Article

J. Mex. Chem. Soc. **2010**, *54(4),* 227-232

ISSN 1870-249X

Synthesis and Antithyroid Activity of Some 8-Substituted Purine Derivatives

Ismat Fatima,[1] Munawar A. Munawar[1]* and Affia Tasneem[2]

[1]Institute of Chemistry, University of the Punjab, Lahore-Pakistan
[2]CENUM, Mayo Hospital, Lahore-Pakistan. mamunawar@chem.pu.edu.pk

Received August 6, 2010; accepted September 9, 2010

Abstract. Some novel as well as known derivatives of 8-sulfanyl-3,9-dihydro-1*H*-purine-2,6-dione were synthesized and their antithyroid activity was measured in rats after administration of a daily dose of 20mg/kg via *i.p.* injection for 15 days. Free thyroxine and triiodothyronine concentrations were determined using radioimmunoassay technique. The assay results showed decrease in the hormonal concentrations for the animals treated with these compounds as compared to the control animals. Similarly, thyroid stimulating hormone level was measured with ELISA method and the results demonstrated a comparative increase of this hormone for the treated animals. The hormonal variations indicated significant ($\alpha \leq 0.05$) activity of the compounds. Histological study of thyroid tissues from the test animals showed cellular modifications in the treated animals like cylindrical shape of follicular epithelium, depletion of colloid and high values of thyroid body indices. All these factors further confirmed the antithyroid effects of the compounds under study.
Keywords: 8-sulfanyl-3,9-dihydro-1*H*-purine-2,6-dione, derivatives, antithyroid activity, thyroid hormones, histology.

Resumen. Se sintetizaron algunos derivados nuevos y ya conocidos de la 8-sulfanil-3,9-dihidro-1*H*-purin-2,6-diona, y se determinó su actividad antitiroidea en ratas, tras la administración de una dosis diaria de 20 mg/kg a través de inyección intraperitoneal durante 15 días. Las concentraciones de tiroxina y triyodotironina libres se determinaron utilizando la técnica de radioinmunoensayo. Los resultados de los ensayos mostraron una disminución en las concentraciones hormonales de los animales tratados con estos compuestos en comparación con los animales control. Del mismo modo, el nivel de la hormona estimulante de la tiroides se midió con el método ELISA y los resultados demostraron un aumento comparativo de esta hormona en los animales tratados. Las variaciones hormonales indicaron actividad significativa ($\alpha \leq 0.05$) de los compuestos. El estudio histológico de los tejidos de la tiroides de los animales de laboratorio mostró cambios celulares en los animales tratados, tales como la forma cilíndrica del epitelio folicular, la reducción en los valores de coloide y elevación de los índices del cuerpo tiroideo. Todos estos factores confirman también los efectos antitiroideos de los compuestos en estudio.
Palabras clave: 8-Sulfanil-3,9-dihidro-1*H*-purin-2,6-dione, derivados, actividad antitiroidea, hormonas tiroideas, histología.

Introduction

Hyperthyroidism is a common endocrinal disorder associated with the over production of thyroid hormones, observed in almost 1.3 percent of the population [1]. Surgery, radioiodine therapy and antithyroid drugs are the options available for the treatment of an overactive thyroid. The major antithyroid drugs namely propylthiouracil (6-propyl-2-thioxo-3,4-dihydropyrimidin-2-(1*H*)-one; PTU) and methimazole (1-methyl-1*H*-imidazole-2-thiol; MMI) are thionamide derivatives [2]. These drugs interfere with the incorporation of iodine into the tyrosine residues of thyroglobulin and therefore ceases the biosynthesis of thyroid hormones namely thyroxine (T_4) and triiodothyronine (T_3) [3, 4]. Unfortunately, the thionamides have been reported to cause many side effects including agranulocytosis (severe decrease in the production of white blood cells), liver damage (more common with PTU), aplastic anemia (failure of the bone marrow to produce blood cells) and vasculitis (inflammation of blood vessels). Up to 15 percent of the patients observe minor side effects like; itching, rash, hives, joint pain and swelling, fever, change in taste, nausea, and vomiting [5]. The scarcity of medicines in this particular area and the problems related to the existing drugs provoked many researchers to synthesize the new antithyroid agents with lesser side effects [6, 7]. Purine derivatives are reported to have antitubercular, fungicidal, antiallergic, antimicrobial, antitumor and antihistamic activities [8-10]. However, no studies were found in the recent years regarding the antithyroid effects of purine derivatives in spite of the fact that in late forties some low level antithyroid activities have been reported in adenine, guanine and some alkyl xanthines [11]. Taking the idea we started work on the antithyroid activity of purine derivatives and synthesized series of compounds as potential antithyroid agents [12]. The present study deals with the synthesis of some 8-substituted purine derivatives including some novel compounds and the measurement of their antithyroid activity in male Wistar rats. The complexation of these compounds with iodine is also studied using spectrophotometric analysis.

