Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 76 (10) 1355–1364 (2011)
JSCS–4210
UDC 547.21.024+547.857+
547.367:611.4+615.252
Original scientific paper

Antithyroid activity of some 6-(alkylsulfanyl)-9*H*-purines

ISMAT FATIMA[1], MUNAWAR A. MUNAWAR[1]*, AFFIA TASNEEM[2], SARWAT JAHAN[3], MISBAHUL A. KHAN[1] and SHAKEEL AHMED[3]

[1]*Institute of Chemistry, University of the Punjab-Lahore,* [2]*CENUM, Mayo Hospital -Lahore and* [3]*Department of Animal Sciences, Quid-i-Azam University Islamabad, Pakistan*

(Received 13 December 2010, revised 4 February 2011)

Abstract: Some alkyl and aryl derivatives of 9*H*-purine-6-thiol were synthesized and evaluated *in vitro* and *in vivo* for potential antithyroid effects. Spectrophotometric studies demonstrated 1:1 charge transfer complexation between iodine and these compounds with quite high values of the formation constants. The blood assays of rats treated with these compounds revealed significant antithyroid activity for almost all the compounds, which was further supported by a histological study of the thyroid tissues of the animals. These compounds are expected to provide less toxic alternative of the existing medicines as the sulfanyl group, which is known to be a cause of toxicity of many drugs, is blocked by alkyl/aryl substituents.

Keywords: 9*H*-purine-6-thiol; derivatives; iodine; antithyroid.

INTRODUCTION

Hyperthyroidism is a major dysfunction of the thyroid gland in which the gland produces more hormones than is normally required by the body for its normal metabolic functions as well as mental and physical growth. Thyroid disorder disturbs not only other glands of endocrine system, but also other organs since they act on nearly every cell in the body.[1] The available treatments for hyperactive thyroid are: thyroidectomy (surgical removal of a part of or the whole gland), radioactive iodine therapy and antithyroid drugs. The former two modes provide permanent treatment but have serious drawbacks. The side effects of radioiodine are the development of tumors, leukemia, thyroid cancer and birth defects in women but the reported incidences are low.[2] On the other hand, surgery may lead to tracheal compression due to bleeding, infection, bilateral vocal fold paralysis and superior laryngeal nerve damage. Perpetual hypothyroidism is common for both these treatments.[3,4] Moreover, certain hyperthyroid conditions

* Corresponding author. E-mail: mamunawar.chem@pu.edu.pk
doi: 10.2298/JSC101209041I

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are not permanent and do not need surgery or radioiodine therapy. The use of antithyroid drug becomes the only choice under such conditions but there is a scarcity of drugs in this particular area. Propylthiouracil, methimazole (MMI) and carbimazole are the only widely used synthetic antithyroid agents (SATs).[5] These drugs have many serious side-effects, such as agranulocytosis, liver damage, aplastic anemia and vasculitis, while minor side-effects, such as itching, rash, hives, pain and swelling in joints, fever, change in taste, nausea and vomiting have been observed in fifteen percent of the patients treated with these drugs.[6–8] The presence of free SH group in these drugs is reported to be the cause of the toxic side effects, as with many other compounds containing the sulfonamide group.[9] For this reason, an earnest need was felt to search for new, less toxic and more effective SATs. Many compounds were evaluated and some were reported to possess recognizable antithyroid activity.[5,10] SATs are supposed to act either through inhibition of the thyroperoxidase enzyme or by making stable charge transfer (CT) complexes with iodine in which iodine acts as a σ-acceptor and the synthetic compound as an *n*-donor.[11] Although, many drugs, such as levamisole, tetramethylthiourea, tetrahydrozoline and phenothiazines, have no effect on peroxidase yet exhibit strong antithyroid activity *in vivo* due to complexation with iodine.[12] Studies of CT complexation of various drugs having a thiazole or imidazole ring with iodine using UV/visible spectroscopy revealed a positive correlation between the formation constant (K_c) and *in vivo* antithyroid activity. Compounds having $K_c \geq 100$ L mol^{-1} were found to exhibit recognizable antithyroid activity.[13] MMI acts predominantly through CT complexation with a K_c value of 23193 L mol^{-1}.[14] Heterocyclic compounds, including purines, form CT complexes with iodine.[15–17] Similarly, the medicinal value of certain purines and purine derivatives, including 6-mercaptopurine (6MP, 9*H*-purine-6-thiol); against HIV-1, cancer, bacteria and miscellaneous microbes was also reported.[18–21] In light of the above, it was proposed to derivatize 6MP and to evaluate the antithyroid potential of the formed compounds. These compounds were expected to form stable CT complexes with iodine, like other heterocyclic compounds, which is a clue to antithyroid activity. Moreover, the suspected toxic effects due to free –SH group were ruled out by blocking this site with alkyl substituents. This also led to 1:1 complexation as the only site for an n–σ complex was available at the N-9 position.

