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Synthesis and Biological Evaluation of 1, 3, 4-Thiadiazole Derivative on Some Parameters of Immunity and Liver Enzymes

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Abstract: In the present study, a series of five members heterocyclic where synthesized by the reaction between isoniazid and various substituted isothiocyanates. The newly synthesized compounds where characterized by IR and 1H-NMR spectral data. The effect of Thiadiazole derivative (2b) in monocyte and lymphocyte in the differential count is 20349 and 11415 cells cu.mm^{-1}, respectively. Prepared compounds (2a and b) where not effective against differential count of eosinophil. In WBCs differential count that significant immune effects were occurring in compound (2b) more than in compound (2a). Liver enzymes, Glutamic Oxaloacetic acid Transaminase (GOT) and Glutamic Pyruvic acid Transaminase (GPT) were chosen to assess liver function. In comparison with control, the administration of Thiadiazole derivatives (2a and b) significantly declined the activity of GOT, GPT and Urea. These results indicate that prepared compound effectively increases of immune system in the animals.

Key words: 1, 3, 4-thiadiazoles derivatives, synthesis, statistical analysis, spectral data, biological evaluation, WBCs, packed cell volume, liver enzymes

INTRODUCTION

In recent years 1, 3, 4-thiadiazole derivatives have received significant attention and have been increasingly investigated due to their diverse range of biological properties. They exhibit for example, antimicrobial (Demirbas *et al*., 2004; Desai and Daxi, 1992), anti-micro bacterial (Matysiak *et al*., 2006), anticancer (Azam *et al*., 2008), anti-inflammatory (Foroumadi *et al*., 2005; Chou *et al*., 2003), carbonic anhydrase inhibiting effect (Siddiqui *et al*., 2009), ant-anxiety, anti-depressant (Clerici *et al*., 2001), anti-oxidant properties (Martinez *et al*., 1999).

The 1, 3, 4-thiadiazole exhibit diverse biological activities, possibly due the present of = N-C-S moiety (Oruc *et al*., 2004). The 1, 3, 4-thiadiazole are very interesting compounds due to their important applications in many pharmaceutical biological and analytical fields (Katritzky and Rees, 1984; Ahmed *et al*., 2002). The therapeutic importance of these rings prompted us to develop selective molecules in which a substituted could be arranged in a pharmacological activity. Derivatives of these nuclei are synthesized from substituted thiosemicarbazides, obtained by reaction isonized and different substituted isothiocyanates. There are five distinctly different kinds of board cells (WBCs), neutrophils, monocytes, lymphocytes, eosinophils and basophils, some have ability to change with needs and situations in the body. So, for example there are different monocytes found in different tissues and different types of lymphocytes with different rules in fighting infections. These cells can leave the bloodstream sliding out through the vessel walls and attacking invaders at the site of an infection (Hinton *et al*., 2003). Liver is in the central organ of metabolism and act as an organ of storage. Many potentially toxic substances are metabolized by cells, metabolic action by the hepatic parenchyma cells have been regarded as an important defense system against toxicants and the transformation involved have been referred to as detoxification.

The great susceptibility of liver to damage by chemical agent is presumably a consequence of its primary rule in metabolism of foreign substances. The rule of liver in metabolic conversion is due to its susceptibility to chemical injury (Rao *et al*., 2006). Liver enzyme such as Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and urea are considered to be biochemical makers for assessing liver function.

MATERIALS AND METHODS

Melting points were determined by Open Capillary Tube Method and are un-corrected. Purity of the compounds was checked in Thin Layer Chromatography (TLC) plates (silica gel G) in the solvent system toluene: ethyl acetate: formic acid (5:4:1, v,v,v) and benzene: acetone (8:2, v, v). The IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). The

H-NMR Spectra were obtained on a Bruker Ac 300MHz spectrometer in (DMSO-D6) using TMS as an internal standard and mass spectra under Electron Impact conditions (EI) were recorded at 70 eV ionizing voltage with AG prospect instrument and are presented as m/z.