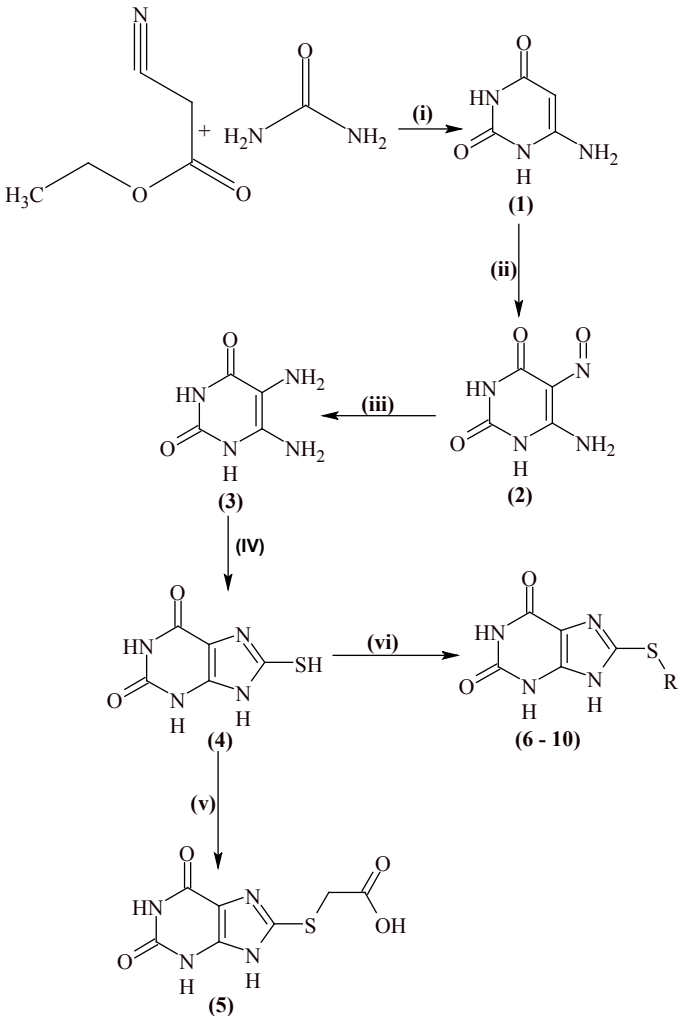
Results and Discussion

The intermediates; 6-aminopyrimidine-2,4(1*H*,3*H*)-dione **(1)**, 6-amino-5-nitrosopyrimidine-2,4-(1*H*,3*H*)-dione **(2)** and 5,6-diaminopyrimidine-2,4(1*H*,3*H*)-dione sulfate **(3)** were synthesized by known methods starting from commercially available ethyl cynoacetate and sodium ethoxide prepared from sodium metal in absolute ethanol [13]. 8-Sulfanyl-5,9-dihydro-1*H*-purine-2,6-dione **(4)** and **6-10** were also synthesized using well established procedures already described in literature [14]. 8-(Methylsulfanyl)-3,9-dihydro-1*H*-purine-2,6-dione **(6)** was already reported. However, compounds **7-10** are novel

(Scheme 1). Compound **5** was synthesized by the reaction of chloroacetic acid with 8-sulfanyl-5,9-dihydro-1*H*-purine-2,6-dione in aqueous sodium hydroxide solution and is also novel. Structures of all new compounds are established on the basis of spectral data and elemental analysis. Compounds **1-4** and **6** have been reported earlier but were characterized by the elemental analysis and mass spectrometry only, the complete spectral analysis of these compounds is being reported for the first time.

Antithyroid Activity

All the compounds showed complexation with iodine but not of 1:1 stiochiometry. There is no easy method available to determine formation constants of such complexes yet the complexation was verified using Beer-Lambert Law. Compounds **5-10** exhibited absorption maxima at 285-90 nm, while **4** at 315 nm in UV region. Two new bands, one at 265-70 nm and the other at 330-35 nm were appeared on mixing the solutions of iodine and the compounds **4** and **6-10**. The former is a characteristic charge transfer band of the complex in UV region. However, **5** did not show complexation with iodine. The complexation of the compounds with iodine provided a clue to the possible antithyroid effects of the compounds.



