

Full length Research paper

***In vitro* and *in vivo* antitumor activity of some synthesized 4-(2-pyridyl)-3-Thiosemicarbazides derivatives**

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In search for potential antitumor drug candidates, a series of thiosemicarbazide derivatives were prepared via 4-(2-pyridyl)-3-thiosemicarbazide with phenyl isothiocyanate, benzoyl isothiocyanate, phenyl isocyanate and 4-pyridyl isothiocyanate. Effects of the synthesized compounds were tested using in vitro growth of a transplantable murine tumor cell line (Ehrlich Ascites Carcinoma) and in vivo-induced hepatocellular carcinoma (HCC) were studied. 1-(amino N-(pyridin-3yl)methanethiol-4-(pyridin-2-yl) thiosemicarbazide (H₂PPY) has remarkably decreased the viable ascitic cell count as indicated by trypan blue dye exclusion assay. This compound prolonged the lifespan of Ehrlich Ascites Carcinoma (EAC) bearing-mice. Tumor inhibitory of this compound was demonstrated by significant improvement in blood picture (hemoglobin, RBC and WBC). The in vivo studies for H₂PPY in HCC on rats showed a substantial improvement on both biochemical and histopathological parameters in comparison to non-treated HCC rats. The obtained data of the tested compound exhibited minimal adverse effects on the treated animals as compared to those of the untreated control groups.

Key words: Thiosemicarbazide derivatives, EAC, HCC, antitumor activity.

INTRODUCTION

Heterocyclic thiosemicarbazones (TSCs) have aroused considerable interest in chemistry and biology due to their antibacterial, antimalarial, antineoplastic and antiviral activities and represent an important series of compounds because of potentially beneficial, biological activity (Scovill et al., 1982). The mechanism of action of TSCs is due to their ability to inhibit the biosynthesis of DNA, possibly by blocking the enzyme ribonucleotide diphosphate reductase; binding to the nitrogen bases of DNA, hindering or blocking base replication; creation of lesions in DNA strands by oxidative rupture (Agrawal et al., 1978; Miller et al., 1998). In our previous work we have found that certain substituted pyridines and their derivatives show antimicrobial and pharmacological properties (Amr et al., 2006) and antitumor activities (Amr

et al., 1999; Attia et al., 2000).

In addition, the biological and analgesic activities of many heterocyclic compounds containing a sulfur atom have been reviewed (Braña et al., 1995; Fahmy et al., 2001). On the other hand, thienopyrimidine and thioxopyrimidine derivatives have promising biological (DeClercq, 1986) and anticancer activities (Hammam et al., 2001; 2003). Recently, some new pyridine, pyrimidine and their derivatives have been synthesized and used as analgesic, anticonvulsant, and anticancer agents (Nehad et al., 2007).

MATERIALS AND METHODS

General consideration

Elemental analyses (C, H, and N) were performed on Perkin-Elmer 2400 analyzer. All compounds were within $\pm 0.5\%$ of the theoretical values. Melting points were determined in open capillaries on electrothermal melting point apparatus (Electrothermal

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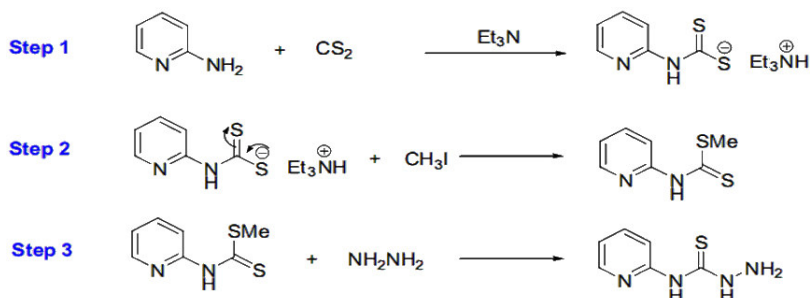


Figure 1. Preparation of 4-(2-pyridyl)-3-thiosemicarbazide (PTC).

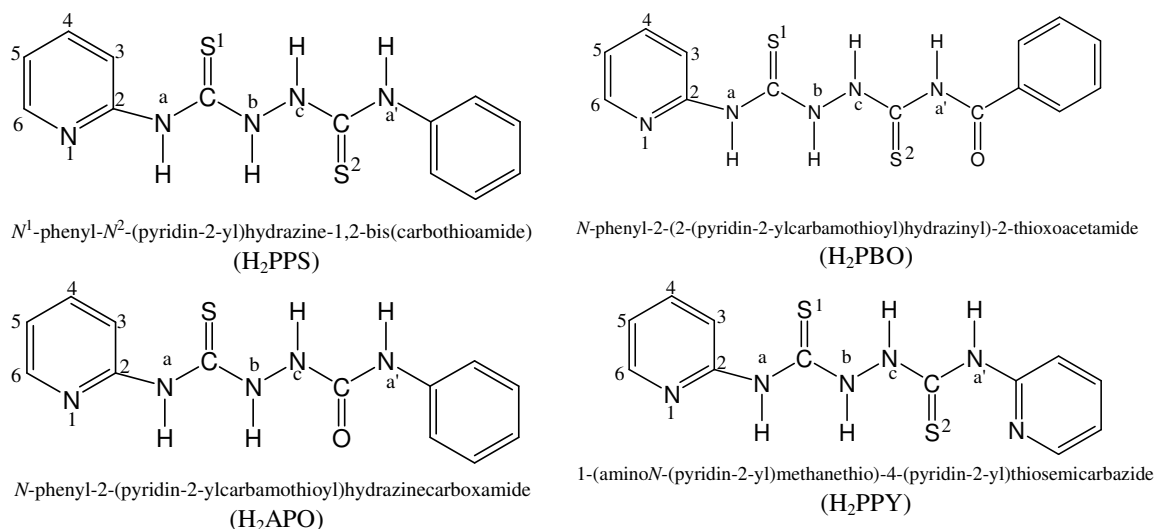


Figure 2. Structure of the synthesized compounds (H₂PPS, H₂PBO, H₂APO and H₂PPY).