Method synthesis of thiosemicarbazide: Substituted phenyl thiosemicarbazide (1) was synthesized by refluxing isonized (0.02 moles) with substituted phenol isothiocyanayes (0.02 moles) in 15 mL ethanol on a boiling water bath for 6 h. After completion of reaction, the reaction mixture was concentrated and kept overnight at room temperature. The needle shaped crystals of thiosemicarbazides.

Method synthesis of 2-(substituted phenyl)-amino-5-(4-pyridyl)-4H-1, 3, 4-thiadiazole(2): The 2-(substituted phenyl)-amino-5-(4-pyridyl)-4H-1, 3, 4-thiadiazole (2) were synthesized by cyclization of substituted phenyl thiosemicarbazide of isonized (0.002 moles) with sulfuric acid at 0-5°C. After completion of reaction, the mixture was poured into crushed ice, the solid separated was filtered, washed with water and re-crystallized from methanol yielded the pure compound.

Blood film methods: This method was done according to Catalovo.

Differential count of leukocytes:

- A small drops of heparinized blood which drawn from mouse was put on the end of clean and dry slide. A pusher slide was place at an angle of 30-45°C to the slide and then moved back to make contact with the drop. The forward movement of the pusher spreads the blood on the slide
- The blood film was allowed to dry in air
- The slides were completely covered with Leishman stains after 3 min. The slides were washed gently and then examined under light microscope and by applying the following equation:

$$\text{No. of cells/(cells nm}^{-3}\text{ blood)} = \frac{\text{Total no. of leukocytes (\%)}}{100}$$

Total count of leukocytes:

- The blood was taken by heart puncher and put into heperinazed tube
- A dilution solution (190 μL) was pipette into test tube
- The heperinazed blood (10 μL) was pipette and mixed well with diluting fluid for at least 2 min
- The hemocytometer was sited up with its cover glass in position and by a pasture pipette; both sides of the hemocytometer were filled with the diluted blood
- The cells were allowed for 2 min to be settled
- The cells were count in the four large squares on both sides of chamber using the 40x objectives and subdued light
- The WBCs were calculated on the bases of cells counted area and the dilution:

$$\text{No. of cells/(cells/nm}^{-3}\text{ blood)} = \text{No. of cells in four square} \times \frac{\text{Correct volume correct dilution}}{4}$$

According to Creskoff (Wood, 1983), blood was collected from the mice by heart puncture. The serum was separated by centrifuge at 2000 rpm for 10 min then the serum was taken and treated as follows: two test tubes were used for each sample; the first one contained the blank reagent and second contains the sample. These samples were tested as in the following: mix wait 5 min measure under condition identical to those used for the standard curve. Wave length: 505 nm (490-520 nm). Activities for these two enzymes in the serum were estimated from the activity table attached with kit of each enzyme.

Statistical analysis: Statistical analysis was performed to compare two different groups by using ANOVA-test. Statistical significance was determined at ($p<0.05$) (Liu *et al*., 2002).

RESULTS AND DISCUSSION

A series of 2-(substituted phenyl) amino-5-(4-pyridyl)-4H-1, 3, 4-thiadiazole was prepared from isoniazed and substituted phenyl isothiocyanates derived thiosemicarbazides (Fig. 1). The structure of newly synthesized compounds was confirmed by spectral and analytical data. In general, the IR spectra of newly synthesized compounds revealed NH, C = N, N-N, C-S-C peaks near 3390, 1620, 1065 and 660 cm^{-1}, respectively. In the 1H-NMR spectra, signal of respectively protons of newly synthesized compounds showed the peaks for $-OCH_3$, NH and aromatic protons near δ3.7, 7.5 and 6.8-8.5, respectively.

