wallac) chart recorder. The readings were recorded at one minute intervals for 14 minutes and the light output was plotted against time. The percentage CL response was calculated from the integrated area under the curve of the CL curve of the tested compounds [(AUC-CL)_i] and that of control [(AUC-CL)_c] according to Eq. 4:

$$\% \text{ CL-Response} = \frac{(AUC-CL)_i - (AUC-CL)_c}{(AUC-CL)_c} \times 100 \quad (4)$$

where the value of Eq. 4 indicates stimulation if positive and indicates inhibition if negative. Stimulation (+), represents the magnitude of oxidant property and inhibition (-), represents the magnitude of antioxidant property.

The results were computed from the CL peak response (mV) and the integral area under the curve of each graph by the LKB computer program. Differences between experiments and control were evaluated with a two-tailed Student's t-test.

Effect of the tested agents on the viability of PMNLs

The effect of the compounds I-III 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 (0.2% w/v) exclusion test (39).

Charge transfer complexation between compounds I,II and III with iodine

Solutions of the investigated compounds in chloroform were made up by accurate dilution of stock solutions prepared gravimetrically. All the reaction mixtures contained fixed concentration of iodine ($4.498 \times 10^{-4} \text{M}$) while varying concentrations of compounds I-III. All solutions were freshly prepared before running each experiment. The absorbances of the solutions were recorded immediately, after iodine mixing, with a Shimodzu UV-160 UV/Vis spectrophotometer at $21 \pm 0.1^\circ \text{C}$ throughout the measurements. Formation constant (K_c) and molar extinction coefficients ($\epsilon_{\lambda}^{\text{AD}}$) were calculated from Benesi-Hildebrand equation (Eq. (5)) as described by Borazan and Ajeena (40).

$$[A_0]/E_{\lambda}^{\text{AD}} = 1/\epsilon_{\lambda}^{\text{AD}} + [1/(K_c^{\text{AD}} \cdot \epsilon_{\lambda}^{\text{AD}} (D_0))] \quad (5)$$

where (A_0) and (D_0) represent the total concentrations of the reactants, iodine and the investigated compounds, respectively. E_{λ}^{A} and $\epsilon_{\lambda}^{\text{A}}$ are the absorbance and the molar extinction coefficient of the complex at the wavelength λ , respectively.

Animal stocks

Normal out-bred Wistar albino rats (220-250gm) and Swiss albino male mice (25-30 gm)

were provided by the Experimental Animal Care Center, College of Pharmacy, King Saud University. The animals were maintained under conditions of controlled temperature, humidity and lighting with free access to food and water. Animals were randomly assigned to the different control and treated groups.

Acute Toxicity

The acute lethality (LD_{50}) of the compounds was determined using male Swiss Albino mice (25-30 gm) after i.p. dosing. The mice were divided into groups of 10 mice each. Drugs were suspended in 15% glycerol solution in 2% cremophore-el in sterile saline using ultrasonic bath. Increasing doses of test compounds were given i.p. at increments of 10, 50, 100 or 200 mg/kg, and then death in each group was scored after 48 h. The mean mortality was calculated and multiplied by the dose increments used. A special formula of Karber as cited in Ghosh (41) was then used to calculate the median lethal dose (LD_{50}) of each compound.

Antithyroid Testing

The antithyroid activity of these compounds was tested and compared to that of propylthiouracil using ^{125}I -thiocyanate discharge technique (42-43). The compounds I-III and propylthiouracil were dissolved (0.00143 moles/100 ml) in dimethylsulfoxide (DMSO). One hundred and fifty adult Wistar albino rats were divided into five groups (30 rats each). The test was performed by administering each of the 3 compounds i.p (0.1 ml) into 3 groups of rats for 30 days in equimolar doses (The total dose is $42.87 \mu\text{moles}$ for each rat / 30 days). A control group of rats received daily intraperitoneal dose of 0.1 ml of DMSO per rat. The normal group of rats was left in a separate cage untreated.

At the end of the 30 day treatment, the test was performed by i.p. administration of a trace dose ($\sim 353460 \text{ cpm}$) of carrier-free Na^{125}I per rat (time of injection was considered as zero time). The same calculated dose ($\sim 353460 \text{ cpm}$) was kept aside to be counted as a standard. Each group of rats was, then, divided into six subgroups of 5 rats each. Rats in subgroup one, two and three were killed at 10, 20 and 30 minutes respectively. The thyroid gland was rapidly removed, and the

where the value of Eq. 4 indicates stimulation if positive and indicates inhibition if negative. Stimulation (+), represents the magnitude of oxidant property and inhibition (-), represents the magnitude of antioxidant property.

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Solutions of the investigated compounds in chloroform were made up by accurate dilution of stock solutions prepared gravimetrically. All the reaction mixtures contained fixed concentration of iodine ($4.498 \times 10^{-4} \text{M}$) while varying concentrations of compounds I-III. All solutions were freshly prepared before running each experiment. The absorbances of the solutions were recorded immediately, after iodine mixing, with a Shimodzu UV-160 UV/Vis spectrophotometer at $21 \pm 0.1^\circ \text{C}$ throughout the measurements. Formation constant (K_c) and molar extinction coefficients ($\epsilon_{\lambda}^{\text{AD}}$) were calculated from Benesi-Hildebrand equation (Eq. (5)) as described by Borazan and Ajeena (40).

$$[A_o]/E_{\lambda}^{\text{AD}} = 1/\epsilon_{\lambda}^{\text{AD}} + [1/(K_c^{\text{AD}} \cdot \epsilon_{\lambda}^{\text{AD}} (D_o))] \quad (5)$$

where (A_o) and (D_o) represent the total concentrations of the reactants, iodine and the investigated compounds, respectively. E_{λ}^{AD} and $\epsilon_{\lambda}^{\text{AD}}$ are the absorbance and the molar extinction coefficient of the complex at the wavelength λ , respectively.

Animal stocks

Normal out-bred Wistar albino rats (220-250gm) and Swiss albino male mice (25-30 gm)

were provided by the Experimental Animal Care Center, College of Pharmacy, King Saud University. The animals were maintained under conditions of controlled temperature, humidity and lighting with free access to food and water. Animals were randomly assigned to the different control and treated groups.

Acute Toxicity

The acute lethality (LD_{50}) of the compounds was determined using male Swiss Albino mice (25-30 gm) after i.p. dosing. The mice were divided into groups of 10 mice each. Drugs were suspended in 15% glycerol solution in 2% cremophore-el in sterile saline using ultrasonic bath. Increasing doses of test compounds were given i.p. at increments of 10, 50, 100 or 200 mg/kg, and then death in each group was scored after 48 h. The mean mortality was calculated and multiplied by the dose increments used. A special formula of Karber as cited in Ghosh (41) was then used to calculate the median lethal dose (LD_{50}) of each compound.

Antithyroid Testing

The antithyroid activity of these compounds was tested and compared to that of propylthiouracil using ^{125}I -thiocyanate discharge technique (42-43). The compounds I-III and propylthiouracil were dissolved (0.00143 moles/100 ml) in dimethylsulfoxide (DMSO). One hundred and fifty adult Wistar albino rats were divided into five groups (30 rats each). The test was performed by administering each of the 3 compounds i.p (0.1 ml) into 3 groups of rats for 30 days in equimolar doses (The total dose is $42.87 \mu\text{moles}$ for each rat / 30 days). A control group of rats received daily intraperitoneal dose of 0.1 ml of DMSO per rat. The normal group of rats was left in a separate cage untreated.

At the end of the 30 day treatment, the test was performed by i.p. administration of a trace dose ($\sim 353460 \text{ cpm}$) of carrier-free Na^{125}I per rat (time of injection was considered as zero time). The same calculated dose ($\sim 353460 \text{ cpm}$) was kept aside to be counted as a standard. Each group of rats was, then, divided into six subgroups of 5 rats each. Rats in subgroup one, two and three were killed at 10, 20 and 30 minutes respectively. The thyroid gland was rapidly removed, and the

radioactivity was measured using a Beckman Gamma 4000 Scintillation Counting system connected to a Texas Instrument Silent 700 printer. After 45 minutes a solution of 15 mg potassium thiocyanate in 0.9% saline was administered i.p. to each of the remaining rats in the three subgroups. The thyroid uptake was again determined at 60, 90 and 120 minutes of each isolated thyroid gland in subgroups four, five and six respectively.

