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Proliferative and Antioxidative Activity of Two Newly Synthesized Antithyroid Drugs, Abouthiouzine and Abouthiouline, as Compared to Propylthiouracil and Methimazole

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The antioxidant and proliferative activity of currently used antithyroid drugs are known to cause agranulocytosis. In our previous work, the E-State approach was used to introduce two new antithyroid drugs Abouthiouzine (ABZ) [1-n-butyl-3(isonicotinamido)-2-thiourea] and Abouthiouline (ABL) [1-cyclohexyl-3(3-quinolyl)-2-thiourea] with modified acyclic thiourylene structure to reduce antioxidative and mutagenic activity. This study was conducted to compare the antioxidant, phagocytic, clastogenic and proliferative effects of our newly designed antithyroid agents with propylthiouracil, methimazole and thyroxine. Different experiments were undertaken on the agents to investigate their antioxidant and proliferative activities. Chemiluminescence's (CL) response and phagocytic activity on isolated polymorphonuclear leukocytes (PMNLs) were used to evaluate antioxidant effects. Micronucleus test on femoral cells of mice as well as protein and nucleic acids level in hepatic cells were also used to investigate proliferative and mutagenic effects. It was found that all compounds except abouthiouzine inhibited the CL response and suppressed the phagocytic activity; however, the intensity was comparatively less than that of propylthiouracil. Cytological studies demonstrated that none of the compounds were clastogenic, however, the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was found to be increasing by the treatment with propylthiouracil, methimazole and thyroxine, whereas this ratio was comparatively less after treatment with Compounds I and IV, Abouthiouline and Abouthiouzine. These results were supported by biochemical analysis. The newly synthesized antithyroid drugs reduced the antioxidative and proliferative activity as compared to propylthiouracil and methimazole. Further studies are warranted to determine the exact mode of action of these compounds before clinical trials are undertaken to suppress agranulocytosis.

Key words: Abouthiouzine, abouthiouline, agranulocytosis, antioxidant, proliferation, chemiluminescence's response and phagocytic activity, Polymorphonuclear leukocytes (PMNLs), Micronucleus test, propylthiouracil, methimazole, thyroxine, e-state indexes

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INTRODUCTION

Agranulocytosis is a severe and life-threatening complication of the currently used antithyroid drugs. The pathogenesis of this complication was attributed to an autoimmune phenomenon caused by circulating anti-neutrophil antibodies and lymphocyte sensitization to antithyroid drugs (Bartalena *et al*., 1996). The antioxidant activity of propylthiouracil (PTU) and methimazole (MTM) as well as the structurally related cyclic thioamide group are involved in the stimulation of anti-neutrophil antibodies that mediate agranulocytosis (Wall *et al*., 1984; Wilson *et al*., 1990). In a large-scale pharmacovigilance study conducted in the United Kingdom, data reported between 1963 and 2003 to the Committee on Safety of Medicines (Yellow Card Scheme) were analyzed to determine the relative frequency and spectrum of adverse drug reactions to carbimazole (the parent drug for MTM) and PTU (Pearce, 2004). The study found that the number of prescriptions for thionamide drugs were 5.23 million prescriptions where 94% of which were for carbimazole. All thionamide medication studied induced significant adverse drug reactions, including agranulocytosis and neutopenia.

The antioxidant property of propylthiouracil and methimazole has been shown to increase interleukin-2 production, proliferative activity of cells, lymphocyte infiltration, production of antibodies, phagocytosis and alter the function of natural killer cells (Wilson *et al*., 1990; Karlsson and Totterman, 1988; Matsunaga *et al*., 1988; Fidelus *et al*., 1987; Hicks *et al*., 1992). The antioxidant activity of thyroid hormones (T4 and T3) and their major role in the proliferation of different cells are also well documented (Giuriato *et al*., 1991; Sterling *et al*., 1988). These hormones were also found to be essential for normal growth and are known to increase the RNA and protein contents of liver and muscle of lata fish (Medda and Ray, 1979).