The general mass fragmentation pattern for the compounds showed the m/z peaks. Both analytical and spectral data (IR, 1H-NMR and mass) of all the synthesized compounds were in full agreement with proposed structures. Physical data of all the synthesized compounds are shown in Table 1.

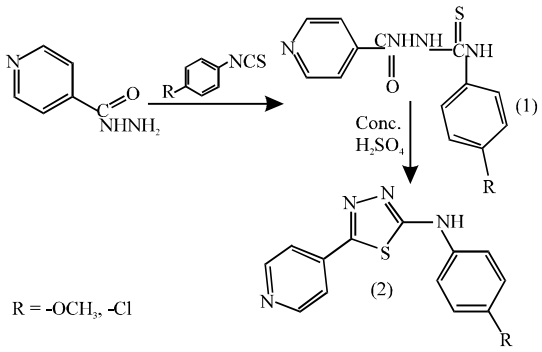

Fig. 1: Synthetic pathways for the preparation of 1, 3, 4-thiadiazole derivatives

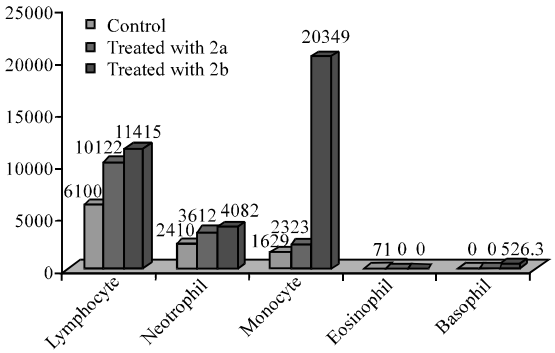

Fig. 2: The relationship between treated of thiadiazole derivatives (2a, b) and differential count of WBCs

Table 1: Physical constant of newly synthesized thiadiazole derivatives

Compound	R group	Yield (%)	m.p. (°C)	Mol. formula	Mol. wt.
2a	-Cl	70	152-54	$C_{13}H_9ClN_4S$	288.75
2b	-OCH_3	81	168-70	$C_{14}H_{12}N_4OS$	284.33

Spectral data

Compoundof2-(4-chlorophenyl)-amino-5-(4-pyridyl)-4H-1, 3, 4-thiadiazole (2a): IR (KBr, cm^{-1}):3390 (NH), 1620 (C = N), 1060 (N-N) 660 (C-S-C); (400 MHz, DMSO-d6): 8 7.4-7.7 (d, 2H, Ar, J = 8.56 Hz), 8.4-8.9 (d, 2H, pyridine, J = 8.71 Hz), 8.0 (S, 1H, NH), MS (m/z):289 (M^++1).

Compoundof2-(4-methoxyphenyl)-amino-5-(4-pyridyl)-4H-1,3,4-thiadiazole (2b): IR (KBr, cm^{-1}):3070 (NH), 1610 (C = N), 1003 (N-N) 700 (C-S-C); (400 MHz, DMSO-d6): 8 3.7 (S, 3H, OCH_3), 6.8-7.5 (d, 2H, Ar, J = 8.25 Hz), 8.2-8.8 (d, 2H, pyridine, J = 5.50 Hz), 7.6 (S, 1H, NH), MS (m/z): 284 (M^+).

Biological evaluation: Its aimed to study effects 1, 3, 4-thiadiazole derivatives on blood film (total and differential) (WBCs) and liver enzymes. Animals were divided into three groups of 6 animals each as follows: none treated mice (control group), mice were injected with compound (2a) and mice were injected with compound (2b).

White Blood Cells (WBCs): The compound (2b) was found to be the most active in Lymphocyte and monocyte in differential count 11415 and 20349 cells cu.mm^{-1}, respectively while compound (2a) showed little activity against all types of White Blood Cells (WBCs) by compared with control. These prepared compounds (2a and b) were not effective against the count of Eosinophil as shown in Table 2 and Fig. 2.