The extent of radioiodine thyroid uptake and its discharge following the administration of 15 mg per rat of KSCN is a measure of the thyroid inhibition effect (37-38). The total area under the curve (AUC) of radioiodine uptake and discharge was calculated up to 120 minutes after ^{125}I administration to each rat in the group used, for all compounds. In addition, the AUC of the ^{125}I -uptake phase and the rate of ^{125}I -discharge were calculated. The comparative % efficacies of compounds I-III with respect to both ^{125}I -uptake and the rate of ^{125}I -discharge in reference to rats treated with propylthiouracil and that of the DMSO control, were calculated according to Eq. 6.

$$\% \text{ Efficacy} = \frac{(\text{PAR})_t - (\text{PAR})_c}{(\text{PAR})_{\text{ptu}} - (\text{PAR})_c} \times 100 \quad (6)$$

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

Statistical analysis

The different parameters studied, iodine uptake, CL maximum peak response (mV) and CL area under the curve were subjected to statistical analysis with Student's t-test.

Results

E-State indexes and selection of compounds:

In order to prepare antithyroid compounds with acyclic thiourea and reduced antioxidant property, E-state indexes (32) of both sulphur and nitrogen skeletal atoms of the thiourylene moiety of N,N-alkylthiourea were utilized as a guideline for the rational selection of these compounds. To set

this guideline, literature data was utilized. The literature search revealed that Imamura *et al.*, (44) studied the inhibitory effect of propylthiouracil, methimazole, 2-thiouracil, imidazole and uracil on PMA-induced chemiluminescence in human neutrophil after 100 $\mu\text{g}/\text{ml}$ and found that propylthiouracil, 2-thiouracil, and methimazole cause CL inhibition with different magnitudes. However, uracil and imidazole did not show any CL inhibitory influence. The peak %CL inhibitory effects on PMA-induced chemiluminescence response with respect to the untreated control were 75.6, 67.7 and 53.6 for 2-thiouracil, propylthiouracil, and methimazole, respectively (44). The analysis of this data with respect to the atom level E-state indexes of sulphur and nitrogen of the thiourylene moiety ($S_{\text{N\&S}}$) reveals the following:

1. The lower inhibitory effect of propylthiouracil ($S_{\text{N\&S}} = 7.48$) as compared to 2-thiouracil ($S_{\text{N\&S}} = 7.254$) is due to the influence of 6-n-propyl moiety on the E-state of sulphur and nitrogen (Scheme 2).
2. The percent CL-Inhibition of methimazole ($S_{\text{N\&S}} = 7.84$) is lower than propylthiouracil, thus it has less antioxidant property.
3. There was a linear relationship between the observed %CL inhibition effect of antithyroid agents on human PMNLs and the E-state of the thiourylene moiety ($S_{\text{N\&S}}$) of these agents as shown in Eq.7 and Fig.1.

$$\% \text{ CL-Inh} = 349.254 (\pm 8.5) - 37.7 (\pm 1.1) (S_{\text{N\&S}}) \quad (7)$$

$[r = 0.999; n = 3; \text{SEM} = 0.47; F = 1108; P = 0.019]$

It is evident that the value of the E-state of thiourylene moiety is related to the antioxidant magnitude of the compounds. The higher the $S_{\text{N\&S}}$ value of thiourylene-type compounds the lower their antioxidant property.

4. Replacement of sulphur in 2-thiouracil ($S_{\text{N\&S}} = 7.254$) by oxygen (as in uracil ($S_{\text{N\&O}} = 10.41$)) and the loss of sulphur from methimazole (as in imidazole) eliminate the CL inhibitory effect on human PMNLs. This suggests that the value of the E-state indexes of atoms in a compound is related to the magnitude of the oxidant/antioxidant property.

On the basis of this analysis, the influence of the different cycloalkyl, aralkyl and aryl moieties

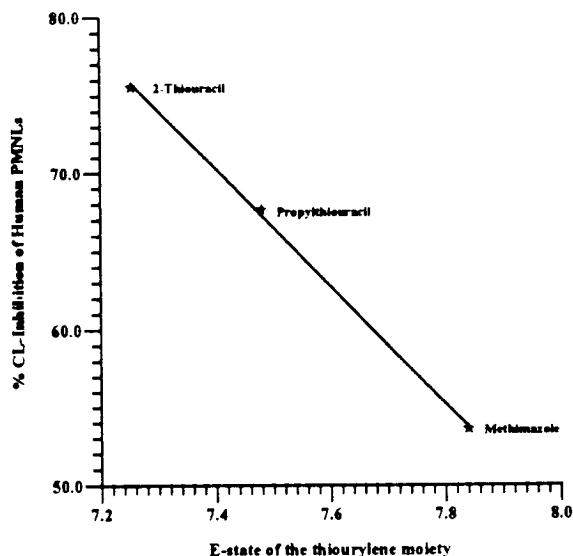


Figure 1. Inhibitory effect of antithyroid agents on PMA-induced chemiluminescence in human neutrophils in relation to their E-state indexes of thiourylene moiety ($S_{N\&S}$). Percent CL-inhibition values were obtained from Imamura et. al. (44) as the % of the peak values of PMA-induced CL response, on human neutrophils, of that of the untreated control.

(Benzyl, 3-pyridyl, cyclohexyl and 3-quinolyl) on the E-state of both sulphur and nitrogen skeletal atoms of thiourylene moiety was calculated to select the compounds to be synthesized. The compounds were selected to have $S_{N\&S}$ value more than 8 as shown in Scheme 1. The selected compounds are synthesized by direct reaction of isothiocyanate derivatives with equimolar quantity of the appropriate amine. Characterization of the prepared N,N-alkylthiourea derivatives (Table 2) was performed using IR and NMR spectrophotometry. The IR and ^1H NMR spectra of the three compounds were in agreement with the proposed structure. Further identification was performed by using the elemental analysis.

Effect of the tested compounds on luminol-dependent CL in human PMNLs stimulated with PMA

The Effects of the investigated compounds on the luminol-dependent CL of human PMNLs (1×10^5 cells/ml), in reference to untreated control and propylthiouracil at $5 \mu\text{g/ml}$, are shown in Fig.2. The degree of inhibition of the maximum CL peak response of propylthiouracil is the high-

Table 2. Physical and Analytical Data of the Prepared Thiourea Derivatives.

Compound	Yield %	Melting Point ^a °C	Chemical Formula	Element	Analysis %		IR ^b	
					Calc.	Found	ν (N-H)	ν (C=S)
I	66	154.45 ± 0.05	$\text{C}_{12}\text{H}_{17}\text{N}_3\text{S}$	C	61.28	61.53	3345	1515
				H	7.23	6.85		
				N	17.87	18.15		
II	78	135.6 ± 3.2	$\text{C}_{13}\text{H}_{13}\text{N}_3\text{S}$	C	64.2	63.8	3230	1535
				H	5.35	5.47		
				N	17.28	16.89		
Abouthiouline (III)	51	173.9 ± 0.6	$\text{C}_{16}\text{H}_{19}\text{N}_3\text{S}$	C	67.37	67.05	3130	1540
				H	6.67	6.3		
				N	14.74	14.34		
NMR Characterisation ^c								
					Cyclohexyl H (11 H,m)		NH (1 H,s)	
							Other H	
I						1.13–2.03	6.37 8.64	7.4–8.4 (4H,m)
II						–	9.71	7.34–8.6 (4H,m) 8.4 (1 H,s,CH ₂)
Abouthiouline (III)						1.23–2.14	6.6	7.25–8.8 (6H,m)

^a Mean of 3–4 runs \pm SD.

^b KBr pellets.

^c Solvent, DMSO- d_6 ; Internal standard, tetramethylsilane.