Engineering Ltd-Essex, UK) uncorrected. ¹H NMR spectra were recorded in DMSO-d₆ JEOL JNM LA 300 WB spectrometer at 300 MHz using TMS (tetramethylsilane) as an internal standard (chemical shift in δ ppm) and the infrared spectra of the ligands were recorded as KBr discs on Mattson 5000 FTIR Spectrophotometer.

General procedure for preparation of H₂PPS, H₂PBO, H₂APO and H₂PPY

Preparation of 4-(2-pyridyl)-3-thiosemicarbazide

4-(2-pyridyl)-3-thiosemicarbazide is prepared in three steps as follow (Figure 1):

Step 1: Preparation of triethylammonium N-2-Pyridylidithiocarbamate

2-aminopyridine (0.2 mol, 19 g), carbon disulfide (0.2 mol, 12 mL) and triethylamine (0.2 mol, 30 mL) were warmed to give a clear solution. Two phases were separated rapidly and the whole mixture was shaken at room temperature for 24 h, the whole had then solidified. The product was filtered and then washed with ether and air-dried. The lemon-yellow plates formed were triethylammonium N-2-Pyridylidithiocarbamate, C₁₂H₂₁N₃S₂, m.p. 85 °C, yield 49.0 g (90.5%).

Step 2: Preparation of Methyl-2-pyridylidithiocarbamate

Methanol (100 ml) was added to triethylammonium N-2-Pyridylidithiocarbamate (49.0 g, 0.18 mol), followed by methyl iodide (13 ml, 0.2 mol). After 1 h, water was added into the solution and pale yellow needles of Methyl-2-pyridylidithiocarbamate were formed, C₇H₈N₂S₂, m.p. 101 °C, yield 25.2 g (76%).

Step 3: Preparation of 4-(2-pyridyl)-3-thiosemicarbazide

A mixture of 0.13 mol (25.0 g) of methyl-2-pyridylidithiocarbamate and 13 ml (0.4 mol) of hydrazine hydrate in 10 ml of absolute ethanol was heated for 5 min. An abundant amount of crystalline precipitate of 4-(2-pyridyl)-3-thiosemicarbazide was formed, m.p. 193 °C, yield 21.2g (97%), C₆H₈N₄S (168.22).

Preparation of H₂PPS, H₂PBO, H₂APO and H₂PPY

An equimolar of 4-(2-pyridyl)-3-thiosemicarbazide (1.6 g, 100 mmol) and compounds phenyl isothiocyanate, benzoyl isothiocyanate, phenyl isocyanate and 4-(2-pyridyl) isothiocyanate was heated under reflux for 2 to 4 h. After cooling, the obtained solids were filtered, dried and crystallized (Figure 2).

H₂PPS: N1-phenyl-N2-(pyridin-2-yl)hydrazine-1,2-bis(carbothioamide)

Yield 78%: m.p 180°C., C₁₃H₁₃N₅S₂ (303.41): calcd C 51.46, H 4.32, N 23.08, S 21.14; found C 51.13, H 4.25 N 22.96 S 21.11- IR (KBr): ν = 861 (C=S), 3100 (NH)a,a', 3160 (NH) b,c, 1569 (C=N)py, 624 δ (C=N)py and 1598 (C=C) - ¹HNMR δ =11.1 , 10.97 (s,1H,NHa,a'), 8.27, 8.25 (d,1H,NHb,c), 15.4 (s,1H,SH), 7.0, 7.35 (m,5H, Ph ring) and 8.26 (d, J= 5.4 Hz, 1H, pyridine H6), 7.85 (dd, J= 6.9, 5.4 Hz, 1H, pyridine H4), 7.37-7.5 (d, J= 5.4 Hz, 1H, pyridine H3), 7.65 (dd, J= 6.9 Hz, 1H, pyridine H5).

H₂PBO: N-phenyl-2-(pyridine-2-ylcarbamothioyl)hydrazinecarboxamide

Yield 81%: m.p 270°C. ,C₁₃H₁₃N₅O₂ (287.34): calcd C 54.34, H 4.56, N 24.37, O 5.57 S 11.16 ; found C 54.15, H 4.45 N 23.98 O 5.78 S 11.11- IR (KBr): ν = 861 (C=S), 3170,3104 (NH)a,a', 3220 (NH) b,c , 1562 (C=N)py ,630 δ (C=N)py, 1672 (C=O) and 1600 (C=C) - ¹HNMR δ =11.9, 11.30 (s,1H,NHa,a'), 8.36, 8.09 (d,1H,NHb,c), 15.8 (s,1H,SH), 7.08 , 7.64 (m, 5H, Ph ring) and 8.01 (d, J= 5.3 Hz, 1H, pyridine H6), 7.66 (dd, J= 6.1 Hz, 1H, pyridine H4), 7.9 (d, J= 6.4 Hz, 1H, pyridine H3), 8.23 (dd, J= 8.7 Hz, 1H, pyridine H5).

H₂APO: 1-(amino(thioformyl)-N-phenylform)-4-(pyridine-2-yl)thiosemicarbazide

Yield 79%: m.p 182°C. ,C₁₄H₁₃N₅O₂S₂ (331.42) : calcd C 50.74, H 3.95, N 21.13, O 4.83 S 19.35 ; found C 50.35, H 3.47 N 21.78 O 4.71 S 19.31- IR (KBr) : ν = 865 (C=S), 3158,3100 (NH)a,a', 3239 (NH) b,c , 1542 (C=N)py ,625 δ (C=N)py, 1675 (C=O) and 1604 (C=C) - ¹HNMR δ =12.57 , 11.09 (s,1H,NHa,a') , 9.54 , 8.20 (d,1H,NHb,c) , 7.12 , 7.23 (m,5H, Ph ring) and 8.02 (d, J= 5.1 Hz, 1H, pyridine H6), 7.55 (dd, J= 6.3 Hz, 1H, pyridine H4), 8.27 (d, J= 8.1 Hz, 1H, pyridine H3), 7.78 (dd, J= 7.3 Hz, 1H, pyridine H5).