Packed cell volume and hemoglobin: These two compounds (2a and b) of 1, 3, 4-thiadiazole derivatives that were prepared in theses research showed high effect in total and differential count of White Blood Cells

Table 2: Method of total count of leukocytes

Methods	GPT	GOT
Reagent 1	1 mL	-
Reagent 1	-	1 mL
Serum	0.2 mL	0.2
Mix and incubate at 37°C	1 h	30 min
Reagent 3	1 mL	1 mL
Mex let stand for 20 min at room temp.	-	-
NaOH 0.4N	10 mL	10 mL

(WBCs), percentage of Packed Cell Volume (PCV %) and Hemoglobin percentage (HB%) as shown in Table 3. Immune mechanisms affected by thiadiazole derivatives in addition to blood film dependent immunity include reduced production of complement by the liver and decreased phagocytosis by neutrophils and microphage (Dugyala and Sharma, 1996). WBCs generally fall into distinct subtypes:

- Polymorph-nuclear leukocytes of granulocytic lineages including neutrophils, eosinophils and basophils
- Lymphomononuclear cells such as lymphocytic and monocytic (Batey and Wang, 2002)

The compound (2b) emerged as the most active against the Packed Cell Volume (PCV%) at (mean±SE) 36.45±3.61 and Hemoglobin (Hb%) 13.51±1.94 as shown in Fig. 3.

Hemoglobin gives red blood cells their color. Hemoglobin carries from the lungs to the tissues and takes carbon dioxide from the tissues to the lungs (Hinton *et al*., 2003).

Liver enzymes: The present study showed that the exposure of 1, 3, 4-thiadiazole drivativies caused decreasing the activity of Glutamic Oxaloacetic acid Transaminase (GOT), Glutamic Pyruvic acid Transaaminase (GPT) and urea.

The compound (2b) was found to be the most active against GOT, GPT and urea while compound (2a) showed moderate activity against these tests.

Table 3: The effect of 1, 3, 4-thiadiazole derivatives on differential and total counts of blood

	Differential count (mean±SE)					
Groups	Total WBCs (mean±SE) (%)	No. of lymphocyte cells cu.mm^{-1} blood	No. of neotrophil cells cu.mm^{-1} blood	No. of monocyte cells cu.mm^{-1} blood	No. of eosinophil cells cu.mm^{-1} blood	No. of basophil cells cu.mm^{-1} blood
Control	A (100.63±11.41)	A (6100±98.4)	A (2410±121.6)	A (1629±200.0)	A (71.0±21.00)	A (0.00±0.00)
A (Treated with 2a)	B (153.5±14.100)	B (10122±100.5)	B (3612±201.3)	B (2323±182.0)	B (0.00±0.00)	A (0.00±0.00)
B (Treated with 2b)	C (171.0±16.200)	B (11415±321.4)	C (4082±141.6)	B (20349±124.6)	B (0.00±0.00)	B (526.3±74.80)

Differences A-C are significant ($p<0.05$) to compression column

Table 4: The effect of 1, 3, 4-thiadiazole derivatives on packed cell volume and hemoglobin

Groups	PCV (mean±SE) (%)	Hb (mean±SE) (%)
Control	A (28.56±3.20)	A (9.64±1.45)
A (Treated with 2a)	AB (31.27±2.98)	B (11.33±2.63)
B (Treated with 2b)	B (36.45±3.61)	C (13.51±1.94)

Differences A-C are significant ($p<0.05$) to compression column

Table 5: Effects of 1, 3, 4-thiadiazole derivatives on activity of liver function represent in Glutamic Oxaloacetic acid Transaminase (GOT), Glutamic Pyruvic acid Transaaminase (GPT) and urea