Scheme 1. Synthesis of 8-(alkylsulfanyl)-3,9-dihydro-1*H*-purine-2,6-diones.
Reagents and conditions: (i) Na, EtOH, 3 hours reflux, CH_3COOH; (ii) $NaNO_2$, H_2O, CH_3COOH, r.t.; (iii) NaS_2O_3, conc. H_2SO_4, H_2O Δ, (iv) $EtOH/H_2O$, KOH, CS_2, Δ in boiling water bath, 1 h; (v) $ClCH_2COOH$, NaOH, CH_3COOH, H_2O, 2 h, r.t., (vi) R-I, NaOH, CH_3COOH, H_2O, r.t.; R = Methyl, ethyl, n-propyl, n-butyl, n-pentyl.

Radioimmunoassay results showed a relative decrease of free T_3 and T_4 levels in the serum of the animal groups treated with the compounds and MMI as compared to control and vehicle control groups. On the other hand, TSH levels were measured with ELISA method and notable increase in the TSH levels was recorded for the treated animals with respect to control and vehicle control groups (Table 1). The hormonal variations indicate that the compounds diminished the biosynthesis of T_3 and T_4 hormones, which decreased their concentration in the serum. In response, the TSH secretion increased than the normal to send a more powerful signal to the thyroid to increase its activity. Though the hormonal variations were quite explicit yet the data was further analyzed statistically (using Student's 't' test) to validate the inferences from the assay results. The statistical analysis of the data demonstrated the hormonal changes to be significant to highly significant ($0.05 \geq \alpha \leq 0.01$). The hormone levels of vehicle control group also showed a mild decrease in FT_3 and FT_4 levels and increase in TSH level as compared to the control. Although these changes were of only suggestive nature at this stage ($\alpha > 0.05$) even then a mild antithyroid effect of the solvent is suspected. Therefore, the hormonal changes of the treated groups were compared with vehicle control to get a more real

Table 1. Mean hormonal levels and thyroid histology as observed in treated and control animals.

Animal Groups	FT_3		FT_4		TSH	
	pmol/L		pmol/L		μi.u./mL	
	Mean	S.D	Mean	S.D	Mean	S.D
Control	9.74	0.86	37.56	4.16	1.86	0.63
Vehicle Control	8.41	1.12	33.51	3.94	2.04	0.35
4	5.32	2.37	19.8	8.74	3.37	0.71
5	6.96	0.86	27.76	6.12	2.82	0.43
6	6.9	1.49	27.75	5.99	2.99	0.63
7	7.23	0.33	29.36	5.21	2.97	0.45
8	6.67	0.7	28.01	7.23	3.25	0.35
9	6.49	2.04	26.36	6.61	3.19	0.93
10	7.33	0.47	29.46	4.48	3.07	0.96
MMI	4.34	1.18	16.64	5.93	4.11	1.07

Treatment time = 15 days, n = 5, FT_3 and FT_4 with RIA technique, TSH with ELISA.
Assays run in doublet.

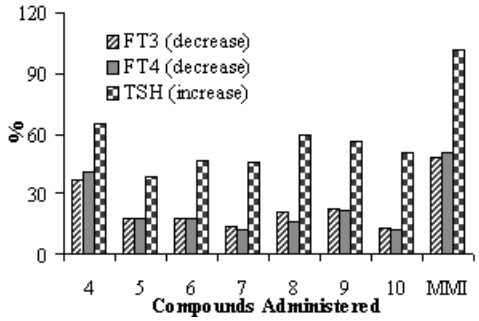


Figure 1. Mean percentage hormonal variations in the treated groups with respect to vehicle control showing fairly good antithyroid effects of the compounds.

picture, which revealed that all the compounds possess appreciable antithyroid activity in the descending order of **MMI > 4 > 8 > 9 > 10 > 6 > 7 > 5** (Fig. 1). On the basis of changes in FT_3, FT_4 and TSH values for **4** was found almost 80% as active as MMI, while antithyroid activity demonstrated by **8** and **9** was 50-60% of that of MMI.