H₂PPY: 1-(aminoN-(pyridine-3-yl)methanethio)-4-(pyridine-2-yl)thiosemicarbazide

Yield 82%: m.p 310°C. ,C₁₂H₁₂N₆S₂ (304.06) : calcd C 47.35, H 3.97, N 27.61, S 21.07 ; found C 47.02, H 4.05 N 27.86 S 21.31- IR (KBr) : ν = 861 (C=S), 3174.3100 (NH)a,a', 3234 (NH) b,c , 1562 (C=N)py 634 δ (C=N)py and 1604 (C=C) - ¹HNMR δ = 11.10(s,1H,NHa,a') , 8.29 , 8.28 (d,1H,NHb,c) , 15.4 (s,1H,SH), and 8.21 (d, J= 6.2 Hz, 1H, pyridine H6), 7.85 (dd, J= 7.3 Hz, 1H, pyridine H4), 8.17 (d, J= 7.4 Hz, 1H, pyridine H3), 7.88 (dd, J= 5.5 Hz, 1H, pyridine H5).

In vitro cytotoxic activity**Materials**

Ehrlich cells were derived from ascetic fluid from diseased mouse purchased from National Cancer institute, Cairo, Egypt). The cells (were grown in suspension culture, partly floating and partly attached, in RPMI 1640 medium (Sigma chemical Co., St. Louis, USA), supplemented with 10% fetal bovine serum (GIBCO, UK). They were maintained at 37°C in a humidified atmosphere with 5% CO₂. The viability of the cells used in control experiments (DMSO only without drug) exceeded 95% as determined with trypan blue. Test compounds were prepared initially at concentration 1 mg/ml DMSO.

In vivo antitumor activity**Induction of hepatocellular carcinoma**

Drinking water with TAA 300 mg/l was administered orally. Harvested tissues were formalin-fixed and paraffin embedded for morphologic and histochemical studies. Thioacetamide (Sigma Co., St. Louis, Mo, USA).

Experimental animals

Male Sprague-Dawley (SD) rats (n = 42), weighing 350 +/- 20 g, were used in this study. Forty two rats were divided into 6 groups (7 rats) as follow:

Group 1: Normal control, Group 2: Thioacetamide (TAA), Group 3: Treated with compound TAA+H₂PPS, Group 4: Treated with compound TAA+H₂PBO, Group 5: Treated with compound TAA+H₂APO, and Group 6: Treated with compound TAA+H₂PPY.

Histopathology

The liver specimen was preserved in formol saline and sections were prepared and stained with Hematoxylin and Eosin.

Statistical analysis

Results were expressed as mean \pm standard deviation (SD). One - way ANOVA with Dunnett's post test was performed using graphed InStat version 3.00 for windows 95, Graph Pad software, San Diego California USA, www. Graph Pad.com and used to computer data between the groups. Significance was assigned to P values \leq 0.05 (Eybl et al., 2004).

RESULTS AND DISCUSSION**Chemistry**

The most important IR bands of thiosemicarbazides are listed in Table 1. The spectra exhibit three bands between 3234 to 3100 cm⁻¹ due to ν (NH) groups. The ν and δ modes of (CN) group of pyridyl rings are found at \approx 1560 and 620 to 635 cm⁻¹. In the spectra of H₂PBO and H₂APO the bands at 1672 and 1675 cm⁻¹ are assigned to ν (CO). The thioamide IV band which has a large contribution from ν (C=S) was found at approximately 860 cm⁻¹. In the spectra of H₂PPS and H₂PBO a strong bands at 1646 and 1642 cm⁻¹ due to azomethine group. Also the bands at 668 and 660 cm⁻¹ assigned to ν (C-S) group. We suggested that (C=S) 1 is present in the thione form and (C=S) 2 exist in the thiole form confirmed by the absence of band assigned to ν (C=N) azomethine in the IR spectrum of (PTC) in addition to the absence of signal referred to SH group in the ¹HNMR spectrum of H₂APO (Figure 11). Moreover, the spectrum of H₂APO exhibits sharp band at 3521 cm⁻¹ due to ν (OH) suggested the keto-enol tautomerism. The ν (C=N) was difficult to assign due to broad band of ν (C=C) at 1604 cm⁻¹ in the spectra of H₂APO and H₂PPY of phenyl ring.

In the spectrum of H₂PPY the ν (C-S) is found at 683 cm⁻¹ and ν (C=N) is difficult to assign because it is overlapped with ν (C=C) of pyridyl ring. The appearance

Table 1. Assignments of the IR spectral bands of H₂PPS, H₂PBO, H₂PBO and H₂PPY.

Compound	$\nu(\text{C}=\text{S})$	$\nu(\text{C}-\text{S})$	$\nu(\text{NH})_{\text{a,a}}$	$\nu(\text{NH})_{\text{b}}$	$\nu(\text{C}=\text{N})_{\text{py}}$	$\nu(\text{C}=\text{C})$	$\delta(\text{C}=\text{N})_{\text{py}}$	$\nu(\text{C}=\text{O})$
H ₂ PPS	861	668	3100	3160	1569	1598	624	-
H ₂ PBO	861	660	3170,3100	3220	1562	1600	630	1672
H ₂ APO	865	-	3158,3100	3239	1542	1604	625	1675
H ₂ PPY	861	683	3174,3100	3234	1562	1604	634	-