EXPERIMENTAL

Materials, instruments and methods

Iodine (suprapur, bisublimed) was obtained from Merck and kept in dark in a dessicator containing P_2O_5. Dimethyl sulfoxide (DMSO) of spectroscopic grade was obtained from Merck. It was dried over calcium hydride, distilled under reduced pressure and stored over type 4Å molecular sieves. 9*H*-Purine-6-thiol monohydrate (6MP) was obtained from Sigma-Aldrich and was used without further purification. 1-Methyl-1*H*-imidazole-2-thiol (MMI, methima-

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zole) of analytical grade was purchased from Sigma-Aldrich and used without further purification for the *in vivo* study. Free T3 and T4 kits of Immunotech (France) and rat-TSH ELISA kit from Cusabio Biotech Co. Ltd. were used.

The spectra were recorded on a double beam UVD-3500 spectrophotometer (Labomed Inc.) and the slides of thyroid tissues were studied under an Olympus BX51microscope fitted with an Olympus DP12 digital camera. The melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The ^{1}H-NMR and ^{13}C-NMR were determined in DMSO-d_6 using TMS as the internal standard on a Bruker Avance 300 (300 MHz for ^{1}H- and 75 MHz for ^{13}C-NMR). The mass spectra were recorded on a Bruker Esquire 300+ ion trap with ESI ionization. Microanalysis for carbon, hydrogen and nitrogen was realized on a Perkin Elmer 2400-CHN analyzer.

*Synthesis of 9*H*-purine-6-thiol derivatives*

9*H*-Purine-6-thiol monohydrate (0.170 g, 1 mmol) in aqueous sodium hydroxide (2 M, 10 mL) was stirred at room temperature with the respective alkyl halides (1 mmol). The clear solution was stirred until the insoluble alkyl halides disappeared. After completion of the reaction, the pH of the solution was adjusted to 5.0 by the addition of glacial acetic acid and the white precipitates were filtered and recrystallized. All the compounds (Fig. 1) were synthesized using literature methods,[22–25] except for (9) which was not found in the literature.

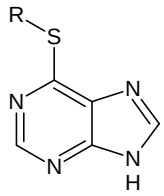

Fig. 1. General structure of the 9*H*-purine-6-thiol derivatives (R: **1**, CH_3; **2**, C_2H_5; **3**, C_3H_7; **4**, C_4H_9; **5**, C_5H_{11}; **6**, C_6H_{13}; **7**, C_7H_{15}; **8**, C_8H_{17}; **9**, C_9H_{19}; **10**, $C_{10}H_{21}$; **11**, $PhCH_2$.

Assessment of antithyroid activity

In vitro. The *in vitro* activity was assessed by studying spectrophotometrically the complexation of the compounds with iodine. Solutions of iodine and the compounds were prepared just before the start of the experiment by diluting accurately prepared stock solutions in DMSO. The iodine concentration was kept constant (2×10^{-5} M), while those of the compounds were varied between 1×10^{-4} M and 1×10^{-3} M. The reactions were performed directly in the spectrophotometric cell by mixing 1.5 mL of the compound solutions and iodine solution and the spectra were recorded immediately. New absorption bands appeared which demonstrated the formation of CT complexes between iodine and the compound(s). The stoichiometry of the complex was ascertained by the method of continuous variations.[26]

In vivo. The *in vivo* study was performed on young male Wistar rats of 175±25 g weight. The animals were divided into fifteen groups, *i.e.*, the control, the vehicle control (solvent treated), the MMI treated and twelve groups for the compounds under study (6MP and **1**–**11**). MMI was used as a positive control, so that the efficacy of the potential compounds could also be compared with the most popular existing drug. Five animals were allocated to each group and were fed chick feed with water *ad libitum*. Solutions of the compounds and MMI in DMSO were administered *via i.p.* injection to the respective groups at a dose rate of 20 mg kg^{-1} per animal daily in the morning for 15 days. The control and vehicle control categories received an equivalent dose of normal saline and the solvent, respectively, for the same duration. On the 16th day, the animals were weighed and carried to the dissection room for blood

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sampling and dissection. Due time was allowed before blood sampling to avoid hormonal changes because of any stress caused by transportation. Blood samples were collected from all the animals by puncturing the abdominal aorta under light diethyl ether anesthesia. Standard animal protocols were adopted for the experimentation.[27] Free T3 and T4 levels were determined using radioimmunoassay technique, while that of TSH by the ELISA method. The animals were subsequently sacrificed on the same day under deep diethyl ether anesthesia and the thyroid was removed, washed and weighed. Sections of the gland were fixed in 4 % paraformaldehyde (PFA) solution for 4–6 h and stained with hematoxylin and eosin to prepare slides for histological studies.