In our previous communications, atom level electrotopological-state indexes of thiourylene moiety ($S_{N\&S}$) were utilized to custom design two new antithyroid drugs with acyclic thiourylene moiety and reduced antioxidant property that might reduce the production of anti-neutrophil antibodies which mediate the life-threatening agranulocytosis (Abou-Shaaban *et al*., 1995, 1996). The new antithyroid agents, Abouthiouzine (ABZ) and Abouthiouline (ABL), have low toxicity ($LD_{50,(ABZ)}$ = 1000 mg kg^{-1}, $LD_{50,(ABL)}$ = 800 mg kg^{-1}), reduced antioxidant activity and higher antithyroid efficacy than propylthiouracil with respect to rate of ^{125}I-discharge. For equimolar doses, the ratio of percent efficacy with respect to rate of ^{125}I-discharge to ^{125}I-uptake of Abouthiouline, Abouthiouzine and propylthiouracil are 2.54, 1.98 and 1.0, respectively. The importance of the rate of thyroid Iodine-discharge in antithyroid therapy in reducing the dose and avoiding the iatrogenic hypothyroidism was also emphasized (Abou-Shaaban *et al*., 1995, 1996). Furthermore, the preclinical toxicology of ABL was determined in mice and rats compared with PTU and MTM following short-term administration (7 days) to mice, ABL had minimal effects on biochemical parameters (Abou-Auda and Abou-Shaaban, 2006). Also long-term studies (30 days) in rats revealed that ABL had no detrimental effects on hematologic parameters (Abou-Auda and Abou-Shaaban, 2006).

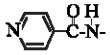
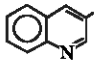
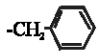
In this report, experiments on chemiluminescence's (CL) response and phagocytic activity of Polymorphonuclear leukocytes (PMNLs) were undertaken to compare quantitatively the antioxidative property of Abouthiouzine and Abouthiouline with propylthiouracil to their E-state indexes of thiourylene moiety. Furthermore, the experiments on the proliferative activity in femoral and hepatic cells of mice were conducted to compare new compounds with propylthiouracil, methimazole and thyroxine which have a proven antioxidative and proliferative activity.

MATERIALS AND METHODS

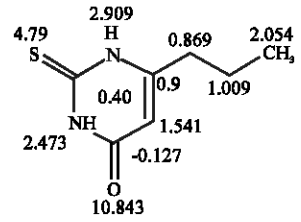
Chemicals: Propylthiouracil (Aldrich Chemical Company, Milwaukee, WI), Methimazole (Sigma Chemical Company, St. Louis, MO), Thyroxine (Galax Laboratory Ltd. Greenford, England), phorbol 12-myristate 13-acetate (PMA) and luminol (Sigma Chemical Company, St. Louis, MO), Nycodenz (Nygaard and Co., Torshov, Norway), Phosphate buffer saline (PBS, Electronucleonics Inc., Columbia, MD) were used as received. The compounds I, IV, Abouthiouzine and Abouthiouline were synthesized (Table 1) in our laboratory (Abou-Shaaban *et al*., 1995, 1996; Abou-Shaaban, 1996). All other reagents and chemicals used in this study were of general grade and obtained from Aldrich Chemical Company (Milwaukee, WI, USA).

Computation of E-state indexes of skeletal atoms and thiourylene moiety ($S_{N\&S}$): The calculations were performed as described elsewhere (Abou-Shaaban *et al*., 1995, 1996; Kier and Hall, 1990). All computations were performed using ABOUFAC-ET program (Abou-Shaaban *et al*., 1996). Scheme I shows the E-state indexes of Abouthiouzine, Abouthiouline and the currently used antithyroid drugs. The $S_{N\&S}$ values of the compounds were calculated from the average of the

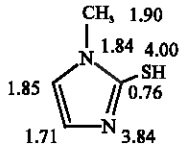
Table 1: The Investigated thiourylene-type compounds, electrotopological-state indexes of sulphur (S = or -SH), nitrogen (>NH or -N =) and the calculated thiourylene moiety ($S_{N\&S}$)

	$R_1HN-\overset{S}{\overset{\|}{C}}-NHR_2$			E-state indexes			
Compounds	R_1	R_2	Chemical Formula	$S_{>NH}$	S_s	$S_{>NH}$	$S_{N\&S}$
Abouthiouzine		n-$(CH_2)_3CH_3$	$C_{11}H_{16}N_4OS$	2.64	4.94	2.98	7.75, 10.25*
Abouthiouline			$C_{16}H_{19}N_3S$	3.24	5.39	3.41	8.715
Propylthiouracil	Cyclic thiourea		$C_7H_{10}N_2OS$	2.909	4.79	2.47	7.48
Methimazole	Cyclic thiourea		$C_4H_6N_2S$	-	4.0¥	3.82¥	
Compound (I)			$C_{12}H_{17}N_3S$	3.15	5.27	3.36	8.525
Compound (IV)			$C_{13}H_{13}N_3S$	3.07	5.18	3.14	8.285