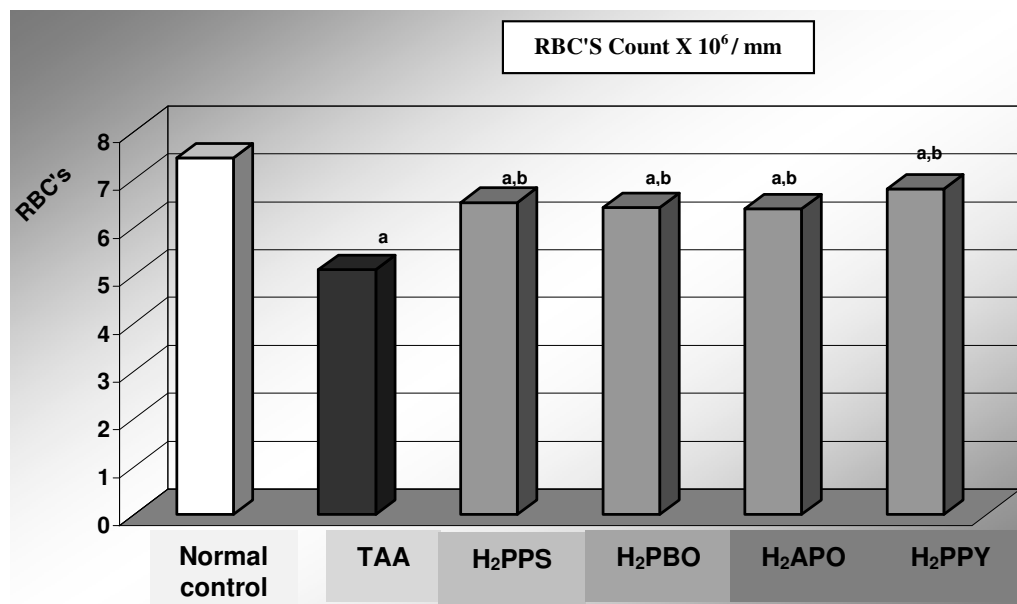


Figure 3. RBC's Count after two month of treatment by H₂PPS, H₂PBO, H₂APO and H₂PPY in TAA-induced HCC Rats. Rats were treated with H₂PPS (100 mg/ kg., orally), H₂PBO, (100 mg/ kg., orally), H₂APO (25 mg/ kg., orally), and H₂PPY (100 mg/ kg., orally) a long 60 successive days against TAA (300 mg/ kg., orally). Hematological and histopathological studies were assessed. Results are expressed as mean \pm standard deviation (S.D.), where n=3 and 4 in some groups. The results were considered statistically significant, when p value \leq 0.05. (a) Significance compared to normal control rats. (b) Significance compared to TAA -toxicated control rats.

of $\nu(\text{C}-\text{S})$ at higher wave number than of H₂PPS and H₂PBO may be due to the pyridyl rings, which acts as electron withdrawing group, at the extremes of structure. The ¹HNMR spectrum of H₂PPS, H₂PBO and H₂PPY in DMSO-d₆ shows two signals at approximately δ 11.10 and 15.4 ppm relative to TMS that disappear upon adding D₂O. These signals are assigned to the amide (NH_{a,a}) and thiol (SH) protons. The signal at δ 8.29 ppm is assigned to the (NH_b), while the multiplets at 7.00 to 7.86 ppm are assigned to the Pyridyl ring protons (Carpignano et al., 1984). The appearance of SH signal in the spectrum of H₂PPY at 15.4 and the signal of OH group at δ 12.57 in the spectrum of H₂APO confirmed the thiol and enol forms in solution.

Biological evaluation

Biochemical results (Hematological results)

Hematological parameters and survival times were among

the most important parameters for evaluation of synthesized compounds: N1-phenyl-N2-(pyridin-2-yl)hydrazine-1,2-bis(carbothioamide) (H₂PPS), N-(2-(pyridin-2-yl) carbamothioyl) hydrazinecarbothioyl)benzamide (H₂PBO), N-phenyl-2-(pyridin-2-yl)carbamothioyl)hydrazinecarboxamide (H₂APO) and 1-(aminoN-(pyridin-2-yl)methanethio)-4-(pyridin-2-yl)thiosemicarbazide (H₂PPY) were evaluated versus control (non treated) and experimentally induced HCC rats. The results showed significant changes when compared with the normal rat as shown in Figures 3 to 8.

Group 1 (Normal control)

Hematological examination of the normal control rats showed RBC's count average (7.45 \pm 0.1), WBC's count average (4.27 \pm 0.1), platelets count average (890 \pm 5), Hb average (28.14 \pm 9.27), HCT (%) average (42.4 \pm 2) and APRI average (aspartate aminotransferase to platelet ratio index) (Loaeza-del-Castillo et al., 2008) was (0.68 \pm