RESULTS AND DISCUSSION

All the compounds (Fig. 1) were synthesized using literature methods except **9**, which is not known to be reported. Previously, confirmation of the structures of the known compounds was established using melting point and elemental analysis only. Complete spectral information, including mass spectrometry, ^{1}H-NMR, ^{13}C-NMR and CHN, were determined in the present study for detailed structure elucidation. The results are given in the Supplementary material.

All compounds absorbed in the UV region between 280 and 330 nm. The compounds **1**–**11** exhibited 1:1 complexation with iodine. 6MP also showed complexation but not of 1:1 stoichiometry. The compounds were soluble only in DMSO, therefore, its use as a solvent was inevitable, apart from its interaction with I_2, like many other aromatic and alkyl halide solvents.[28] A solution of iodine in DMSO gave sharp peaks at 295 and 365 nm, which are characteristic of the I_3^-.[29] Occasionally other peaks around 250–255 nm were also observed. The interaction of DMSO with iodine is a slow process resulting in a weak complex.[30,31] It was observed that in the presence of strong *n*-donors, its interaction with I_2 becomes very restrained. The CT band generally appears at 265–270 nm. The reaction mixture exhibited negligible absorption for the compound at concentrations below 2×10^{-4} M. On increasing the concentration of compounds ($\geq 3\times10^{-4}$ M), two peaks appeared usually at 300 and 270 nm, the latter being characteristic of complex formation.

The formation constants and molar extinction coefficients of the complexes were determined using the Lang method.[32] This method derives a mathematical expression to calculate K_c for 1:1 stiochiometric complexes:

$$K_c = [C]/([I_0]–[C])([P_0]–[C]) \quad (1)$$

where, $[I_0]$ and $[P_0]$ are the initial concentrations of iodine and the compound(s), respectively, whilst [C] is the concentration of the complex. Now, according to Beer–Lambert Law:

$$[C] = \varepsilon_c d_c$$

where d_c and ε_c are the absorbance and extinction coefficient of the complex, respectively. The value of d_c was calculated by subtracting the absorbance due to

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free iodine present in the mixture from the observed absorbance of the reaction mixture. The solution of pure compound of same concentration is kept in the reference cell of the spectrophotometer. Now Eq. (1) can be re-written in the form:

$$Y = (1/\varepsilon_c)\cdot X + 1/(K_c\varepsilon_c) \quad (2)$$

where $Y = [I_0][P_0]/d_c$ and $X = [I_0] + [P_0] - d_c/\varepsilon_c$

Equation (2) is an equation of a straight line with slope $1/\varepsilon_c$ and *Y*-intercept $1/(K_c\varepsilon_c)$. First, the equation was solved using an assumed value of ε_c. This gave not only a value for K_c, but also a new value of ε_c was obtained. This new value differed from the old assumed value of ε_c. Now, the new value of ε_c was used to solve the equation and so forth until both ε_c and K_c converged to discrete values (Table I). This is a lengthy process and was performed by developing a computer-based iterative algorithm, which not only calculated the ε_c and K_c values, but also the values of *X* and *Y*. A linear regression curve gave the best fit for the *XY* scatter ($R^2 > 0.99$), thus confirming 1:1 complexation between iodine and the compounds **1**–**11**.

TABLE I. Spectrophotometric properties of the CT complexes of the studied compounds with I_2

Compound	*CTB*[a] / nm	K_c / 10^4 L mol^{-1}	ε_c[b] / 10^5 L mol^{-1} cm^{-1}
1	265	1.031	0.552
2	270	0.250	1.52
3	315	0.458	1.301
4	265–70	0.562	1.129
5	315	0.642	1.205
6	265–70	0.279	1.121
7	265–70	0.479	0.876
8	265–270	0.256	1.255
9	265–70	0.536	1.0452
10	265–70	0.962	0.635
11	265–70	2.335	0.794

[a]Charge transfer band; [b]molar extinction coefficient

The antithyroid effects of potential compounds can be ascertained *in vivo* by a decrease in the thyroid hormones, which is further confirmed by a corresponding increase in the pituitary hormone (TSH) levels. Similarly, regular exposure to SATs is known to increase the thyroid weight and change the thyroid histology.[33] In the present study, due consideration was given to all these aspects.