This exposition implies that **4** has a free SH group owing to which it showed more antithyroid potential. Presence of free SH group in MMI has been described to be the reason for its antithyroid activity [15]. Moreover, it is also known that iodine makes more stable complex with *n*-donating sulfur atoms [16]. However, free SH group is suspected to be a cause of toxic side effects in many drugs, it was blocked by alkyl substituents in the derivatives **5-10**. This substitution though decreased the activity but the derivatives **5-10** still exhibited notable antithyroid effects and toxic side effects of free SH are supposed to decrease. Iodine complexation might occur at nitrogen atoms in the purine ring. Complexation with iodine as well as antithyroid activity of 6-chloropurine has already been demonstrated and the compound exhibited antithyroid activity without having a sulfur atom [17]. Nevertheless, less potent but these derivatives, especially **6-10** may serve as less toxic alternatives. The presence of carboxylic group however diminished the activity.

Histology of the thyroid from control group showed cuboidal shape of follicular epithelium with sufficient quantity of colloid (Fig. 2a). In hyperactive gland colloid is consumed and the follicular epithelium becomes cylindrical. Moreover, the number as well as size of follicular cells are increased, the conditions termed as follicular hyperplasia and hypertrophy respectively. These modifications are brought upon to meet the increased demand of hormones and are natural response of the body (gland) in hyperactive situation. The thyroid tissues of the vehicle control group were also observed to be cuboidal but with mild symptoms of hyperactivity. On the other hand, thyroid from the treated animals showed moderate to severe colloid depletion and cylindrical shape of epithelium (Fig. 2c, d, e). The microscopic study of the thyroid tissues also revealed mild to moderate follicular hyperplasia and hypertrophy for the treated animals, the conditions were found to be severe for the animals treated with MMI (Table 2). Thyroid body indices (TBIs) were calculated using final body weights of the animals and the weight of thyroid gland after washing. TBI may be defined as weight of thyroid tissues (mg) per 100 g of final body weight of the animal. Higher values of TBIs of the treated animals as compared to the control groups also depicted the antithyroid effects of the compounds (Table 2). In fact increase in the number of tissue cells as well as cell enlargement increase overall weight of the thyroid. No major deformation of cells (atrophy) was observed. Similarly, no causality was observed among the animals during the study period, which though not establishes but indicates low toxicity of the compounds.

Conclusions

8-Sulfanyl-5,9-dihydro-1*H*-purine-2,6-dione **(4)** and its alkyl derivatives represent a new class of compounds, which possess significant antithyroid activity. The in vivo findings are quite encouraging and warrant further research to synthesize different new derivatives of this class as well as to assess the cytotoxic effects of these compounds for use as alternate drug.

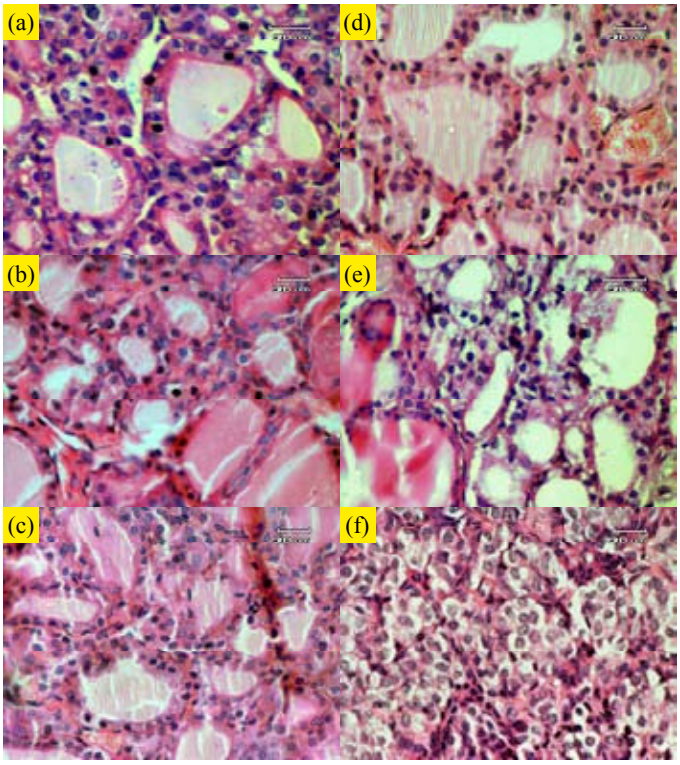


Figure 2. Thyroid sections (a) control shows normal cuboidal epithelium with sufficient colloid material, (b) vehicle control, depletion of colloid material is started (c) treated with 4, cylindrical epithelium is visible as well as lining in the epithelium depicts hyperactivity of gland. Quantity of colloid is less as compared to control (d) treated with 6 also indicates the hyperactivity and a transition towards cylindrical epithelium, (e) treated with 7 also indicates cylindrical epithelium, (f) treated with MMI shows severe depletion of colloid and some of follicular nuclei are quite thick, which indicates generation of tumor.