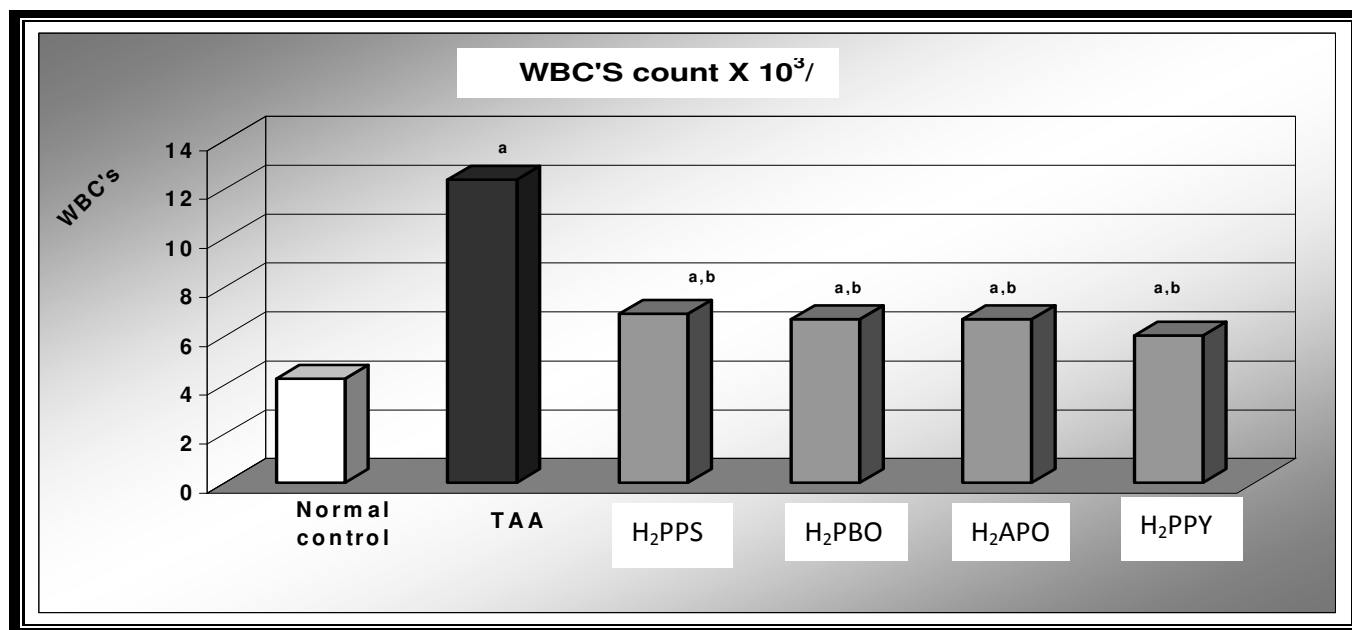


Figure 4. WBC's Count after two month of Treatment by H₂PPS, H₂PBO, H₂APO and H₂PPY in TAA-Induced HCC Rats. Rats were treated with H₂PPS (100 mg/ kg., orally), H₂PBO, (100 mg/ kg., orally), H₂APO (25 mg/ kg., orally), and H₂PPY (100 mg/ kg., orally) a long 60 successive days against TAA (300 mg/ kg., orally). Hematological and histopathological studies were assessed. Results are expressed as mean ± standard deviation (S.D.), where n=3 and 4 in some groups. The results were considered statistically significant, when p value ≤ 0.05. (a) Significance compared to normal control rats. (b) Significance compared to TAA -toxicated control rats.

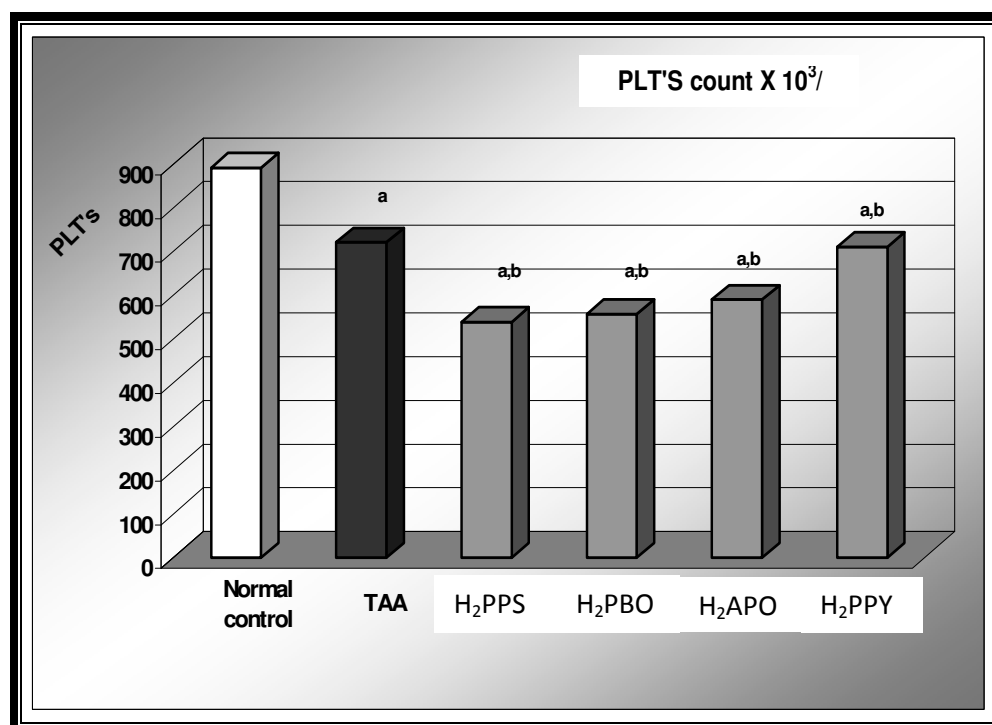


Figure 5. PLT's Count after two month of Treatment by H₂PPS, H₂PBO, H₂APO and H₂PPY in TAA-Induced HCC Rats. Rats were treated with H₂PPS (100 mg/ kg., orally), H₂PBO, (100 mg/ kg., orally), H₂APO (25 mg/ kg., orally), and H₂PPY (100 mg/ kg., orally) a long 60 successive days against TAA (300 mg/ kg., orally). Hematological and histopathological studies were assessed. Results are expressed as mean ± standard deviation (S.D.), where n=3 and 4 in some groups. The results were considered statistically significant, when p value ≤ 0.05. (a) Significance compared to normal control rats. (b) Significance compared to TAA -toxicated control rats.