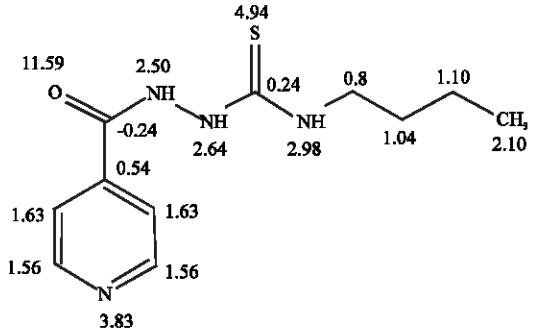
* Sum of the E-state of nitrogen and thiourylene moiety ($S_{N\&S} + S_N = 7.75 + 2.5 = 10.25$) of abouthiouzine. ¥The vibrational frequencies exists in thiourylene moiety is (=N–C–SH ↔ =NH^+–C=S^-). This vibration causes exchange repulsion perturbation



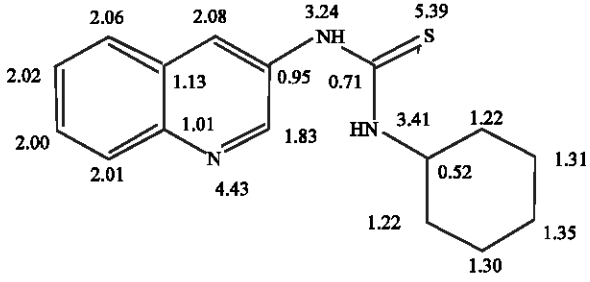
Propylthiouracil



Methimazole



Abouthiouzine



Abouthiouline

Scheme 1: Atom level electrotopological-state indexes and structure of abouthiouline, abouthiouzine and the currently used antithyroid drugs

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. Table 1 shows the investigated thiourylene-type compounds, chemical formula and the E-state indexes of sulphur (S = or -SH), nitrogen (>NH or -N =) and the calculated thiourylene moiety ($S_{N\&S}$). E-state of thiosemicarbazone of abouthiouzine [HN-NH-C(= S)] is equal to the sum of the E-state of nitrogen and thiourylene moiety ($S_{N\&S}+S_N = 7.75+2.5 = 10.25$).

Isolation of Polymorphonuclear Leucocytes (PMNLs): PMNLs were isolated from the blood of healthy human donors (Abou-Shaaban *et al*., 1996a). Each 5 mL of heparinized blood were mixed with 1 mL of 6% (w/v) Dextran T 500 in saline and incubated at room temperature for 30 min. The leukocyte-rich plasma layer was separated and mixed with 3 mL Nycodenz solution before centrifugation at 400 g for 15 min. The PMNL-rich portion 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.

Chemiluminescence's (CL) activity and viability testing: The effect of the tested agents on luminol-dependent CL of PMA-stimulated PMNLs was studied as described (Abou-Shaaban *et al*., 1995; Abou-Shaaban *et al*., 1996; Abou-Shaaban, 1996) using an LKB-Wallac 125I Luminator. The principle of oxidation of luminol, 5-amino-2,3-dehydro-1,4-phthalazinedione, by the Reactive Oxygen Species (ROS) produced during phagocytosis in PMNLs, was applied to increase the amount of measurable light in the luminol-dependent CL measurements (Allen and Loose, 1976). To 10 μL of each of the compounds (in DMSO) of variable concentration, 0.9 mL PBS medium containing 10^{-3} M luminol and 2 μg of PMA were added. After shaking, 0.1 mL 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 recorded at one min intervals for 14 min and plotted against time. 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 [$(AUC\text{-}CL)_c$]. The resulting positive or negative value indicates stimulation or inhibition of oxidant and antioxidant property, respectively. The results were evaluated by two-tailed Student's t-test for independent samples and linear regression equations were generated using the Statistical Package for Social Sciences (SPSS) version 13.0 for Windows (SPSS Inc., Chicago, Illinois). The effect of compounds I and IV on the viability of PMNLs was tested at 10 and 30 min following incubation at 37°C. The percentage of viable cells was estimated by trypan blue exclusion test (Abou-Shaaban *et al*., 1995).

Studies on the effect on phagocytic activity in the human PMNLs: The effect of different compounds under analysis was evaluated on the phagocytic activity in the human PMNLs according to the procedure described by Tawfik *et al*. (1990). Baker yeast was killed by suspending in PBS and boiling for 30 min. Autologous human serum (100 μL) was added to 900 μL of yeast suspension. The mixture was then incubated at 37°C for 30 min. The opsonized yeast was washed with PBS and resuspended in Hepes RPMI 1640 medium. Human PMNLs were now mixed with the Hepes RPMI 1640 medium containing 10 μL of the drug (solvent DMSO) in the ratio of 1:10. The final concentration of human PMNLs was 5×10^6 cells mL^{-1} and that of yeast was 2×10^5 particles mL^{-1}. The control in each experiment contained opsonized yeast, PMNLs and 10 μL of DMSO. The mixture was incubated at 37°C for 30 min. One hundred viable PMNLs were stained with trypan blue. The phagocytic activity of PMNLs was evaluated by screening the yeast ingested cells under hemocytometer.