The radioimmunoassay results showed a decrease of free T_3 and T_4 levels in the serum of the treated groups, as compared to the control and vehicle control animals. In addition, a relative increase was observed in the TSH levels for the treated groups (Table II), which is a clear sign of the antithyroid potential of the studied compounds.

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The hormonal variations were quite explicit, yet the data was analyzed statistically using the Dunnett test to further validate the results. This is a popular and useful hypothesis testing method used for comparing the treatments with a control when the sample size is the same in the control and the treatments. The Dunnett *D* value is:

$$D = d_{\alpha}(k,v)(2S_w^2/n)^{1/2}$$

where, $d_{\alpha}(k,v)$ is the critical Dunnett value at a given significance level (α), the number of non-control treatments (k) and the degree of freedom (v); n is the sample size and S_w^2 is the combined estimate of the common variance.[34]

TABLE II. Hormonal variations observed after 15 day dose administration (n = 5, dose rate = = 20 mg kg^{-1})

Animal group	Mean hormone levels		
	FT_3 / pmol L^{-1}	FT_4 / pmol L^{-1}	TSH / μ i.u. mL^{-1}
Control	8.41[a]±0.64[b]	35.86±2.14	1.64±0.32
Vehicle control	8.15±0.81	34.09±1.67	1.70±0.23
	Treatment[c]		
6MP	5.86±0.33	24.49±2.48	2.99±0.27
1	5.01±0.65	24.32±2.31	2.93±0.45
2	6.78±0.90	26.66±2.82	2.40±0.58
3	6.01±0.97	25.92±2.23	2.59±0.71
4	5.82±0.92	26.67±2.34	2.62±0.34
5	5.88±0.71	25.16±3.26	2.68±0.39
6	6.69±0.81	26.96±2.58	2.40±0.56
7	5.90±0.93	26.77±2.39	2.56±0.38
8	6.79±0.85	27.50±2.40	2.38±0.56
9	5.89±1.17	26.99±2.14	2.40±0.26
10	5.37±0.97	25.00±2.08	2.88±0.25
11	3.33±1.05	16.08±2.00	3.38±0.30
MMI	3.36±0.96	17.28±1.98	3.53±0.39

[a]Mean values of assay results run in duplicate; [b]standard deviation; [c]$p \leq 0.05$ (with respect to the vehicle control)

The research hypotheses were:

H_a: $\mu_i < \mu_c$; will be true if $(y_i - y_c) \leq -D$ (for comparison of the FT_3 and FT_4 levels) and H_b: $\mu_i > \mu_c$; will be true if $(y_i - y_c) \geq D$ (for comparison of the TSH levels). Here, i and c stand for the treatment sample and the control, while μ and y for the population and sample means, respectively.

The hormone levels for all the treatments were compared with the controls using this method at $\alpha = 0.05$. The test results confirmed the efficacy of the compounds in lowering the serum free T_3 and T_4 levels with a corresponding simultaneous increase in the TSH levels. This clearly demonstrates the antithyroid potential of these compounds. However, **11** was proved to be the most potent among the whole series. It exhibited antithyroid potential almost equivalent to MMI.

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Comparison between the K_c values and the pertaining hormonal variations revealed a positive correlation between K_c and the *in vivo* antithyroid activity. The relationship was observed to be fairly linear for moderate values of K_c. All the compounds demonstrated *in vivo* antithyroid effects; the higher activity of **11** can be attributed to resonance, which resulted in a more stable equilibrium, evident from the high K_c value of the **11**:I_2 complex.

SATs are known to increase in the weight of the thyroid gland. Since thyroid weight also depends on the size of the animal, a useful index called "thyroid body index" (*TBI*) was introduced to account for the effect of body weight on the thyroid weight. *TBI* can be defined as the weight of clean thyroid tissues (mg) per 100 g of body weight. These indices for treated animals were found to be higher than those of the controls (Table III). Moreover; the *TBI* values, like those of K_c, were also found proportional to the change in hormone levels. This depicts that the *TBI* can also be used as an empirical parameter for the assessment of anti-thyroid activity. Sections from thyroids of the treated animals showed marked difference in the shape of the glandular cells and quantity of intracellular fluid (colloid). Follicular cell hyperplasia and hypertrophy of various degrees were also observed in the treated animals (Table III).