Experimental Section

Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR spectra were taken in DMSO-d_6 and recorded at 400 M*Hz* on a Brucker/ XWIN-NMR instrument. Chemical shifts are given in ppm relative to $(Me)_4Si$ as internal standard (abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet; chemical shifts in ppm). Mass spectra were recorded from Jeol MS Route. Elemental analyses were carried out in HEJ Research Institute of Chemistry Karachi University. Reagent grade materials were purchased from Aldrich Chemical Co. and were used without further purification. Intermediates were synthesized using reported methods and were characterized using NMR and mass spectrometry.

6-AMINOPYRIMIDINE-2, 4(1*H*, 3*H*)-DIONE(1)

The title compound was synthesized using already reported method [13]. The product was purified and subjected to the analysis.

Amorphous powder: mp > 300°C; ^{1}H NMR (DMSO-d_6, 300Mz) δ 10.05 (1H, s, NH), 10.03 (1H, s, NH), 6.15 (2H, s, NH_2), 4.40 (1H, s, CH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 164.2 (C=O), 155.1 (C),150.9 (C=O),74.1 (CH); EIMS *m/z* (rel. int.): 128 [M+1] (10), 127 $[M]^+$ (100).

6-AMINO-5-NITROSOPYRIMIDINE-2,4 (1*H*, 3*H*)-DIONE(2)

Red powder: mp > 300°C; ^{1}H NMR (DMSO-d_6, 300MHz,) δ 11.27 (2H, s, NH_2), 10.48 (1H, s, NH), 7.98 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz,) δ 162.7 (C=O), 152.8 (C=O), 149.5 (C), 140.1 (C); EIMS *m/z* (rel. int.): 151 [M-2] (14), 149 (71), 147 (14), 57 (100).

5, 6-DIAMINOPYRIMIDINE-2, 4 (1H, 3H)-DIONE SULPHATE(3)

White powder: mp > 300°C; ^{1}H NMR (DMSO-d_6, 300MHz) δ 10.53 (1H, s, NH), 8.53 (1H, s, NH), 5.92(2H, s, NH_2), 4.37 (2H, s, NH_2); ^{13}C NMR (DMSO-d_6, 75 MHz,) δ 160.8 (C=O), 149.0 (C=O), 145.5 (C), 90.4 (C); EIMS *m/z* (rel.int.): 143.15 [M+1] (4), 142 $[M]^+$(86), 127 (7), 97(25), 64 (100).

8-SULFANYL-3, 9-DIHYDRO-1*H*-PURINE-2, 6-DIONE(4)

The title compound was synthesized using already reported method [14]. Brown needles: mp> 300°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.75 (1H, s, SH), 11.25 (1H, s, NH), 11.10 (1H, s, NH), 10.92 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 100 MHZ) δ 163.8 (C), 152.5 (C=O), 150.2 (C=O), 139.2 (C), 103.6 (C); EIMS *m/z* (rel.int.) 183 [M-1] (21), 182 [M-2] (100.0)

Anal. C 32.62%, H 2.14%, N 30.42%, Calculated for ($C_5H_4N_4O_2S$) C 32.61%, H 2.173%, N 30.43%.

[(2, 6-DIOXO-2, 3, 6, 9-TETRAHYDRO-1*H*-PURIN-8-YL) SULFANYL] ACETIC ACID(5)

Chloroacetic acid (0.188 g, 2 mmol) was added to a solution of 8-sulfanyl-5,9-dihydro-1*H*-purine-2,6-dione (0.368 g, 2 mmol) in aqueous NaOH solution (10 mL, 2N) and stirred for 2 hours at room temperature. Progress of the reaction was monitored by TLC using silica gel plates. Clear solution was acidified with glacial acetic acid (pH 5.0) and the white precipitates obtained were filtered off, washed with water and dried in oven at 110°C. Product was recrystallized with water. TLC solvent system was isopropyl alcohol: water (80:20 v/v).

Table 2. Histological observations of the thyroid tissues from control, vehicle control and treated rats.

Description		Follicular Hypertrophy/ Hyperplasia	Colloid Depletion	TBI (mg/100g)
Control		Nil	Nil	5.43
Vehicle Control		Nil	±	5.91
Treated	4	++	++	11.56
	5	+	+	8.56
	6	+	++	9.63
	7	+	++	9.25
	8	++	++	10.51
	9	++	++	9.93
	10	+	++	10.05
	MMI	+++	+++	16.23

Treatment duration = 15 days, n = 5, dose rate = 20 mg/kg daily via *i.p.* injection ± Slight; + mild; ++ moderate; +++ severe.