Studies on the proliferative activity of femoral cells and quantification of nucleic acids and proteins in hepatic cells

Animal stocks: Male Swiss albino mice (SWR), aged 5-6 weeks, weighing 20-25 g were obtained from Experimental Animal Care Center, King Saud University, Riyadh, Saudi Arabia. The animals were fed on a purina chow diet and water *ad libitum* and were maintained under standard conditions of humidity, temperature and light. The principles of the laboratory care (INH#85-23) were followed in undertaking different experiments.

Experimental design: Equimolar dose of the different compounds were given to mice except thyroxine dose was based on human therapeutic dose adjusted according to mice surface area. A total of 50 mice were randomly assigned to different control and treatment groups (5 mice in each group). The experimental groups of mice consisted of group 1, control (1% Tween 80); group 2, Thyroxine (77.40 μg kg^{-1} day^{-1}); group 3, Propylthiouracil (10 mg kg^{-1} day^{-1}); group 4, Methimazole (6.71 mg kg^{-1} day^{-1}); group 5, Compound I (14.0 mg kg^{-1} day^{-1}); group 6, Abouthiouline (16.70 mg kg^{-1} day^{-1}); group 7, Compound IV (14.28 mg kg^{-1} day^{-1}) and group 8, Abouthiouzine (14.80 mg kg^{-1} day^{-1}). The treatment in each case was given orally for 7 days. The animals were

sacrificed 30 h after the last treatment. The femurs were used for micronucleus test and the liver was excised and stored at -20°C until analyzed for protein and nucleic acid concentrations in hepatic cells.

Micronucleus test: The micronucleus test procedure described by Schmid (1975) was used in the present study. The femoral cells were collected in fetal calf serum. After centrifugation, the cells were spread on slides and air dried. Coded slides were fixed in methanol and stained in May-Gruenwald solution followed by Giemsa stain. The polychromatic erythrocytes (PCE/1000 per mouse) were screened for micronuclei and the reduction of the mitotic index was evaluated on the basis of polychromatic to normochromatic erythrocytes (PCE/NCE) ratio.

Estimation of protein and nucleic acids: Total protein was determined by the method of Lowry *et al.* (1951). The method of Bregman (1983) was also used to determine the levels of nucleic acids. Tissues were homogenized and the homogenate was extracted in different concentrations of cold and hot trichloroacetic acid (TCA) and absolute ethanol. After the final extraction in TCA, incubation and centrifugation; the supernatant was used to determine the levels of DNA and RNA. The levels of DNA were determined by treating the nucleic acid extract with diphenylamine reagent and reading the intensity of the blue color at 600 nm. For the quantization of RNA, the nucleic acid extract was treated with orcinol and the green color was read at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

RESULTS AND DISCUSSION

Antioxidant/oxidant properties of the investigated agents on PMNLs: In order to study the antioxidant property, the effect of the investigated compounds (Scheme 1) on luminol-dependent CL-response was examined on stimulated PMNLs. When stimulated by PMA, the PMNLs secrete Reactive Oxygen Species (ROS) into the reaction mixture respiratory burst, which rapidly increases the luminol-dependent CL-response to reach a peak before gradual decline. The integrated area under CL curves (AUC) produced for the different compounds were used to calculate the percentage of stimulation or inhibition of CL-response. The percentage CL-stimulation and CL-inhibition represents the magnitude of oxidant and antioxidant property, respectively. The results indicate that Propylthiouracil inhibited the PMNLs as assessed by luminol-dependent CL-peak response of PMNLs. The percent inhibition of CL by propylthiouracil at different concentrations was dose-dependent and varied in the range of 41.71-74.99% (Table 2). The inhibitory effect of the investigated compounds is also dose dependent, but the magnitude of the antioxidative effect is less than that of propylthiouracil. Contrary to propylthiouracil, abouthiouline and abouthiouzine stimulate the CL-response at 40 μg mL^{-1} by 19.24% and 15.04%, respectively. Table 2 summarizes the effect of the different concentrations of the investigated compounds on %CL-response of human PMNLs (1×10^5 cells), with reference to propylthiouracil. The experiments on human PMNLs viability did not show any significant effect of any of these compounds at different concentrations.

Quantitative relationship between the % CL-response and their E-state indexes of thiourylene moiety ($S_{N\&S}$): The E-state value reflects the consequences of the structural changes of each atom that lead to a mosaic of E-state values throughout the molecule in a given series. Scheme 1 shows the influence of structural changes on the E-state indexes of thiourylene moiety ($S_{N\&S}$) of the new drugs and the currently used antithyroid drugs due to the addition of different alkyl or functional groups, or tautomeric changes.