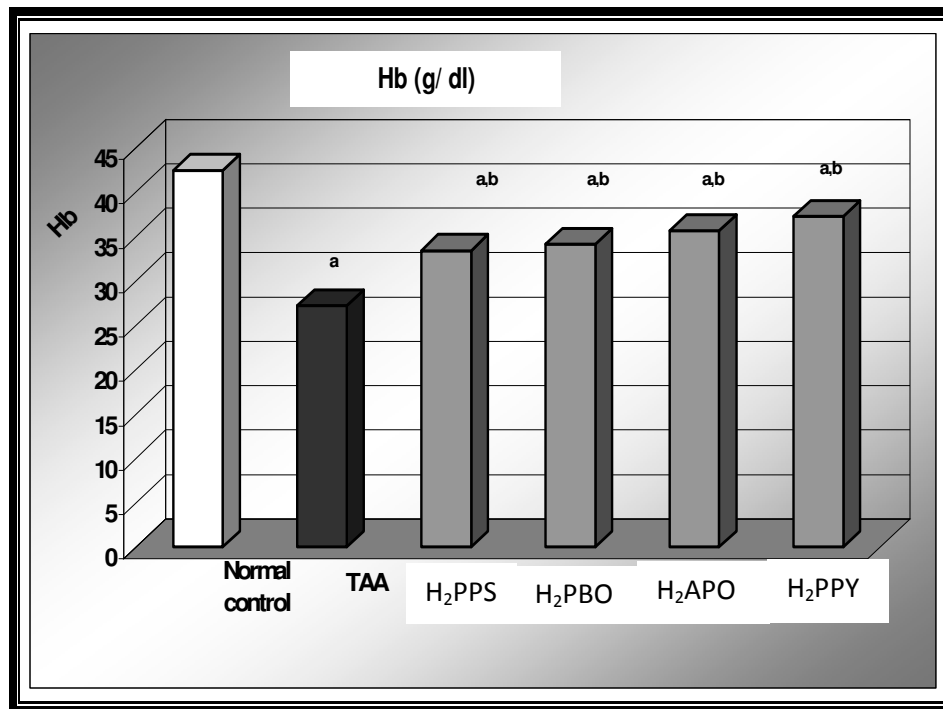


Figure 6. Hb Count after two month of Treatment by H₂PPS, H₂PBO, H₂APO and H₂PPY in TAA-Induced HCC Rats. Rats were treated with H₂PPS (100 mg/ kg., orally), H₂PBO, (100 mg/ kg., orally), H₂APO (25 mg/ kg., orally), and H₂PPY (100 mg/ kg., orally) a long 60 successive days against TAA (300 mg/ kg., orally). Hematological and histopathological studies were assessed. Results are expressed as mean \pm standard deviation (S.D.), where n=3 and 4 in some groups. The results were considered statistically significant, when p value \leq 0.05. (a) Significance compared to normal control rats. (b) Significance compared to TAA -toxicated control rats.

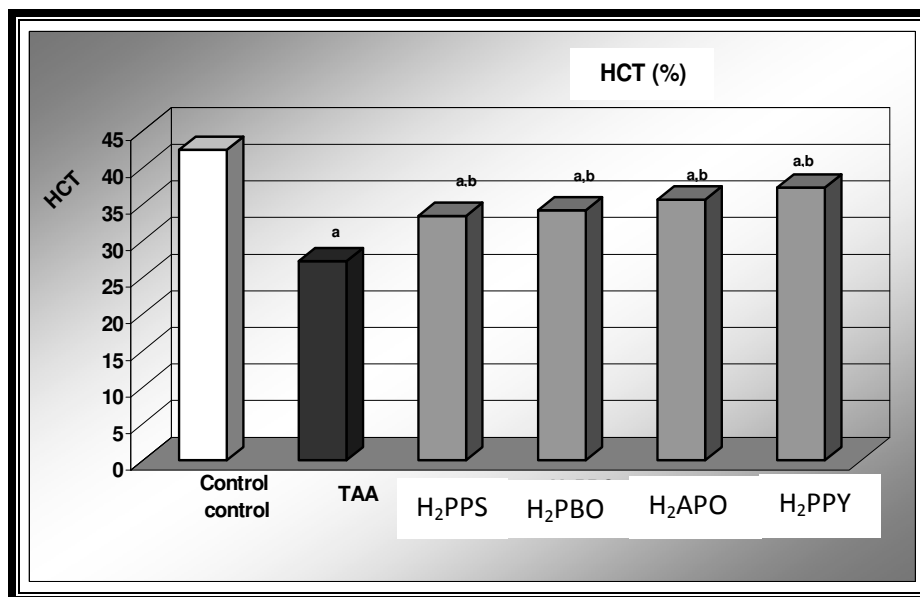


Figure 7. HCT count after two month of treatment by H₂PPS, H₂PBO, H₂APO and H₂PPY in TAA-Induced HCC Rats. Rats were treated with H₂PPS (100 mg/ kg., orally), H₂PBO, (100 mg/ kg., orally), H₂APO (25 mg/ kg., orally), and H₂PPY (100 mg/ kg., orally) a long 60 successive days against TAA (300 mg/ kg., orally). Hematological and histopathological studies were assessed. Results are expressed as mean \pm standard deviation (S.D.), where n=3 and 4 in some groups. The results were considered statistically significant, when p value \leq 0.05. (a) Significance compared to normal control rats. (b) Significance compared to TAA -toxicated control rats.