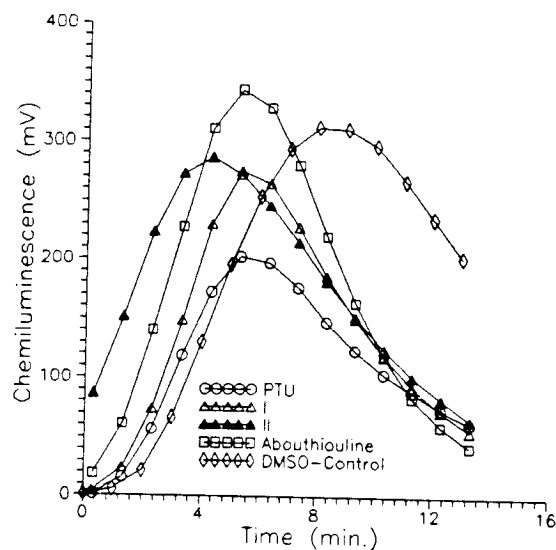


Figure 2. Effect of the investigated compounds on luminol-dependent chemiluminescence response of PMA-induced human PMNLs (1×10^5 cells/ml), in reference to DMSO-control and propylthiouracil (PTU) at $5 \mu\text{g/ml}$.

est and that of compound III (abouthiouline) is the lowest.

The integrated areas under the CL curves (AUC) produced for the different tested agents and different concentrations are summarized in Table 3. Propylthiouracil was found to inhibit the PMNLs "respiratory burst" at all doses ($5\text{-}40 \mu\text{g/ml}$), whereas compounds I, II and abouthiouline slightly inhibited the CL production at the lower doses only ($5\text{-}20 \mu\text{g/ml}$). However the intensity of inhibition was higher in compounds II and I as compared to abouthiouline. In addition, abouthiouline caused significant stimulation of "respiratory burst" at the highest dose level ($40 \mu\text{g/ml}$).

The percentage of CL-stimulation (+) and the CL-inhibition (-) calculated according to Eq. (5), represents the magnitude of oxidant and antioxidant property, respectively. The percentage inhibition of CL by propylthiouracil was dose-dependent and varies between 41.71% and 74.99% for the dose levels of $5\text{-}40 \mu\text{g/ml}$ (Figure 3). The minimum CL inhibitions at $5 \mu\text{g/ml}$ for compounds I and II were 7.32% and 26.81%, respectively and the maximum CL inhibitions at $40 \mu\text{g/ml}$ for compound I and II were 24.07% and 39.66%, respectively. On the other hand, the percent inhibition of CL after treatment with

abouthiouline varied between 14.9 and 2.34 for the dose levels in the range of $5\text{-}20 \mu\text{g/ml}$ which is almost close to the untreated control. Furthermore at higher dose ($40 \mu\text{g/ml}$), abouthiouline stimulates the PMNLs "respiratory burst" by 19.24%.

The antioxidant and oxidant activity of the investigated compounds as depicted by their effect on luminol-dependent CL response of PMA-induced human PMNLs, in relation to the E-state of thiourylene moiety ($S_{N\&S}$) are also shown in Fig.3.

Effect of the tested compounds on the viability of PMNLs

The results on the viability of isolated human PMNLs after treatment with propylthiouracil, compound I, II and abouthiouline are presented in Table 4. Incubation of all tested compounds with human PMNLs for 10 and 30 min at 37°C and for varying concentrations, as judged by trypan blue exclusion test, did not have any significant effect on human PMNLs viability.

Charge transfer complexation with iodine as a preliminary criterion for antithyroid activity

The formation constant of the charge transfer complexes with iodine have been used as a criterion for the preliminary evaluation of their potential antithyroid activity. Absorption spectroscopy was used in the study of such complexes. The spectroscopic results in Figures 4, 5 and 6A show the visible spectra of the three investigated compounds at concentration ranging from $2.0 \times 10^{-5} \text{ M}$ to $2.16 \times 10^{-4} \text{ M}$. The compounds displayed no absorbance between 600 nm and 350 nm. The addition of a solution of iodine to a solution of the investigated compound in the same solvent (chloroform) shifted the visible region of the iodine band (513 nm) towards shorter wavelengths. This was attributed to the formation of iodine charge transfer complex. The intensity of this new band was found to increase with increasing compound concentrations at any given concentration of iodine. The spectra crossed at the isobestic points of 469 nm, 477 nm and 474 nm for compounds I, II and abouthiouline, respectively. The absorption peak of the complex was measured by placing a solution of iodine at the same concentration in the reference beam. The

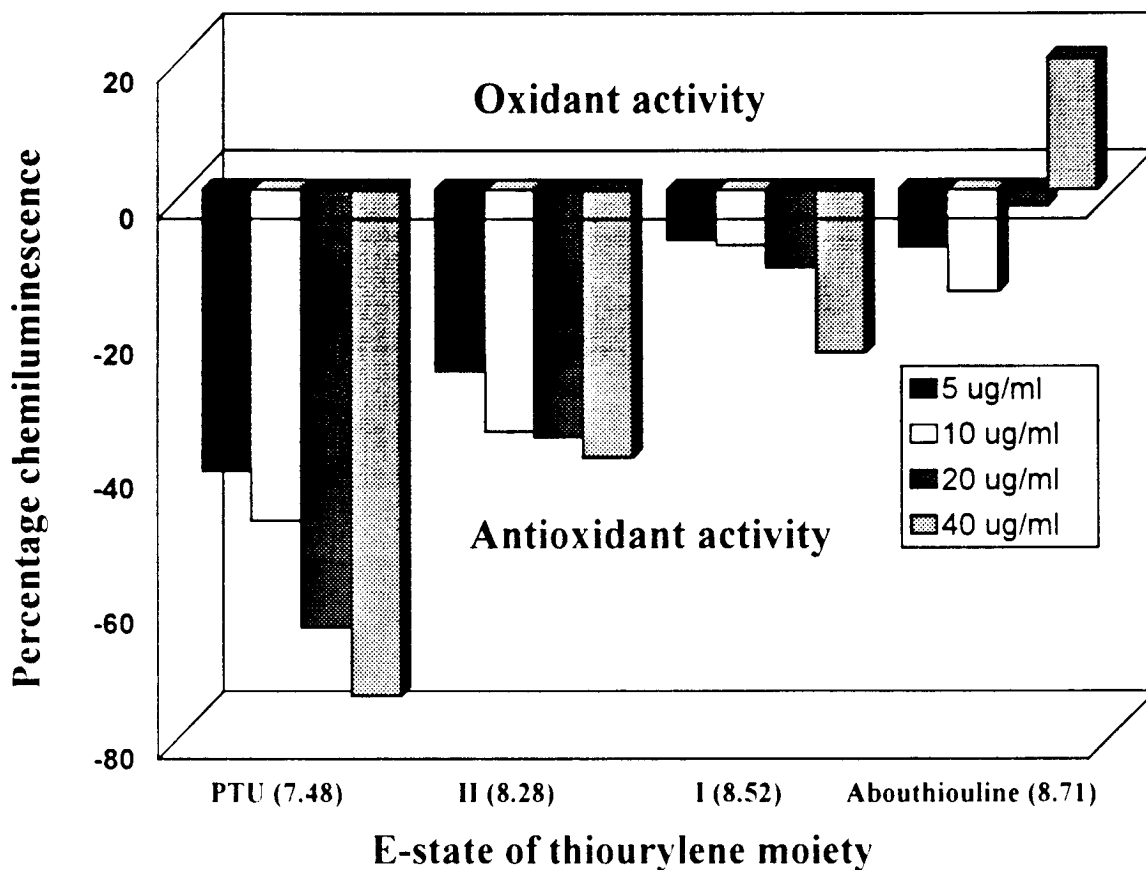


Figure 3. Antioxidant and oxidant activity of the investigated compounds as determined by their effect on percentage inhibition (-) or stimulation (+) of the PMA-induced chemiluminescence. Percentage CL was calculated according to Eq. (4).

Table 3. Effect of the Investigated Compounds on the Area Under the Curve of the Chemiluminescence of PMA-Stimulated Human Polymorphonuclear Leukocytes (1×10^5 Cells/ml).

	Chemiluminescence Area Under the Curve \pm SD* (min. mV)			
	PTU	I	II	Abouthiouline (III)
Control	2590.5 ± 131	2590.5 ± 131	2590.5 ± 131	2590.5 ± 131
5 μ g/ml	1510 ± 76	2401 ± 97	1896 ± 94	2373 ± 70
10 μ g/ml	1321 ± 80	2382 ± 118	1663 ± 62	2204 ± 65
20 μ g/ml	911 ± 46	2296 ± 68	1638 ± 48	2530 ± 77
40 μ g/ml	648 ± 52	1967 ± 102	1563 ± 78	3089 ± 185

*Area under the curve from 0 – 14 min. (Mean \pm Standard deviation); n = 2 – 6 runs.