Figure 1 shows the relationship between the %CL-response of human PMNLs and the E-state indexes of thiourylene moiety ($S_{N\&S}$) of the investigated antithyroid compounds with respect to propylthiouracil. Therefore, the magnitude of antioxidant and oxidant activities of antithyroid drugs depends on the value of the E-state indexes of thiourylene moiety ($S_{N\&S}$) and the concentration of the drug. The quantitative relationship between the antioxidant/oxidant activities and these variables are shown in Fig. 2. The linear regression parameters and analysis of variance of the quantitative relationship are

Table 2: The effect of the investigated compounds on %CL-response (mean±SEM) of human PMNLs (1×10^5 Cells), stimulated with PMA and with reference to propylthiouracil at different concentrations

Drug	Stimulation (+) or Inhibition (–)				
Concentration	PTU	Abouthiouzine	Abouthiouline	(I)	(IV)
5 μg mL^{-1}	-41.71±2.95	+49.55±3.17	-8.40±2.70	-7.32±3.74	-26.81±3.63
10 μg mL^{-1}	-49.01±3.09	+35.73±2.78	-14.92±2.51	-8.05±4.56	-35.80±2.39
20 μg mL^{-1}	-64.83±1.78	+36.81±4.09	-2.34±2.97	-11.37±2.62	-36.77±1.85
40 μg mL^{-1}	-74.99±2.01	+15.04±4.59	+19.24±7.14	-24.07±3.94	-39.66±3.01

The % CL response was calculated from the integrated area under the CL curve of tested compounds [$(AUC\text{-}CL)_t$] and from that of the control [$(AUC\text{-}CL)_c$] according to % CL = 100 [$(Avc\text{-}cL)_t$-$(AUC\text{-}CL)_c$]/$(AUC\text{-}CL)_c$]; where stimulation = (+), represents the magnitude of oxidant property and inhibition = (–), represents the magnitude of antioxidant property

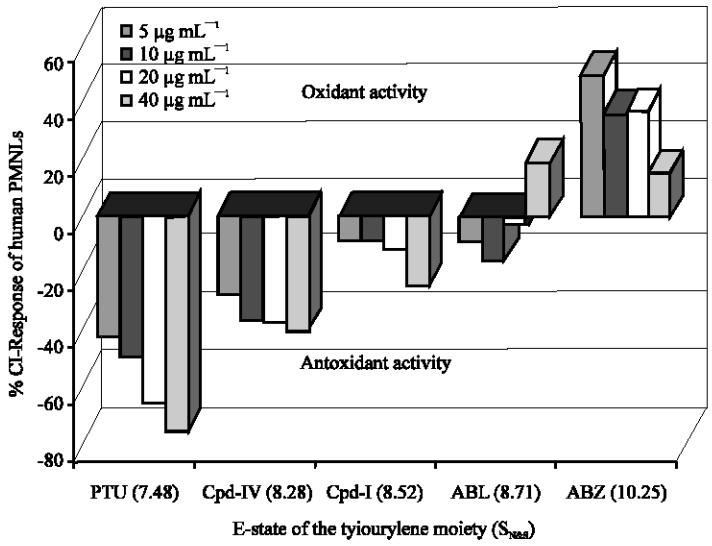


Fig. 1: Antioxidant and oxidant activity (expressed as %CL-response of human PMNLs) of the thiourylene-type compounds in Table I in relation to their E-state indexes ($S_{N\&S}$). (PTU) is propylthiouracil; (ABZ) is abouthiouzine and (ABL) is abouthiouline

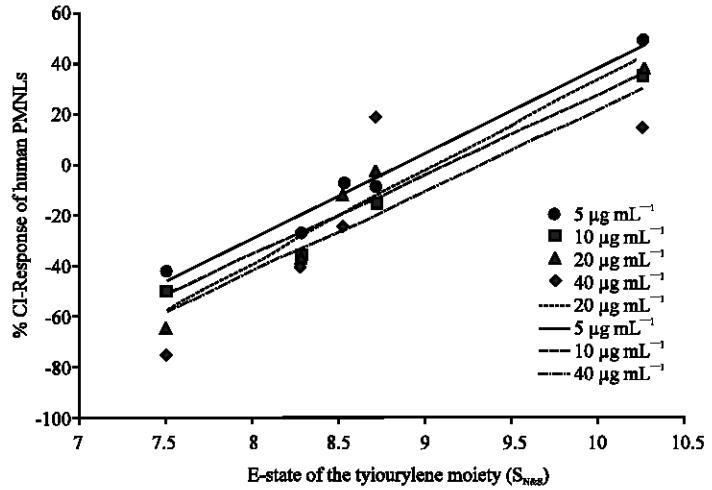


Fig. 2: Quantitative relationship between the %CL-response of human PMNLs treated with the thiourylene-type compounds in Table 1 and their E-state indexes ($S_{N\&S}$). The E-state of thiosemicarbazone moiety [($S_{N\&S}$)+(S_{NH})] of Abouthiouzine was utilized