White amorphous: mp 258-262°C (dec.); ^{1}H NMR (DMSO-d_6, 400MHz) δ 13.25 (1H, s, NH), 11.39 (1H, s, NH), 10.60(1H, s, NH), 8.31(1H, s, OH),4.65 (2H, s, CH_2); ^{13}C NMR δ (75 MHz, DMSO-d_6) 170.0 (COOH),154.6 (C), 151.11 (C), 149.9 (C=O), 149.3 (C=O), 108.8 (CH), 35.1 (CH_2); MSEI *m/z* (rel.int.) 238 [M-4] (30), 225 (6), 225 (10), 223 (100); Anal. C 34.42%, H 2.442%, N 23.124%, Calculated for $C_7H_6N_4O_4S$ C 34.71%, H 2.50%, N 23.13%.

8-(ALKYLSULFANYL)-3,9-DIHYDRO-1*H*-PURINE-2,6-DIONES(6 - 10)

General Procedure: 8-sulfanyl-5,9-dihydro-1*H*-purine-2,6-dione (0.184 g ,1 mmol) in aqueous NaOH (20 mL, 1N) was stirred at room temperature with 1mmol of alkyl iodide (1 mmol) and the reaction was monitored by TLC. The pH of the reaction mixture was adjusted (5.0) by adding glacial acetic acid; white precipitates were filtered, washed with water and dried in oven at 110 °C. The product was recrystallized in distilled water.

8-(METHYLSULFANYL)-3,9-DIHYDRO-1*H*-PURINE-2,6-DIONE(6)

The title compound was synthesized from 8-sulfanyl-5,9-dihydro-1*H*-purine-2,6-dione (0.184 g, 1 mmol) and methyl iodide (0.062 mL, 1 mmol) [18].

White amorphous: mp >300°C; ^{1}H NMR (DMSO-d_6, 300MHz) δ 13.22 (1H, s, NH),11.54 (1H, s, NH), 10.75 (1H, s, NH), 1.22 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.3 (C), 151.1 (C=O),149.4 (C=O),148.9 (C), 65.9 (CH),15.0 (CH_3); EI MS *m/z* (rel.int.) 198 $[M]^+$ (53), 197 (3), 183 (7), 85 (100);Anal. C 36.32%, H 2.99%, N 28.25%, Calculated for $C_6H_8N_4O_2S$, C 36.36%, H 3.05%, N28.27%.

8-(ETHYLSULFANYL)-3,9-DIHYDRO-1*H*-PURINE-2,6-DIONE(7)

The title compound was prepared from 8-sulfanyl-5,9-dihydro-1*H*-purine-2,6-dione (0.184 g, 1 mmol) and ethyl iodide (1 mmol, 0.794 mL, 0.155 g) while stirring at room temperature.

White powder: mp 290 °C, ^{1}H NMR (DMSO-d_6, 300MHz) δ 11.49 (1H, s, NH),10.72 (1H, s, NH), 3.18 (2H, q, J = 7.20, CH_2), 1.30 (3H, t, J = 7.20, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.33 (C), 151.0 (C=O), 149.41 (C=O), 148.89 (C), 107.9 (CH), 25.2 (CH_2), 15.0 (CH_3); EIMS *m/z* (rel.int.) 212 $[M]^+$ (23), 197 (15), 184 (10), 179 (15), 44 (100); Anal. C 39.62%, H 3.78%, N 26.43 %, Calcd for $C_7H_8N_4O_2S$, C 39.61%, H 3.80 %, N 26.40 %, O 15.08%, S 15.11%.

8-(PROPYLSULFANYL)-3,9-DIHYDRO-1*H*-PURINE-2,6-DIONE(8)

The title compound was prepared using 8-sulfanyl-5,9-dihydro-1*H*-purine-2,6-dione (0.184 g, 1 mmol) and 1-iodopropane (0.0969 mL, 1 mmol) by the same general procedure.

White amorphous: mp 288°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.23 (1H, s, NH), 11.49 (1H, s, NH), 10.71 (1H, s, NH), 3.13 (2H, t, J = 7.20, SCH_2), 1.67 (2H, q, J = 7.20 CH_2CH_3), 0.96 (3H, t, J = 7.2, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 154.3 (C), 151.0 (C=O), 149.3 (C=O), 148.9 (C), 107.9 (CH), 33.3 (CH_2), 22.6 (CH_2), 12.9 (CH_3); EI MS *m/z* (rel.int.) 226 $[M]^+$ (69), 198 (8), 184 (100); Anal. C 42.46%, H 4.39%, N 24.73%

Calculated for, $C_8H_{10}N_4O_2S$, C 42.47 %, H 4.45 %, N 24.76 %, O 14.14 %, S 14.17%.