Table 4. Effect of Various Concentrations of the Investigated Agents on the Viability of Isolated Human Polymorphonuclear Leukocytes (PMNLs= 5×10^6 cells/ml) in PRMI Media (w/o NaHCO_3)* Incubated for 10 or 30 minutes; with Reference to Propylthiouracil (PTU).

Drug ($\mu\text{g/ml}$)	PTU*		I*		II*		Abouthioulone*	
	%S	%I	%S	%I	%S	%I	%S	%I
Control (1% DMSO)	96.43 ($\pm .73$) ^a	-	96.43 ($\pm .73$) ^a	-	96.43 ($\pm .73$) ^a	-	96.43 ($\pm .73$) ^a	-
40	94	2.52	95	1.48	94	2.52	94	2.52
20	93	3.56	95	1.48	94	2.52	91	5.63
10	93	3.56	95	1.48	95	1.48	92	4.59
5	94	2.52	96	0.45	94	2.52	93	3.56

Drug ($\mu\text{g/ml}$)	PTU**		I**		II**		Abouthioulone**	
	%S	%I	%S	%I	%S	%I	%S	%I
Control (1% DMSO)	96.86 ($\pm .35$) ^a	-	96.86 ($\pm .35$) ^a	-	96.86 ($\pm .35$) ^a	-	96.86 ($\pm .35$) ^a	-
40	96	0.89	96	0.89	95	1.92	93	3.99
20	95	1.92	95	1.92	96	0.89	91	6.05
10	95	1.92	96	0.89	95	1.92	91	6.05
5	95	1.92	96	0.89	96	0.89	92	5.02

* Incubated for 10 min. at 37°C and the percentage of viable cells was estimated by the trypan (0.2m/w) exclusion test. ** Incubated for 30 min. at 37°C and the percentage of viable cells was estimated by the trypan blue (0.2m/w) exclusion test.

a = Results were expressed as mean \pm SEM of 7 experiments. S = Survival of PMNLs, I = Inhibition of PMNLs.

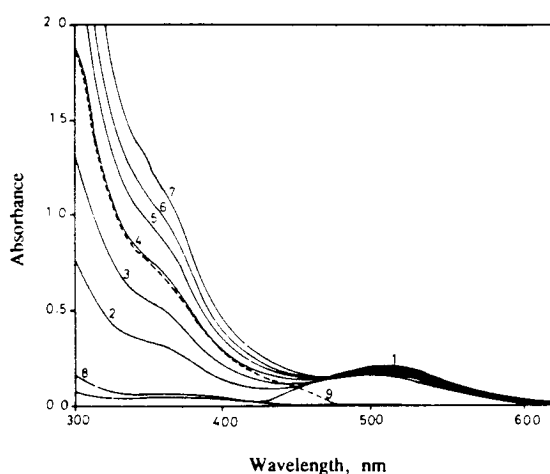


Figure 4. Visible spectra of compound I-iodine charge transfer complex in chloroform at $21.0 \pm 0.1^\circ\text{C}$. Key: Iodine concentrations in solution 1-7 was constant at 4.498×10^{-4} M. Molar concentration of compound 1 were (1) 0.0 M; (2) 3.595×10^{-5} M; (3) 7.19×10^{-5} M; (4) 1.078×10^{-4} M; (5) 1.438×10^{-4} M; (6) 1.797×10^{-4} M; (7) 2.157×10^{-4} M; (8) 1.078×10^{-4} M and (9) Absorbance curve of complex obtained for solution 4 by placing iodine solution (4.498×10^{-4} M) in the reference beam.

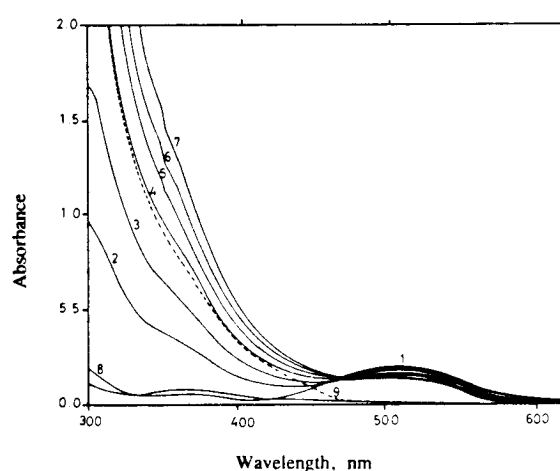


Figure 5. Visible spectra of compound II-iodine charge transfer complex in chloroform at $21.0 \pm 0.1^\circ\text{C}$. Key: Iodine concentration in solution 1-7 was constant at 4.498×10^{-4} M. Molar concentrations of compound II were (1) 0.0 M; (2) 4.225×10^{-5} M; (3) 8.449×10^{-5} M; (4) 1.267×10^{-4} M; (5) 1.69×10^{-4} M; (6) 2.112×10^{-4} M; (7) 2.535×10^{-4} M; (8) 8.449×10^{-5} M and (9) Absorbance curve of complex obtained for solution 4 by placing iodine solution (4.498×10^{-4} M) of iodine solution in the reference beam.

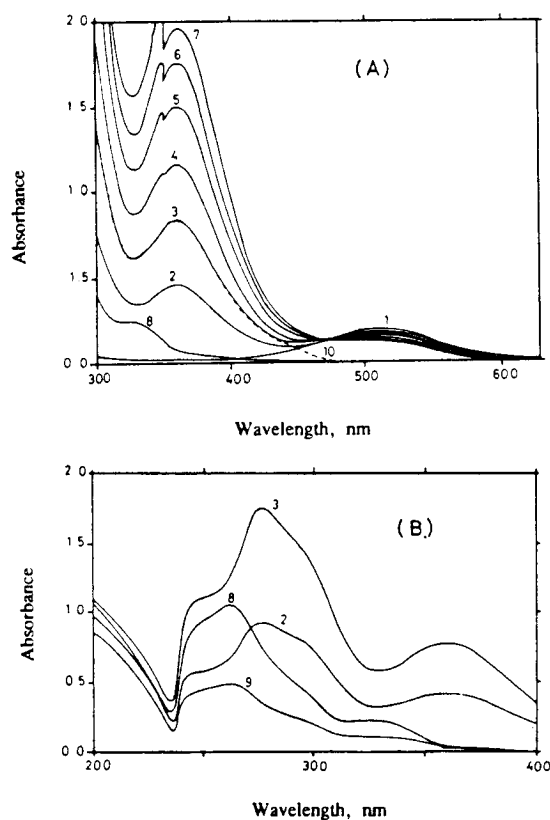


Figure 6. Absorption spectra of aboutthiouline-iodine charge transfer complex in chloroform at $21.0 \pm 0.1^\circ\text{C}$ for (A) visible band (B) ultraviolet band. Key: Iodine concentration in solutions 1-7 was constant at 4.498×10^{-4} M. Molar concentrations of aboutthiouline were (1) 0.0 M; (2) 2.032×10^{-5} M; (3) 4.064×10^{-5} M; (4) 6.097×10^{-5} M; (5) 8.129×10^{-5} M; (6) 1.016×10^{-4} M; (7) 1.219×10^{-4} M; (8) 4.064×10^{-5} M of aboutthiouline; (9) 2.032×10^{-5} M and (10) Absorbance curve of complex obtained for solution 3 by placing iodine solution (4.498×10^{-4} M) in the reference beam.

various spectral parameters of both the pure compounds and their iodine complexes in chloroform at $21.0 \pm 0.1^\circ\text{C}$ are summarized in Table 5.

In the ultraviolet region, The appearance of absorption bands between 290nm and 360 nm indicate the formation of I_3^- and cause the shift of the peak to a higher wavelength as shown in Fig. 6B. The formation of aboutthiouline-iodine complex cause the shift in λ_{max} of the pure compound (262 nm) to a higher wavelength (276 nm) and the absorbance was also increased.