summarized in Table 3. Such quantitative relationship could be used as a predictive guideline for the antioxidant/oxidant properties of the thiourylene type compounds. From the linear regression equation for the relationship between the %CL-response of human PMNLs and the E-state indexes of thiourylene moiety ($S_{N\&S}$) of the five investigated antithyroid compounds (r^2 = 0.976), 97.6% of the oxidant/antioxidant data can be explained.

Studies on the phagocytic activity in the human PMNLs: These results clearly show that the phagocytic activity induced by propylthiouracil at different con-centrations had not been significantly altered compared with the control value. On the other hand, our compounds (I, IV, abouthiouline and abouthiouzine) were found to induce a dose-dependent suppression of the phagocytic activity of PMNLs (Table 4).

Studies on the frequency of micronuclei and the proliferative activity in femoral cells in mice: The micronucleus test is one of the important *in vivo* techniques for a rational and expeditious approach to

Table 3: Linear regression parameters and analysis of variance of the quantitative relationship between the antioxidant/oxidant magnitude (expressed as %CL-response of Human PMNLs) and the E-state of $S_{N\&S}$ of the new compounds and PTU at various concentrations (μg mL^{-1})

Drug Conc. (μg mL^{-1})	Regression parameters and analysis of variance						
	Constant	Slope	r	SEM	n	F	p (sig)
5	-299.8±26.15	33.86±3.01	0.988	6.09	5	126.6	0.001 (S)
10	-284.8±36.39	31.27±4.19	0.974	8.46	5	55.8	0.005 (S)
20	-332.16±45.81	36.59±5.27	0.97	10.65	5	48.3	0.006 (S)
40	-297.19±111.6	31.95±12.8	0.82	25.95	5	6.2	0.089 (NS)

* E-state of thiosemicarbazone moiety of Abouthiouzine (E-state of $S_{N\&S}$ +S_{NH} = 10.25) was utilized in the regression. S = Significant, NS = Not Significant

Table 4: Effect of abouthiouzine, abouthiouline and synthetic thiourea compounds (I and IV) on the phagocytic activity of human PMNLs (*in vitro*) as compared with propylthiouracil

Drug	Control	Phagocytic Yeast uptake (Mean±SEM) at different concentrations[a] (μg mL^{-1}) (n = 8)				
		40	20	10	5	2.5
Propylthiouracil	46.71±2.41	41.2±2.92	41.1±2.81	43.3±2.35	44.9±2.36	42.0±2.32
Compound I	46.71±2.41	13.6±1.48**	13.8±1.61**	21.7±2.1**	25.8±2.12**	31.2±2.56**
Abouthiouline	46.71±2.41	17.4±1.57**	22.1±1.98**	31.0±2.07**	35.9±2.3*	36.6±2.08*
Compound IV	46.71±2.41	24.7±1.8**	28.8±1.88**	29.0±1.8**	29.2±2.86**	35.2±2.12**
Abouthiouzine	46.71±2.41	35.5±2.5*	35.9±2.19**	37.6±2.56*	35.4±2.11**	37.6±1.97*

Results are expressed as percentage of cell population showing ingestion. [a]Final concentration of the drug in the reaction mixture, Opsonized yeast = 2×10^5 particles/ml and PMNLs = 5×10^6 cells/mL * $p<0.01$, ** $p<0.001$. (Student's t-test)

carcinogenicity and oxidative stress testing as well as it will identify a high proportion of human carcinogens and proliferative agents (WHO, 1991). In this test, the polychromatic erythrocytes (PCE) were screened for micronuclei as evidence of clastogenic activity. The ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) is used to analyze for bone marrow depression due to cytotoxic or proliferative activities.

Results in Table 5 clearly reveal that the treatment with thyroxine, propylthiouracil, methimazole, compound I, compound IV, abouthiouline and abouthiouzine failed to induce any significant effect on the frequency of micronuclei in PCE as compared with the control (group 1). This indicates that all tested agents are not clastogenic. However, the number of normochromatic erythrocytes was found to decrease after treatment with thyroxine, propylthiouracil and methimazole indicating a significant increase in the ratio of PCE/NCE in femoral cells compared to the control group, whereas the newly synthesized thiourea derivatives failed to significantly increase the ratio of PCE/NCE. The PCE/NCE ratio obtained after treatment with compound I, compound IV, abouthiouline and abouthiouzine was comparatively less than that induced by thyroxine, propylthiouracil and methimazole. These differences were statistically significant ($p<0.05$) compared with thyroxine and methimazole.