8-(BUTYLSULFANYL)-3,9-DIHYDRO-1*H*-PURINE-2,6-DIONE(9)

The title compound was prepared using 8-sulfanyl-5,9-dihydro-1*H*-purine-2,6-dione (0.184 g, 1 mmol) and 1-iodobutane (0.113 mL, 1 mmol) by the same general procedure.

White amorphous: mp 281 °C; ^{1}H NMR (DMSO-d_6, 300MHz) δ 13.25 (1H, s, NH), 11.49 (1H, s, NH), 10.71 (1H, s, NH), 3.16 (2H, t, J = 7.20, SCH_2), 1.94 (2H, t, J = 7.20 SCH_2CH_2), 1.65 (2H, qu, J = 7.20, $SCH_2CH_2CH_2$), 1.42 (3H, t, J = 7.20, CH_3,); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.36 (C), 151.0 (C=O), 149.4 (C=O), 148.9 (CS), 108.0 (CH), 31.2 (CH_2), 31.1 (CH_2), 21.1 (CH_2), 13.4 (CH_3);

EIMS *m/z* (rel.int.) 240 $[M]^+$ (68), 193 (34), 184 (100) Anal. C 44.96%,H 5.01%, N 23.36%, Calculated for $C_9H_{12}N_4O_2S$, C 44.99%, H 5.03%, N 23.32%, O 13.32%, S 13.35%.

8-(PENTYLSULFANYL)-3,9-DIHYDRO-1*H*-PURINE-2,6-DIONE(10)

The title compound was prepared using 8-sulfanyl-5,9-dihydro-1*H*-purine-2,6-dione (0.184 g, 1 mmol) and 1-iodopantane (0.130 mL, 1 mmol) by the same general procedure.

White amorphous: mp 277 °C**,** ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.25 (1H, s, NH), 11.51(1H, s, NH), 10.76 (1H, s, NH), 3.15 (2H, t, J = 7.20, CH_2,), 2.49 (2H, p, J = 7.20 SCH_2CH_2), 1.63 (2H, p, J = 7.20, $SCH_2CH_2CH_2$), 1.29 (2H, q, J = 7.20, CH_2CH_3,), 0.85 (3H, t, J = 7.20, CH_3)**;** ^{13}C NMR (DMSO-d_6, 100 MHz) δ 154.2 (C), 151.0 (C=O), 149.3 (C=O), 148.9 (CS), 107.8 (CH), 31.3 (CH_2), 30.0 (CH_2), 28.8 (CH_2), 21.5 (CH_2), 13.7 (CH_3); EI MS *m/z* (rel.int.) 254 $[M]^+$ (69), 226 (11), 184 (100); Anal. C 46.99%, H 5.52%, N 22.04%, Calculated for $C_{10}H_{14}N_4O_2S$, C 47.23%, H 5.55%, N 22.03% .

In vitro study

Charge transfer complexation with molecular iodine is a clue to the potential antithyroid activity of compounds. Therefore, compounds were evaluated for their antithyroid potential in vitro by studying complexation of iodine with compounds spectrophotometrically. The reactions were carried out directly in the spectrophotometric cell by mixing 1.5 ml of purine (donor) with 1.5 ml of iodine (acceptor) solutions. The respective solutions were prepared in DMSO as the purine derivatives showed solubility only in this solvent. Spectra were recorded immediately on double beam UV visible spectrophotometer. The compound and iodine concentrations were adjusted by trial and error method. The iodine concentration was fixed at 2×10^{-5} M, while those of the compounds were varied between 10^{-4} and 10^{-3} M. For higher concentrations spectra crossed the scale of the instrument. Iodine has λ_{max} in visible region near 510 nm unlike the purine derivatives, which exhibited the absorption maxima in UV region. Therefore, as a strategy the solutions of pure compounds and iodine were placed in the reference cell of the instrument while taking spectra in UV and visible regions respectively. This helped in nullifying the effect (absorbance) due to iodine and the compounds in the respective regions.