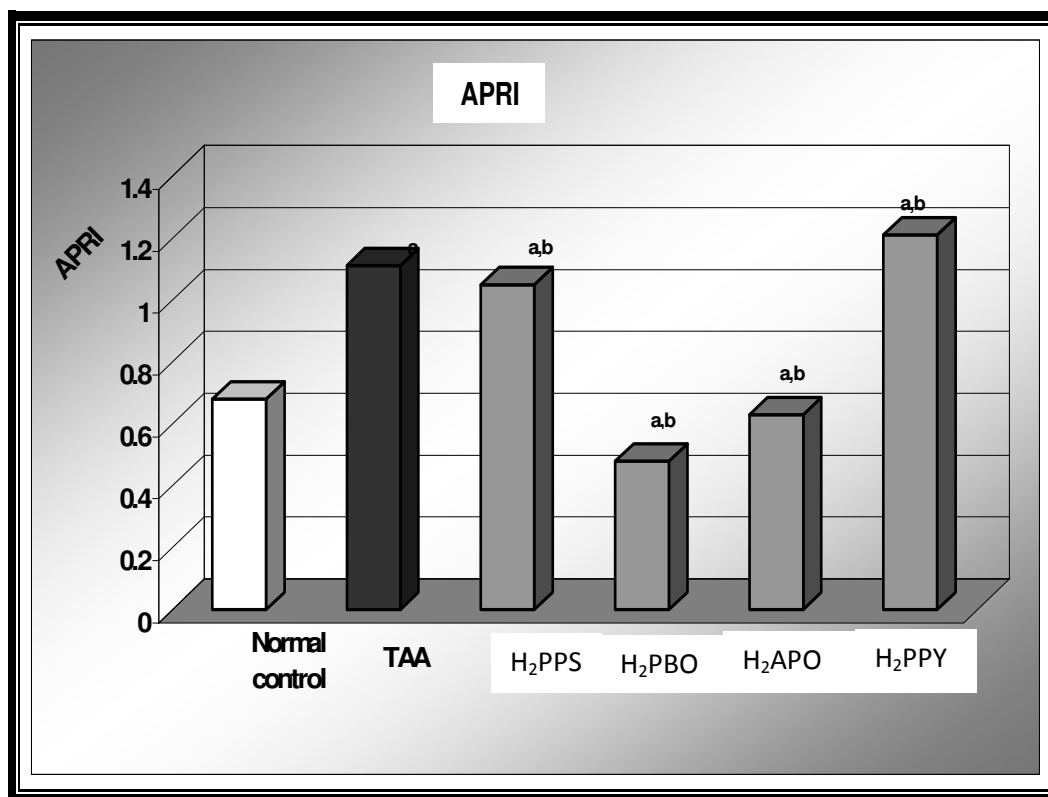


Figure 8. APRI count after two month of treatment by H₂PPS, H₂PBO, H₂APO and H₂PPY in TAA-Induced HCC Rats. Rats were treated with H₂PPS (100 mg/ kg., orally), H₂PBO, (100 mg/ kg., orally), H₂APO (25 mg/ kg., orally), and H₂PPY (100 mg/ kg., orally) a long 60 successive days against TAA (300 mg/ kg., orally). Hematological and histopathological studies were assessed. Results are expressed as mean \pm standard deviation (S.D.), where n=3 and 4 in some groups. The results were considered statistically significant, when p values \leq 0.05. (a) Significance compared to normal control rats. (b) Significance compared to TAA -toxicated control rats.

0.03).

Group 2 (Thioacetamide)

Hematological examination of the positive control rats showed an extremely significant decrease in RBC's count average (p value $<$ 0.0001), with change % (+ 30.07%). WBC's count average showed an extremely significant increase (p value 0.0002) with change % (+ 190.4%). Platelets count average showed an extremely significant decrease (p value $<$ 0.0001) with change % (+ 19.1%). Hb average showed significant increase (p value 0.0086) with change % (+ 76.72%). HCT (%) average showed significant decrease (p value 0.0003) with change % (+ 35.85%). APRI average showed significant increase (p value 0.0020) with change% (+ 63.24%).

Group 3 (Thioacetamide and H₂PPS)

Hematological examination of the H₂PPS treated rats showed significant decrease in RBC's count average (p value $<$ 0.0001), with change % (- 12.48%). WBC's count

average showed significant increase (p value 0.0008) with change % (- 61.59). Platelets count average showed significant decrease (p value $<$ 0.0001) with change % (- 39.55%). Hb average showed significant increase (p value 0.0007) with change % (- 7.25%). HCT (%) average showed significant decrease (p value 0.0006) with change % (- 21.23%). APRI average showed no significant increase with change% (- 54.41%).

Group 4 (Thioacetamide and H₂PBO)

Hematological examination of the H₂PBO treated rats showed significant decrease in RBC's count average (p value $<$ 0.0001), with change % (- 8.32%). WBC's count average showed significant increase (p value 0.0041) with change % (- 62.3%). Platelets count average showed significant decrease (p value $<$ 0.0001) with change % (-0.67%). Hb average showed significant increase (p value 0.0452) with change % (- 25.98%). HCT (%) average showed significant decrease (p value 0.0010) with change % (- 22.17%). APRI average showed significant increase (p value 0.0004) with change %

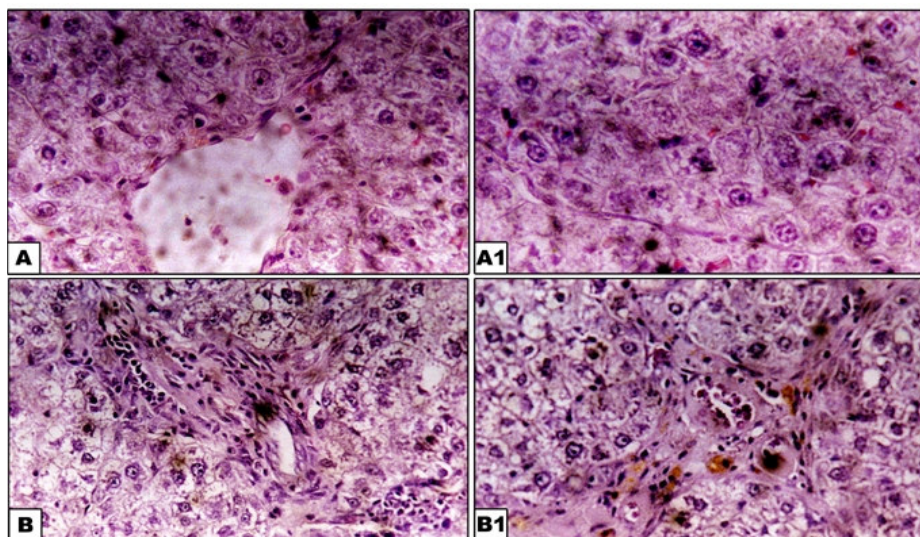


Figure 9. A Photomicrograph of Liver Control rat (A, A1) and those received Thiocetamide (B), Thiocetamide + H₂APO (B1) stained with Hematoxylin and Eosin (x=40).

Table 2. *In vitro* cytotoxicity results of tested compounds.

Tested compounds	% Dead cells	IC ₅₀ (μ g/ml)
H ₂ PPS	25.6	72.5 \pm 0.32
H ₂ PBO	35.9	68.7 \pm 0.6
H ₂ APO	42.1	66.5 \pm 0.5
H ₂ PPY	88.3	18.7 \pm 0.4
5-FU	100	5.1 \pm 0.3

(-29.41%).

Group 5 (Thioacetamide and H₂APO)

Hematological examination of the H₂APO treated rats showed significant decrease in RBC's count average (p value < 0.0001), with change % (- 8.19%). WBC's count average showed significant increase (p value 0.0007) with change % (-59.95). Platelets count average showed significant decrease (p value < 0.0001) with change % (- 0.67%). Hb average showed no significant increase with change % (-19.76%). HCT (%) average showed significant decrease (p value 0.0050) with change % (- 23.82%). APRI average showed significant decrease (p value 0.0012) with change % (- 47.06%).