Studies on the biochemical effects on hepatic cells in mice: These results demonstrate that the levels of nucleic acids after treatment with propylthiouracil, methimazole, thyroxine and compound IV increased significantly as compared with the values obtained for the control (group 1). However, abouthiouline and abouthiouzine showed no adverse effect on the level of nucleic acids (Table 6). The differences were statistically significant as compared with propylthiouracil, methimazole and thyroxine.

The levels of protein after treatment with propylthiouracil, methimazole, thyroxine, compound I and abouthiouline have shown significant reduction as compared with control group. Comparison with thyroxine revealed a statistically significant reduction in the levels of nucleic acids after treatment with compound I, compound IV, abouthiouline and abouthiouzine. The levels of proteins and nucleic acids observed after treatment with these compounds were also significantly less than propylthiouracil and methimazole.

Comparative analysis of antioxidant and proliferative activities: The results obtained in the present study on chemiluminescence's production clearly demonstrated the inhibitory effects of propylthiouracil on PMNLs respiratory burst, thus confirming the earlier reports on propylthiouracil free radical scavenging property. Compound I, compound IV and abouthiouline also inhibited the CL-response at low doses; however the intensity of inhibition was comparatively less than that of propylthiouracil. Conversely, Abouthiouzine (at all concentrations) and abouthiouline (at high concentration, 40 μg mL^{-1}) were found to stimulate PMNLs' respiratory burst which leads to the production of ROIs that cause the depletion of the intracellular glutathione (GSH) and might probably cause decrease in lymphatic proliferation (Abou-Shaaban, 1995; Fidelus and Tsan, 1986; Suthanthiran *et al*., 1990).

Table 5: Effect of abouthiouzine, abouthiouline and synthetic thiourea compounds (I and IV) on the frequency of micronuclei in femoral cells of mice as compared with propylthiouracil, methimazole and thyroxine

Treatment and dose, (mg kg^{-1} daily)	Polychromatic erythrocytes (PCE) screened	Percentage of micronucleated PCE (mean±SEM)	Normochromatic erythrocytes (NCE) screened	PCE/NCE ratio (mean±SEM)
1. Control (1% Tween 80)	5456	0.30±0.05	6011	0.91±0.08
2. Thyroxine (77.40 μg kg^{-1})	5083	0.29±0.03	3829	1.33±0.05***
3. Propylthiouracil (10)	4845	0.28±0.04	4037	1.20±0.09*
4. Methimazole (6.71)	5256	0.30±0.04	4452	1.20±0.07*
5. Compound I (14.00)	5056	0.29±0.03	5006	1.01±0.06¶¶¥
6. Abouthiouline (16.70)	6192	0.32±0.04	5954	1.04±0.04¶¶¥¥
7. Compound IV (14.28)	5717	0.26±0.04	5445	1.05±0.09¶
8. Abouthiouzine (14.80)	4908	0.35±0.04	4746	1.04±0.04¶¶¥¥

Groups 2-8 were statistically compared with group 1; groups 5-8 were statistically compared with group 2 and 4. * p<0.05, ** p<0.01, *** p<0.001. (compared with group 1). ¶ p<0.05, ¶¶ p<0.01, ¶¶¶ p<0.001. (compared with group 2). ¥ p<0.05, ¥¥ p<0.01, ¥¥¥ p<0.001. (compared with group 4). Student's t-test was used for all comparisons

Table 6: Effect of abouthiouzine, abouthiouline and synthetic thiourea compounds (I and IV) on proteins and nucleic acid contents in liver of mice as compared with propylthiouracil, methimazole and thyroxine

Treatment and dose, (mg kg^{-1} daily)	Proteins (mg/100 mg)	RNA (μg/100 mg)	DNA (μg/100 mg)
1. Control (1% Tween 80)	19.77±0.94	695.47±17.21	141.82±6.14
2. Thyroxine (77.40 μg kg^{-1})	16.24±0.45**	1101.53±21.72***	199.90±15.97**
3. Propylthiouracil (10)	22.69±1.03*	848.63±28.93**	225.57±9.69***
4. Methimazole (6.71)	22.82±0.93*	796.24±4.11***	191.48±13.58**
5. Compound 1 (14.00)	16.47±0.44*§§§¥¥¥	630.48±34.30¶¶¶§§§¥¥¥	147.28±3.51¶¥
6. Abouthiouline (16.70)	16.83±0.17*§§§¥¥¥	707.73±21.82¶¶¶§§¥¥	141.13±1.89¶¶§§¥¥¥
7. Compound 1V (14.28)	18.08±0.81§§¥¥	855.47±22.67***¶¶¶¥	223.05±13.78***
8. Abouthiouzine (14.80)	17.58±0.76§§¥¥	724.61±9.89¶¶¶§§¥¥¥	148.30±2.91¶§§¥