For the charge transfer complexes of the three investigated compounds, 1:1 stoichiometry was confirmed by method of continuous mole fraction variation (Job's method)(45) as shown in Fig.7. The position of the maximum and the perfect symmetry of the curves confirm the presence of a complex with 1:1 stoichiometry and exclude the presence of high-order complexes. In addition the presence of a single isobestic point (Table 5) and the exact fit of the experimental points to a linear regression of Eq.(5) (Fig.8), also confirms the 1:1 complex formation.

Equation 5 is based on the assumption that 1:1 complex is formed and the complexation take place via a reversible reaction between the reactants and the complex. Figure 8 shows sample representations of the three investigated compounds to Eq.5, where plots of $[A_0/E_\lambda^{\text{AD}}]$ versus $[1/D_0]$ were used to calculate the formation constant ($K_c \text{ M}^{-1}$) and the absorption coefficients ($\epsilon_\lambda^{\text{AD}} \text{ M}^{-1} \text{ cm}^{-1}$) of the three donor-iodine complexes from the slope and the intercept of the corresponding plot. Iodine concentration (A_0) was kept constant

Table 5. Spectral parameters of the pure investigated compounds, their iodine charge transfer complexes and the formation constants (K_c) in chloroform.

Compounds	Pure Compounds		Complexes with iodine at $21 \pm ^\circ\text{C}$			
	λ_{max} (nm)	$\epsilon_\lambda^{\text{D}}$ ($\text{M}^{-1} \text{ cm}^{-1}$)	isobestic point (nm)	λ_{max} (nm)	$\epsilon_\lambda^{\text{AD}}$ ($\text{M}^{-1} \text{ cm}^{-1}$)	K_c^* (M^{-1})
I	258	11381.1 (± 307.3)	469	375.5	7243.1 (± 96.0)	3125.1 (± 124.6)
II	255	15093 (± 818.3)	477	375.5	3031.7 (± 221.4)	4721.5 (± 64.7)
Aboutthiouline (III)	262	26357.6 (± 718.5)	474	361.5	12969.1 (± 276.1)	4047.4 (± 57.3)

*Mean of 6 runs \pm SD

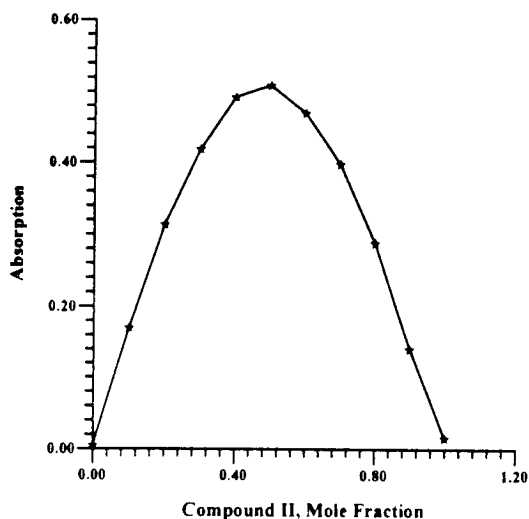


Figure 7. Determination of stoichiometry of compound II-iodine complex ($\lambda_{max} = 357.5 \text{ nm}$) by method of continuous variation (45) (in chloroform and at $21.0 \pm 0.1^\circ\text{C}$). For each complex solution, the sum of compound II and iodine concentrations was constant and equal to $4.498 \times 10^{-4} \text{ M}$.

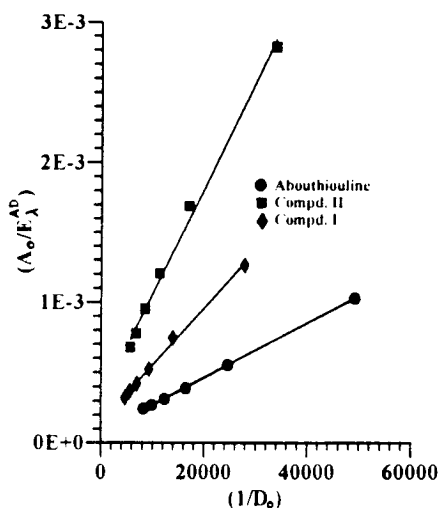


Figure 8. Plots of (A_0/E_λ^{AD}) versus $(1/D_0)$ of the charge transfer complexes of the investigated compounds (D_0) and iodine (A_0). The molar concentration of (A_0) was $4.498 \times 10^{-4} \text{ M}$ for all reaction mixtures and the concentration of the investigated compounds was $2.032 \times 10^{-5} \text{ M}$ to $2.585 \times 10^{-4} \text{ M}$. E_λ^{AD} represents the absorbance of the complex at the λ wave length.

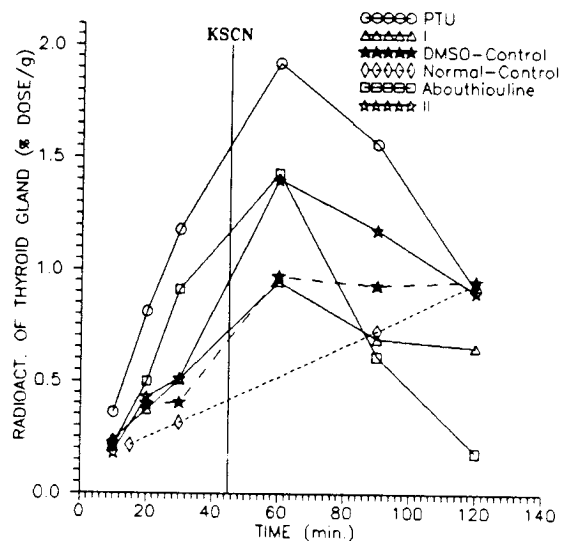


Figure 9. Radioactivity of thyroid gland ($\% \text{ }^{125}\text{I}$ dose/g) in rats treated with the investigated compounds in reference to propylthiouracil. Equimolar doses of all compounds were injected i.p. daily for 30 days before Na^{125}I administration to rats. Each point is an average of 5 runs. At 45 minutes, all remaining rats were injected i.p. with KSCN [SEM is included in the calculated area under the curve (Table 6)].

($4.498 \times 10^{-4} \text{ M}$) for all reaction mixtures. This is consistent with the assumption made in deriving Eq. 5. The formation constants of the investigated compounds with iodine were 3125.1 M^{-1} , 4721.5 M^{-1} and 4047.4 M^{-1} for compounds I, II and abouthioulone respectively.

Toxicity

The acute lethality of the compounds I, II and abouthioulone were determined in male Swiss albino mice after i.p. dosing. The approximate median lethal dose (LD_{50}) of the compounds were 510, 570 and $> 800 \text{ mg/kg}$, respectively. Abouthioulone has higher LD_{50} than the reference compound; propylthiouracil ($\text{LD}_{50} 580 \text{ mg/kg}$). Thus, higher doses of abouthioulone can be administered safely to produce a comparable efficacy to propylthiouracil. It should be noted that the equimolar dose ($1.429 \mu\text{mole/day}$) in rat (200 gm.) corresponds^a to human dose (70 kg man) of

^a Rat (200 gm) to man (70 kg) dose conversion was done according to the surface area ratio (= 56). Value stated = $[(\text{Dose in rat (mg/kg)} / 5) \times 56]$.

11 and 18.3 mg/day of propylthiouracil and abouthiouline respectively.

Antithyroid Activity

The observed radioactivity of the thyroid gland (% ^{125}I dose/g) in rats after treatment with equimolar doses of the tested compounds is shown in Figure 9. The ^{125}I -thyroid uptake in normal control occurred at a constant rate of the entire 2 hr period of the experiment (7.432×10^{-3} (%dose/g)/min, $r = 0.994$, $n = 4$ [4 groups using mean value of 5 rats in each group]). Whereas the ^{125}I -uptake in case of DMSO-control upto 60 min. (^{125}I -uptake phase) was found to be followed by a constant rate of 3.66×10^{-4} (%dose/g)/min ($r = 0.87$, $n = 3$). Furthermore, the curves of the test compounds were found to be biphasic, while the curves of both the normal control (untreated) and the DMSO-control were not. The ^{125}I -uptake phase occurred upto 60 min. and the ^{125}I -discharge phase was between 60-120 min. Table 6 shows the total AUC of ^{125}I -uptake and rate of ^{125}I -discharge calculated after ^{125}I administration to the different groups used for compounds I, II and abouthiouline compared to that of propylthiouracil. Furthermore the total area under the biphasic curve, the AUC of ^{125}I -uptake phase and the rate of ^{125}I -discharge are also listed in Table 6.