Group 6 (Thioacetamide and H₂PPY)

Hematological examination of the H₂PPY treated rats showed significant decrease in RBC's count average (p value < 0.0001), with change % (- 8.72%). WBC's count average showed significant increase (p value 0.0004) with change % (-40.52). Platelets count average showed no significant decrease, change % was (- 20.34%). Hb

average showed significant increase (p value 0.0003) with change % (-10.8%). HCT (%) average showed significant decrease (p value < 0.0001) with change % (- 12.26%). APRI average showed no significant decrease with change % (- 77.94%).

In vitro evaluation of the cytotoxic activity

EAC cells were used because they have a very well known established model (Karrer et al., 1965). The results of *in vitro* Ehrlich ascites of the tested compounds are presented in Table 2. Compound H₂PPY showed a strong cytotoxic activity in comparison to a well known cytotoxic antitumor agent like 5-FU.

In vivo evaluation of the anti-tumor activity

To confirm the *in vitro* results, an *in vivo* study was carried out using EAC on rats. The reliable criteria for judging the value of any anticancer drug are prolongation of decrease of WBC and increase hemoglobin (Clarkson et al., 1965). Histopathological investigation of rat liver treated with thioacetamide (300 mg/kg b.wt daily oral doses for two months) revealed the presence of periportal fibrosis and massive breakdown of hepatic tissues. Many of the hepatocytes possessed pleomorphic nuclei associated with increased of mitotic index (Figure 9).

In other groups received thioacetamide plus either H₂PPS or H₂PBo or H₂APO or H₂PPY, a significant amelioration of hepatic tissue was observed except in case of H₂APO which showed hepatic lesions in the form of diffused pattern of hepatic fibrosis (Figure 10) in comparison to healthy control rats (Figure 9).

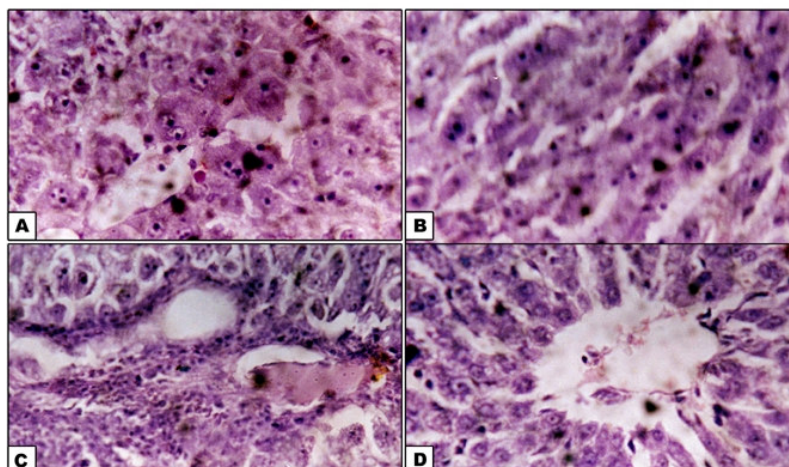


Figure 10. A Photomicrograph of Liver rat received Thiacetamide (C), Thioacetamide+H₂PBO (B), Thiacetamide + H₂PPS (A) and Thiacetamide + H₂PPY (D) stained with Hematoxylin and Eosin (x=40).

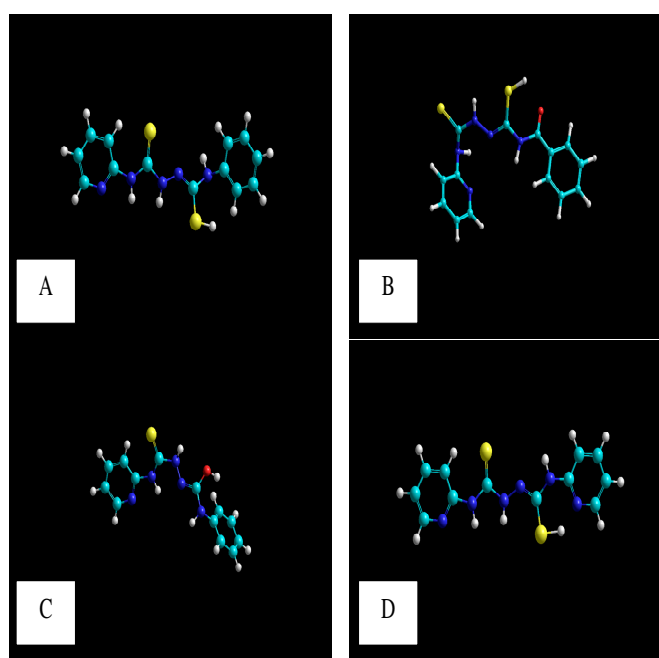


Figure 11. Geometric optimization and conformational analysis of A-H₂PPS, B-H₂PBO, C-H₂APO and D- H₂PPY using (HyperChem version 8 Hypercube, Inc., 2002).

Conclusion

A series of thiosemicarbazide derivatives were prepared via 4-(2-pyridyl)-3-thiosemicarbazide with phenyl isothiocyanate, benzoyl isothiocyanate, phenyl isocyanate and 4-pyridyl isothiocyanate were synthesized. All compounds were tested for their anticancer activity against EAC bearing rat were found to increase the life span the tumor hosts also found to bring the altered hemoglobin

and RBC values of the EAC bearing rat to near normal values. In the EAC bearing rats, cells are present in the peritoneal cavity and the compounds were administered directly into the peritoneum. The presence of two –SH equivalent groups (C=S) as shown in compounds H₂PPs and H₂PPY contribute to the activity of the tested compounds. However, the two pyridine moieties and 2 SH groups (H₂PPY) significantly enhance the antitumor activity as demonstrated in the improvement of

biochemical and histological parameters of intoxicated rats.

Hence, Fe chelation therapy represents a new avenue of chemotherapy, especially due to the rise of resistance to established chemotherapeutics and due to the presence of two SH groups which show a high chelation power towards Fe other than O, so we will recently discuss these compounds as Fe chelators especially H₂PPS and H₂PPY.

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