Groups 2-8 were statistically compared with group 1; groups 5-8 were statistically compared with group 2, 3 and 4. * p<0.05, ** p<0.01, *** p<0.001. (compared with group 1). ¶ p<0.05, ¶¶ p<0.01, ¶¶¶ p<0.001. (compared with group 2). § p<0.05, §§ p<0.01, §§§ p<0.001. (compared with group 3). ¥ p<0.05, ¥¥ p<0.01, ¥¥¥ p<0.001. (compared with group 4). Student's t-test was used in all comparisons

It is evident in literature that antioxidant property of a compound is known to increase the endogenous levels of Interleukin II and GSH and stimulate phagocytosis (Wilson *et al*., 1990; Abou-Shaaban, 1995; Wu *et al*., 1989). Hence it appears to be logical that the increase in the intracellular GSH and consequent lymphocyte proliferation induced by compound I, compound IV and abouthiouline will be comparatively less than that of propylthiouracil. In addition, results on the CL-response are further supported by the phagocytic activity of different compounds on the human PMNLs. This study clearly shows that propylthiouracil did not alter the phagocytic activity of PMNLs, whereas our agents, namely, compound I, compound IV, abouthiouline and abouthiouzine were found to suppress this activity. The stimulation of phagocytosis by propylthiouracil observed in the present study may be due to its antioxidant nature.

The results obtained in these studies on the frequency of micronuclei and the proliferative activities in femoral cells demonstrated the lack of genotoxic effects in any of the compounds under investigation. However, the PCE/NCE ratio in the femoral cells was significantly increased after treatment with thyroxine, propylthiouracil and methimazole, thus confirming earlier reports on the proliferation of cells and antioxidant property of these compounds (Wilson *et al*., 1990; Van-Haaster *et al*., 1992). The thionamide group of goitrogens like carbimazole (methimazole as the metabolite), thiouracil and propylthiouracil are known to increase thyrotropin (TSH) output from the anterior pituitary body. Earlier studies have shown thyrotropin to mediate the mutagenic stimulation (Redmond and Tuffery, 1981). Whereas compound I, compound IV, abouthiouline and abouthiouzine failed to significantly increase the ratio of PCE/NCE in the femoral cells as compared with control. The PCE/NCE ratio obtained after treatment with these compounds was significantly less than that obtained in the thyroxine and methimazole groups. These results are supported by the studies on biochemical analysis where the treatment with thyroxine, propylthiouracil and methimazole were found to increase the levels of nucleic acids. Furthermore, earlier studies revealed that compounds known for their antioxidant property are also known to increase the levels of nucleic acids and proteins (Matty *et al*., 1982). A comparison of the effect on nucleic acids and proteins between thyroxine, propylthiouracil and methimazole and compound I, compound IV, abouthiouline and abouthiouzine revealed significantly lower values of nucleic acids and proteins in the latter. In the absence of related studies, it is difficult to interpret the exact mechanism. However, it appears that reduced antioxidant property of these compounds may be responsible for the lower levels of nucleic acids and proteins and reduced proliferative activity.

Agranulocytosis is described to be an autoimmune function which involves antineutrophil-antibodies and lymphocyte sensitization to antithyroid drugs (Wall *et al*., 1984). Propylthiouracil and methimazole-induced

agranulocytosis are reported to be related to their dose and concentration in the granulocytes (Lamm and Lindsay, 1979). The agranulocytosis caused by propylthiouracil and methimazole appears to be due to the free radical scavenging property of these drugs (Abou-Shaaban, 1995). Earlier studies have shown antioxidant property of drugs to be related to proliferation of cells and production of antibodies (Fidelus and Tsan, 1986; Cooper, 1984). Present investigation was based on the hypothesis that both the cyclic thioamide structure (Wall *et al*., 1984) as well as the antioxidative and proliferative activity (Wilson *et al*., 1990) may be responsible for antithyroid drugs induced agranulocytosis. We have introduced new antithyroid drugs with cyclic thioamide structure that have reduced antioxidant properties by structural manipulation (Abou-Shaaban *et al*., 1995, 1996) in order to decrease the production of antibodies and consequent reduction of agranulocytosis. Our compounds showed reduction of antioxidative potential and mutagenic activity indicating a comparatively lesser possibility of the production of antibodies that are responsible for the agranulocytosis conditions. Further studies are warranted to determine the exact mode of action of these compounds before clinical trials to suppress agranulocytosis.

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