The % efficacy of the tested compounds was calculated according to Eq. 6 in relation to both the ^{125}I -uptake and the rate of its discharge from the thyroid gland. A comparative antithyroid effi-

cacy of the tested compounds in reference to DMSO-control and propylthiouracil treated rats is shown in Table 6. When rats were treated with equimolar doses, the % efficacy of abouthiouline, compounds II and I, with respect to the rate of ^{125}I -discharge, were 131.13, 49.86 and 29.21 of that of propylthiouracil, respectively. Whereas the % efficacy of ^{125}I -uptake of abouthiouline, compounds II and I were found to be 51.72, 23.17 and 6.24 of that of propylthiouracil, respectively.

Discussion

Earlier reports in the literature reveal high quality QSAR models for variety of measured activities and demonstrated that E-state indexes of a molecular structure are very appropriate for QSAR (34-37). The first investigation to utilize this approach in the development of antithyroid compounds was undertaken in our laboratory (46) by developing a linear relation between the formation constants of thiourylene-type compounds with iodine (K_c) and their E-state indexes of thiourylene moiety (Eq. 8).

$$-\ln(K_c) = -17.98 (\pm 0.7) + 1.02 (\pm 0.09) (S_{N\&S}) \quad (8)$$

[$r = 0.985$; SEM = 0.07; F = 133.7; n = 6, p = 0.0003]

In addition, this *in vitro* QSAR model emphasized the importance of thiourylene moiety as the site of complexation as well as its importance in anti-thyroid activity. In the present study we show the

Table 6. 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

Compounds	Total AUC* (AUC. \pm SE: (%Dose/g).min.)	^{125}I -Uptake (AUC. \pm SE: (%Dose/g).min.) up to maximum ^x	Rate of ^{125}I -Release \parallel (%Dose/min.) ($\times 10^4$)	% Efficacy ^c with respect to	
				^{125}I -Uptake	^{125}I -Release
Control - Normal ^a	61.275 \pm 7.54	-	-	-	-
-DMSO ^b	85.45 \pm 8.37	45.76 \pm 3.95	3.66 ($r=0.870$)	-	-
PTU	153.35 \pm 11.63	105.58 \pm 8.33	-159.43 ($r=0.988$)	100	100
I	76.95 \pm 9.39	49.49 \pm 5.84	-49.17 ($r=0.919$)	6.24	29.21
II	107.22 \pm 9.80	59.62 \pm 6.51	-81.33 ($r=0.998$)	23.17	49.86
Abouthiouline (III)	89.2 \pm 10.10	76.70 \pm 9.27	-207.42 ($r=0.984$)	51.72	131.13

*Area under the curve from 0-120 min. ^x From 0-60 min (uptake phase); \parallel From 60-120 min (release phase). $n=5$ rats: ^a untreated rates; ^b rates were treated with 0.1 ml DMSO for 30 days before radioiodine administration; ^c % efficacy = $100 \{ (\text{PAR}_i - \text{PAR}_c) / (\text{PAR}_{\text{ptu}} - \text{PAR}_c) \}$; 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)

importance of the E-state indexes of thiourylene moiety as a projection of the relative antioxidant/oxidant property. Utilizing Imamura *et al.* data (44) and $S_{N\&S}$ indexes of antithyroid compounds in scheme 2, a linear relationship between the inhibitory effect of the antithyroid agents on luminol-dependent CL response of PMA-induced human PMNLs and the E-state of thiourylene moiety ($S_{N\&S}$) was developed. It is evident, from this relation, that the value of $S_{N\&S}$ is related to the antioxidant magnitude of the compounds. Therefore, this relationship is utilized as a guideline for the rational selection of thiourylene-type compounds under investigation.

In this study the effect of the investigated compounds on luminol-dependent CL response of PMA-induced human PMNLs revealed that the intensity of the inhibition of respiratory burst of all compounds is dose dependent and inversely proportional to their $S_{N\&S}$ value (Fig. 3). This suggests that the electron accessibility on thiourylene moiety ($S_{N\&S}$) has influence on the free radical scavenging property of thiourylene-type compounds to the reactive oxygen species (ROS) generated in PMNLs. Thus, it is apparent that compounds I, II and abouthiourline have reduced antioxidant properties as predicted by their high E-state value of $S_{N\&S}$. These results also agree with our depicted relation extracted from Imamura *et al.*, (44) data as presented in Eq. 7 and Fig. 1. Furthermore, the effect of compounds I, II and abouthiourline on PMNLs viability suggests that the compounds have no toxic effect on PMNLs due to direct lysis of the cell membrane. Thus, the CL response is due to respiratory burst.

In contrast to propylthiouracil (12,23,44) abouthiourline has low magnitude of antioxidant property at low concentrations (5-20 $\mu\text{g/ml}$) and minor oxidant property at high concentrations (40 $\mu\text{g/ml}$).

In order to assess the potential activity of the investigated compounds towards the thyroid gland, the complexation with iodine was undertaken, and the formation constant of their charge transfer complexes with iodine were used as a criterion for the preliminary evaluation of their potential antithyroid activity.

Mulliken (47,48), who developed the concept of charge transfer complexation through his quantum mechanical treatment was the first to use

absorption spectroscopy in the study of such complexes. The spectroscopic results in the visible and UV region indicate strong donor-acceptor interactions and confirm the n- δ character of the charge transfer complexes of the investigated compound with iodine. The intermolecular electronic transitions that lead to changes in the electronic structures of the investigated compounds and iodine resulted in modification of their normal absorption spectra and the appearance of new band. This was referred to by Mulliken (47,48) as the "blue shift" of the visible iodine band. The "blue shift" was explained as being due to increased exchange repulsion perturbation (49), existence of vibrational frequencies in thiourylene moiety, N-C=S, (50) and as being due to dissociation between shallow "outer complex" and deeper "inner complex" with an "activated complex" lying between them (47). The correlation of K_c of the investigated drugs with their $S_{N\&S}$, showed that compound II ($K_c = 4721.5 \text{ M}^{-1}$, $S_{N\&S} = 8.28$) has higher K_c and lower $S_{N\&S}$ than that of compound I ($K_c = 3125.1 \text{ M}^{-1}$, $S_{N\&S} = 8.52$) which agrees with Eq. 8. On the other hand, abouthiourline ($K_c = 4047.4 \text{ M}^{-1}$, $S_{N\&S} = 8.71$) has higher K_c and $S_{N\&S}$ than that of compound I which suggests that the formation constant of abouthiourline with iodine deviates from the linear *in vitro* QSAR model (Eq. 8).

The difference in the magnitude of the value of the formation constant of abouthiourline with respect to the E-state of thiourylene moiety could be explained on the basis of the exchange repulsive perturbation and acceleration of the rotation on C-S or C-N covalent bond of thiourylene moiety. As expected by Kier and Hall (42), E-state values are related to the effects which are dependent on electron density as influenced by molecular topology. Thus, the force of repulsion between sulfur and nitrogen atoms of thiourylene moiety [$k(S_{\vec{c}} \times S_{\vec{s}}) / r^2$] is equal to the sum of the force due to the acceleration of atom rotation and the force of resistance to atom rotation in the field of other atoms and in the media. Thus, it is related to the atomic mass (m_a), acceleration of covalent bond C-S or C-N with respect to time ($d^2\vec{x}/dt^2$), rate of shear as one atom rotation becomes closer to the other atoms (dv/dr) and the viscosity of the media (η) as related to concentration of thiourylene-type compounds. Equation 9 was derived to describe this relationship (46).

$$\frac{k(S_N^- \times S_S^-)}{r^2} = m_a \frac{d^2\bar{x}}{dt^2} + \eta \frac{dv}{dr} \quad (9)$$

where "r" is the distance between S and N atoms and k is a unit conversion constant. Although, the increase in electron accessibility on both sulfur and nitrogen skeletal atoms of thiourylene moiety ($S_N^- \times S_S^-$) will accelerate rotation and cause oscillative repulsion kicks to iodine molecule, the atom mass and the steric hindrance of 3-quinolyl moiety cause resistance to atom rotation in the field of other atoms and slows the oscillative repulsive kicks to iodine. Therefore, the formation constant of complexation of abouthiouline with iodine will be higher than expected. This result also agrees with earlier reports on the theoretical aspects of the complexation of iodine with thiourylene-type compounds (47-50), and provide quantitative explanation for the magnitude of K_c of the charge transfer complexes of these compounds with iodine.