Table III. Histological observations of the thyroid tissues from different animal groups (±: slight; +: mild; ++: moderate; +++: severe)

Animal group/description	Follicular cell hypertrophy/hyperplasia	Colloid depletion	*TBI*×100 / mg g^{-1}
Control	Nil	Nil	5.21
Vehicle control	±	+	6.01
6MP	++	++	10.35
1	+++	+++	11.23
2	+	++	7.41
3	++	++	7.83
4	++	++	9.26
5	+	++	8.61
6	+	++	7.37
7	++	++	8.79
8	+	++	7.03
9	++	++	7.58
10	++	+++	11.26
11	+++	+++	14.27
MMI	+++	+++	16.11

The thyroid of the control and vehicle control animals showed a cuboidal follicular epithelium with ample quantities of colloid. On the other hand, severe colloid depletion with a semi-cylindrical to cylindrical-shaped epithelium was observed for the treated animals (Fig. 2). Very slight atrophy was noted in the follicular cells, which demonstrates the smaller toxicity of these compounds.

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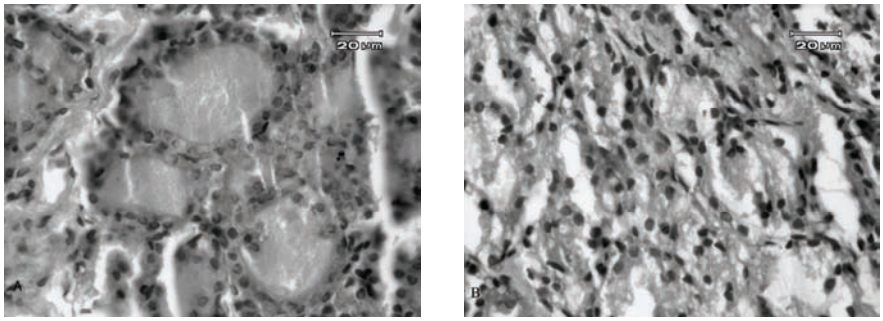
Obesity in the treated animals was also seen in the second week of treatment, without any sign of intoxication because eating and drinking habits remained normal. No animal died during the study period.



(A) (B)

Fig. 2. Microscopic view of thyroid sections: A) control with epithelium full of colloid and normal nuclei of follicular cells, B) **11**-treated animal, severe colloid depletion is visible with follicular cell hypertrophy and hyperplasia.

CONCLUSIONS

The studied derivatives of 9*H*-purine-6-thiol form 1:1 charge transfer complexes with iodine and were found to possess highly significant antithyroid activity *in vivo*, which was in good correlation with the K_C values. 6-(Benzylsulfanyl)-9*H*-purine showed the maximum antithyroid effects, comparable to that of methimazole and may have less side effects due to the blockage of the free SH group. Further research on these compounds could lead to the discovery of new drugs.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of synthesized compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgements. The authors are obliged to the Higher Education Commission of Pakistan for providing the financial assistance to realize the research. The cooperation of Mr. Syed Abbas Sultan, Muhammad Ashraf Mughal and Muhammad Rehan Adil of AEMC-Lahore (Pakistan) during the study is also acknowledged by the authors.

ИЗВОД

АНТИТИРОИДНА АКТИВНОСТ 6-(АЛКИЛСУЛФАНИЛ)-9*H*-ПУРИНА

ISMAT FATIMA[1], MUNAWAR A. MUNAWAR[1], AFFIA TASNEEM[2], SARWAT JAHAN[3], MISBAHUL A. KHAN[1] и SHAKEEL AHMED[3]

[1]*Institute of Chemistry, University of the Punjab-Lahore,* [2] *CENUM, Mayo Hospital – Lahore и* [3]*Department of Animal Sciences, Quid-i-Azam University Islamabad, Pakistan*

Синтетисани су алкил и арил деривати 9*H*-пурин-6-тиола и испитивана је њихова *in vitro* и *in vivo* антитироидна активност. Спектрофотометријска анализа је показала да се ства-

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рају комплекси између јода и ових једињења у односу 1:1, уз високу константу асоцијације. Анализа крви пацова третираних овим једињењима је показала да сва једињења имају значајну антитироидну активност, што је даље потврђено хистолошким налазима тироидног ткива. Могућа предност ових једињења у односу на постојеће антитироидне лекове је у смањеној токсичности, пошто је сулфанил група, иначе позната као узрочник токсичности многих лекова, блокирана алкил/арил супституентима.

(Примљено 13. децембра 2010, ревидирано 4. фебруара 2011)

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