In vivo study

The in vivo study was performed on young male albino Wistar rats of 125 to 200 g weight. The animals were divided into control, vehicle control, treated groups. The treated groups were administered with the solutions of respective compounds (including MMI) in DMSO, while the control and vehicle control groups obtained equivalent doses of normal saline and the solvent respectively. Five animals were allocated to each group and were fed with chick feed with water *ad libitum*. Daily dose of 20 mg/kg was administered *via i.p.* injection to respective groups daily in the morning for 15 days. The animals were weighed before sampling and blood samples were collected from all animals by puncturing abdominal aorta under light diethyl ether anesthesia. Standard animal protocols were adopted for the experimentation. Free T_3 and T_4 levels were determined using *radioimmunoassay* technique, while that of TSH with ELISA method.

The animals were sacrificed afterwards on the same day under deep diethyl ether anesthesia and thyroid glands were removed for histological studies. The dissected thyroid from each animal was placed in Petri dishes containing saline solution to clear organ from fats and blood. The weight of thyroid was determined with sartorius balance to calculate “thyroid body index”, which may be defined as weight of thyroid per 100 gram of final body weight. The tissues were then fixed in

4 % PFA for 4-6 hours. Hematoxylin and Eosin stains were used for staining. Five μm thin sections of thyroid were studied under Olympus BX51 microscope fitted with Olympus DP12 digital camera at 20 and 60 μm magnifications. Slides of all groups were photographed and changes at cellular level were noted.

Acknowledgements

The authors highly acknowledge Higher Education Commission of Pakistan for providing funds and initiating the indigenous doctoral fellowship programme under which this study was carried out. The cooperation of Dr. Asmatullah of Department of Zoology, University of the Punjab Lahore and Mr. Syed Abbas Sultan, Mr. Muhammad Rehan Adil, Mr. Abu Turab Faruqi of Atomic Energy Minerals Centre, Lahore during the study is also acknowledged with thanks.

References

1. Trzepacz, P. T.; Klein, I.; Roberts, M.; Greenhouse, J.; Levey, G. S. *Am. J. Med*. **1989**, *87*, 558-561.
2. Cooper, D. S. *Endo. Metab. Clin. North Am.* **1998**, *27*, 225-247.
3. Raby, C.; Lagorce, J. F.; Jambut-Absil, A. C.; Buxeraud, J.; Catanzano, G., *Endocrinol.* **1990**, *126*, 1683-1691.
4. Buxeraud, J.; Jambut-Absil, A. C.; Raby, C. *J. Pharm. Sci.* **1984**, *73*, 1687-1690.
5. Bandyopadhyay, U.; Biswas, K.; Banerjee, R. K. *Toxicol. Lett.* **2002**, *128*, 117-127.
6. Jambut-Absil, A. C.; Buxeraud, J.; Claude, J.; Raby, C. *Fr. Arzneimittel-Forschung* **1987**, *37*, 772-777.
7. Abou-Shaaban Rafiq, R. A.; Al-Khamees, H. A.; Abou-Auda, H. S.; Simonelli, A. P. *Saudi Pharm. J.* **1995**, *3*, 156-175.
8. Dalton, M. E.; Pegg, A. E. *Clin.Cancer. Res.* **1997**, *3*, 837-847.
9. Hahn, H.; Boop, *M. Biol. Unserer. Zeit.* **1981**, *11*, 113-120.
10. Jacobson, K. A.; Van Galen, P. J. M.; Williams, M. *J. Med. Chem.* **1992**, *35*, 407-422.
11. Mann, F. G.; Porter, J. W. G. *J. Chem. Soc.* **1945**, 751-760.
12. Fatima, I.; Munawar, M. A., Khan, M. A.; Sohail, N.; Amjad, R. *Acta Cryst.* **2009**, *E65*, 02994.
13. Sariri, R.; Khalili, G. *Russ. J. Org. Chem.* **2002,** *38*, 1053-1055.
14. Loo, T. L.; Marvin, E.; Arthur, J.; Garceau, J.; Reid, C. *J. Am.Chem. Soc.* **1959**, *81*, 3039-3041.
15. Lagorce, J. F.; Comby, F.; Rousseau, A.; Buxeraud, J.; Raby, C. *Chem. Pharm. Bull.* **1993,** *41*, 1258-1260.
16. Shamsipur, M.; Mashhadizadeh, M. H. *J. Inc. Phenom. Macrocycl. Chem.* **2000**, *38*, 277-286.
17. Fatima, I.; Munawar, M. A.; Khan, M. A.; Asmatullah; Tasneem, A.; Khalil, M. *J. Braz. Chem. Soc.* **2010**, *21*, 1699-1703.
18. Bergmann, F.; Gorvin, H. K.; Waron, H. U.; Kalmus, A. *Biochem, J.* **1963**, *86*, 567-574.