Since the complexation of antithyroid compounds with iodine represents the major step in the mechanism of inhibition of biogenesis of thyroid hormone (10), the results in this study indicated strong compound-iodine interaction with formation constant exceeding the limited criteria for the potential antithyroid activity ($K_c = 100 \text{ M}^{-1}$) drawn by Raby and coworkers (10,27,31). In addition, the resulted formation constants were higher than that of potassium thiocyanate ($K_c = 94 \text{ M}^{-1}$), whose antithyroid action is well known. Thus, compounds I, II and abouthiouline are of potential value as antithyroid agents and should be subjected to an *in vivo* antithyroid screening for further confirmation and to compare their efficacy with propylthiouracil.

The *in vivo* relative efficacy of the investigated compounds with respect to both ^{125}I -uptake and its rate of ^{125}I -discharge was determined by using ^{125}I -thiocyanate discharge technique in rats. In this method, the extent of radioiodine thyroid uptake and its rate of discharge following the administration of KSCN is considered a measure of thyroid inhibition effect (42,43). Untreated rats were shown to have no ^{125}I -discharge after administration of KSCN, whereas the rats treated with antithyroid agents were found to exhibit ^{125}I -discharge (42). The importance of the rate of ^{125}I -discharge after KSCN

or perchlorate administration, in screening for antithyroid agents, was also emphasized by other investigators (10,51,52). Results of ^{125}I -thiocyanate discharge technique in rats revealed that abouthiouline is of potential value as antithyroid agent. It has high relative efficacy (131%) than propylthiouracil with respect to the rate of ^{125}I -discharge but lower (51.7%) relative efficacy with respect to ^{125}I -uptake. Whereas compounds II and I have lower relative efficacy with respect to both the rate of ^{125}I -discharge (49.86% and 29.2%) and ^{125}I -uptake (23.17% and 6.24%), respectively. Therefore, abouthiouline has more tendency to inhibit the accumulation of iodine by thyroid in rats than propylthiouracil and the ratio of the % efficacy with respect to the rate of ^{125}I -discharge to ^{125}I -uptake of abouthiouline is 2.54. These results provide an advantage for abouthiouline similar to that of the combined antithyroid therapy of ionic inhibitors with propylthiouracil or methimazole that is well documented (53-56) and used to reduce the dose and duration of therapy. In addition, the liberation of iodide during ^{125}I -discharge phase plays an important role in the regulation of thyroid peroxidase (E) and the *in vivo* formation of molecular iodine in thyroid gland (10,57-59). The liberation of iodide during the reversible process will tend to protect thyroid peroxidase from complete inactivation and enhance the generation of molecular iodine according to the following reactions (57-59).



Thus, the reversible effect of excess iodide on thyroid peroxidase (Eq. 12 and Eq. 13) will not completely block the biosynthesis of the vital thyroid hormones and avoids the iatrogenic hypothyroidism. The generation of molecular iodine (Eq. 13), also, ensures the continuity of the formation of the charge transfer complex with the antithyroid drugs.

In conclusion, abouthiouline has lower toxicity ($\text{LD}_{50} > 800 \text{ mg/kg}$), reduced antioxidant property as well as higher antithyroidal efficacy than propylthiouracil with respect to the rate of ^{125}I -discharge.

In view of the toxic implication of the antioxidant property of the currently used antithyroid agents on interleukin-2 production, natural killer cells (14,15), proliferative activity of cells and production of antibodies (5,17-23,60), the reduction in the antioxidant property of abouthiouline is advantageous. This suggests that abouthiouline will be beneficial in reducing the severity of the immune reaction of both the Graves' disease and the drug-induced agranulocytosis. Furthermore, by virtue of its reduced antioxidant property and having acyclic thioamide moiety, abouthiouline, may possess an immunosuppressive effect (by reducing lymphocytic infiltration) and reduced sensitization of anti-neutrophil-antibodies. This might cause suppression of agranulocytosis and reduce the rate of the relapse of Graves' disease after discontinuation of medication. Further supportive investigations are in progress.

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References

- Tajiri J, Noguchi S, Murakami T, Murakami T. Anti-thyroid drug-induced agranulocytosis: the usefulness of routine white blood cell count monitoring. *Arch Intern Med*, 1990; 150:621-624.
- Tajiri J, Noguchi S, Okamura S, Morita M, Tamaru M, Murakami N, Niho Y. Granulocyte colony-stimulating factor treatment of antithyroid drug-induced granulocytopenia. *Arch Intern Med*, 1993; 153:509-514.
- Retsagi G, Kelly JP, Kaufman D.W. Risk of agranulocytosis and aplastic anaemia in relation to use of antithyroid drugs: international agranulocytosis and aplastic anaemia study. *BMJ*, 1988; 297:262-265.
- Biswas N, Ahn Y, Goldman JM, Schwartz JM. Case report: aplastic anemia associated with antithyroid drugs. *Am J Med Sci*. 1991; 301:190-194.
- Cooper DS. Antithyroid drugs. *N Engl J Med*, 1984; 311:1353-1362.
- Utiger DR. The pathogenesis of autoimmune thyroid disease. *N Engl J Med*. 1984;325:278-279.
- Strakozch CR, Weazel BE, Row VV, Volp R. Immunology of autoimmune thyroid diseases. *N Engl J Med*. 1982; 307:1499-1507.
- Wang PW, Luo SF, Huang BY, Lin JD, Huang MJ. Depressed natural killer activity in Graves' disease and during antithyroid medication. *Clin Endocrinol*, 1988; 28:205-214.
- Haynes Jr., RC. Thyroid and antithyroid drugs. In: Gilman AG, Rall TW, Nies AS, and P. Taylor P. eds., Goodman and Gilman's The pharmacological basis of therapeutics, New York: Pergamon press. 1991; 1361-1383.
- Lagorce JF, Thomes JC, Catanzano G, Buxeraud J, Raby M, Raby C. Formation of molecular iodine during oxidation of iodide by the peroxidase/H₂O₂ system. *Biochem Pharmacol*. 1991; 42:S89-92.
- Wall JR, Fang SL, Kurocki T, Lagbar SH, Braverman LE. 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* 1984; 58:868-872.
- Wilson R, McKillop JH, Travers M, Smith J, Smith E, Thomson JA. The effects of antithyroid drugs on intracellular mediators. *Acta Endocrinol Copenh*. 1990; 122:605-609.
- Heufelder AE, Wenzel BE, Bahn RS. Methimazole and propylthiouracil inhibit the oxygen free radical-induced expression of a 72 kilodalton heat shock protein in Graves' retroocular fibroblasts. *J Clin Endocrinol Metab*. 1992; 74:737-742.
- Karlsson FA, Totterman TH. Immunomodulation by methimazole therapy in Graves' disease: rapid changes in activation stage of circulating regulatory T cell subsets, B cells and NK cells. *Clin Exp Immunol*, 1988; 74:258-263.
- Totterman TH, Karlsson FA, Bengtsson M, Mendel-Hartvig I. Induction of circulating activated suppressor-like T cells by methimazole therapy for Graves' disease. *N Engl J Med*, 1987; 316:15-22.
- Matsunaga M, Eguchi K, Fukuda T, Tezuka H, Ueki Y, Kawabe Y, Shimomura C, Otsubo T, Ishikawa N, Ito K, Nagataki S. The effects of cytokines, antithyroidal drugs and glucocorticoids on phagocytosis by thyroid cells. *Acta Endocrinol (Copenh)*. 1988; 119:413-419.
- Aguayo J, Iitaka M, Row VV, Volpe R. Studies of HLA-DR expression on cultured human thyrocytes: Effect of antithyroid drugs and other agents on interferon-gamma-induced HLA-DR expression. *J Clin Endocrinol Metab*. 1988; 66:903-908.
- Fidelus RK, Tsan MF. Enhancement of intracellular glutathione promotes lymphocyte activation by mitogen. *Cell Immunol*. 1986; 97:155-163.
- Fidelus RK, Ginouves P, Lawrence D, Tan MF. Modulation of intracellular glutathione concentrations alters lymphocyte activity and proliferation. *Exp Cell Res*. 1987; 170:269-275.
- Fidelus RK. The generation of oxygen radicals: a positive signal for lymphocyte activation. *Cell Immunol*, 1988; 113:175-182.

21. Suthanthiraa M, Anderson ME, Sharma VK, Meister A. Glutathione regulates activation-dependent DNA synthesis in highly purified normal human T lymphocytes stimulated via the CD2 and CD4 antigens. *Proc Natl Acad Sci USA*, 1990; 87:3343-3347.
22. Weetman AP. Effect of the antithyroid drug methimazole on interleukin-1 and interleukin-2 levels in vitro. *Clin Endocrinol*, 1986;25:133-142.
23. Hicks M, Wong LS, Day RO. Antioxidant activity of propylthiouracil. *Biochem Pharmacol*, 1992; 43:439-444.
24. Ren YZ, Song ZG. The association of Graves' disease with serum soluble interleukin 2 receptor. *Chung. Hua. I. Hsueh. Tsa Chih*, 1993; 73:264-265.
25. Paschke R, Vogt M, Swillens S, Usadel K.H. Correlation of microsomal antibodies with the intensity of the intrathyroidal autoimmune process in Graves' disease. *J Clin Endocrinol Metab*, 1993; 77:939-943.
26. Wenzel KW, Lente JR. Similar effects of thionamide drugs and perchlorate on thyroid-stimulating immunoglobulins in Graves's disease: Evidence against an immunosuppressive action of thionamide drugs. *J Clin Endocrinol Metab*, 1984; 58:62-69.
27. Raby C, Buxeraud J. Antithyroidiens: relation structure-activit. I. Formation de complexes molculaires iods. *Eur J Med Chem*, 1980; 15:425-430.
28. Buxeraud J, Absil AC, Raby C. Secondary antithyroid action of drugs in relation to structure. *J Pharm Sci*, 1984; 73:1687-1690.
29. Buxeraud J, Absil AC, Raby C, Catanzano G, Beck C. Anti-thyroidiens: relation structure-activit. Interpretation du mcanisme d'action des ATS par complexation de transfert de charge. *Eur J Med Chem Chim Ther*, 1985; 20:43-51.
30. Jambut-Absil AC, Buxeraud J, Lagorce JF, Raby C. Charge transfer complexes of drugs with iodine investigation by UV/visible spectroscopy. *Int J Pharm*, 1987; 35:129-137.
31. Raby C, Lagorce JF, Jambut-Absil AC, Buxeraud J, G. Catanzano G. The mechanism of action of synthetic antithyroid drug: iodine complexation during iodide oxidation. *Endocrinol*, 1990; 126:1683-1691.
32. Kier LB, Hall LH. An electrotopological state index for atoms in molecules. *Pharm Res*, 1990; 7:801-807.
33. Kier LB, Hall LH, Frazer JW. An index of electrotopological state for atoms in molecules. *J Math Chem* 1991, 7:229-241.
34. Hall LH, Mohny B, Kier LB. The electrotopological state: An atom index for QSAR. *Quant Struct Act Relat* 1991; 10:43-52.
35. Kier LB, Hall LH. An atom description in QSAR models: Development and use of an atom level index. In Testa B. (ed.), *Advances in drug design*, Academic Press Limited, New York, 1992: pp. 1-38.
36. Hall LH, Kier LB. Binding of salicylamides: QSAR analysis with electrotopological state indexes. *Med Chem Res* 1992; 2:497-502.
37. Hall LH, Mohny B, Kier LB. Comparison of electrotopological state indexes with molecular orbital by hydrazides. *Quant Struct Act Relat* 1993; 12:44-48.
38. Tono-oka T, Ueno N, Matsumoto T, Ohkawa M, Matsumoto S. 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*, 1983; 26:66-75.
39. Allen RC, Loose LD. Phagocytosis of luminol-dependent chemiluminescence in rat alveolar and peritoneal macrophages. *Biochem Biophys Res Commun*, 1976; 69:245-252.
40. Borazan HN, Ajeena YH. Charge transfer complexes of indole:catechol Type II. *J Pharm Sci*, 1988; 77:544-547.
41. Ghosh MN. *Fundamental of experimental pharmacology*. Scientific Book Agency, Calcutta, 1984:189-190.
42. Brown J, Solomon DH. Mechanism of antithyroid effects of sulfonylureas in the rat. *Endocrinol*, 1958;63:473-480.
43. Aboul-Enein HY, Loutfy MA., Abdulla MA. Synthesis and antithyroid activity of 6-n-propyl-5-arylazo 2-thiouracils. *Toxicol Environ Chem*, 1987; 13:257- 263.
44. Imamura M, Aoki N, Saito T, Ohno Y, Maruyama Y, Yamaguchi J, Yamamoto T. Inhibitory effects of antithyroid drugs on oxygen radical formation in human neutrophils. *Acta Endocrinologica*, 1986; 112:210-216.
45. Job P, Formation and stability of inorganic complexes in solution., *Ann Chim*, 1928; 9:113-203.
46. Abou-shaaban RRA, Al-Khamees HA, Abou-Auda HS, Simonelli AP. Atom level electrotopological-state indexes in QSAR: Designing and testing antithyroid agents. *Pharm Res*, 1996;3(1): .
47. Mulliken RS. Molecular compounds and their spectra. III. The interaction of electron donors and acceptors. *J Phys Chem*, 1952; 56:801-823.
48. Mulliken RS. Molecular complexes and their spectra. VI. Some problems and new developments. *Rec Trans Chim Pays-Bas* 1956; 75:845-852.
49. Lang RP. Molecular complexes and their spectra. XIV. Iodine complexes with thiourea and thioacetamide. *J Am Chem Soc* 1962; 84:1185-1192
50. Bhaskar KR, Gosavi RK, Rao CNR. Spectroscopic studies on n-donor + δ -acceptor systems: Alkylthioureas and thiocarbanilides. *Trans Faraday Soc* 1966; 62:29-38.
51. Abdulla ME, Aboul-Enein HY, Hassan MMA, and Taha SA. Application of the ¹²⁵I-perchlorate discharge technique in the evaluation of the potential antithyroid activity of some fluorinated thiouracil derivatives. *Proceedings of the international symposium on applications and technology of Ionizing Radiation*. Riyadh, March 12-17, 1982; 1: 531.
52. Friis J. The perchlorate discharge test with and without supplement of potassium iodide. *J Endocrinol Invest* 1967; 10:581-584.
53. Connell JMC. Long-term use of potassium perchlorate. *Postgrad Med J* 1981; 57:516-517.
54. Rokke K, Vogt, J. Combination of potassium perchlorate and propylthiouracil in the treatment of thyrotoxicosis. *Acta Endocrinol* 1968; 57:565-577.
55. Dal Fabbro S, Dalle Mule I, Bridda A. More on KCIO4 and amiodarone associated thyrotoxicosis (letter). *J Endocrinol Invest* 1988; 11:691.

56. Reichert LJM, and de Rooy HAM. Treatment of amiodarone induced hyperthyroidism with potassium perchlorate and methimazole during amiodarone treatment. *BMJ* 1989; 298:1547-1548.
57. Magnusson RP, Gestautas J, Taurog A, Rapoport B. Molecular cloning of the structural gene for porcine thyroid peroxidase. *J Biol Chem* 1987; 262:13885-13888.
58. Pommier J, Deme D, Nunez J. Effect of iodide concentration on thyroxine synthesis catalysed by thyroid peroxidase. *Eur J Biochem* 1973; 37: 406-414.
59. Magnusson RP, Taurog A, Dorris ML. Mechanism of thyroid peroxidase and lactoperoxidase-catalysed reaction involving iodide. *J Biol Chem* 1984; 259:13783-13790.
60. Abou-Shaaban RRA. Oxidative stress, HIV and AIDS: The basis for antioxidant- oriented antiretroviral nucleoside analogues. *Saudi Pharm J*, 1995; 3:1-22.