Groups	GOT IU mL^{-1} (mean±SE) (%)	GPT IU mL^{-1} (mean±SE) (%)	Urea mg dL^{-1} (mean±SE) (%)
Control	A (210.4±16.8)	A (68.5±10.60)	A (15.6±4.30)
A (Treated with 2a)	B (198.6±14.5)	B (62.3±6.400)	B (12.2±3.80)
B (Treated with 2b)	C (188.7±12.2)	B (58.7±4.810)	B (10.4±2.83)

Differences A-C are significant ($p<0.05$) to compression column

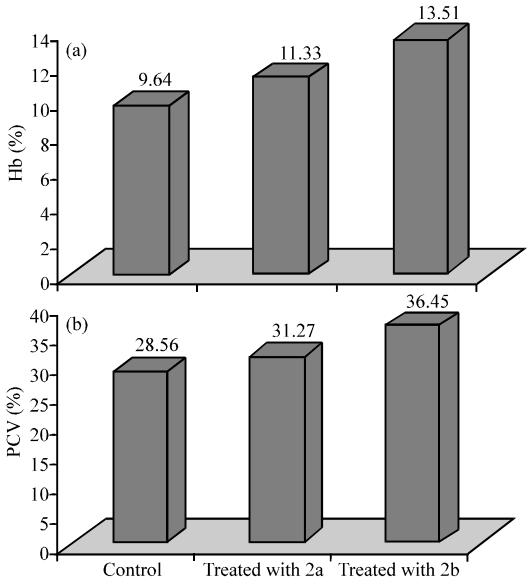

Fig. 3: a) The percentage of packed cell volume and b) relationship between treated of thiadiazole derivatives and percentage of hemoglobin

A significant decrease in the serum GOT (188.7±12.2) and GPT (58.7±4.81) IU mL^{-1} levels were seen in the compound (2b) treated mice by compared with control, the results of such studies are shown in Table 4 and 5.

The marked elevation of urea level in the serum of mice were significantly decreases in the thiadiazole, the GOT and GPT levels respectively dropped from 210.8±16.8-198±14.5 IU mL^{-1} regarding compound (2a)

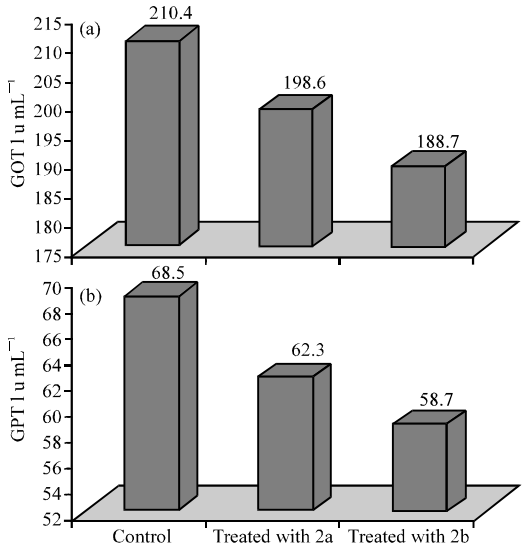

Fig. 4: Effect of prepared compound (2a and b) in liver enzymes; a) Glutamine Oxaloacetic acid Transminase (GOT) and b) Glutamine Pyruvic acid Transaaminase (GPT)

and from 210.4±16.8-188.7±12.2 IU mL^{-1} regarding compound (2b) as shown in Fig. 4. Liver enzymes, GOT and GPT were chosen to assess liver function. The enzyme is absorbed by the organism and passes through the blood to fulfill a systemic activity. Thus, it inhibits the production of prostaglandins which provoke inflammations. The decrease in inflammatory phenomenon results in the retrogression of liver deterioration. The results establish the decrease in those two enzymes and urea. On the whole, the use of Thiadiazole derivatives made the GPT and GOT rates increase the liver function.

CONCLUSION

A series of 1, 3, 4-thiadiazole derivatives were synthesized and their structures were elucidated by spectral data.

The biochemical revealed that the 1, 3, 4-thiadiazole caused activator effects on GOT, GPT and urea enzymes activities and increase the differential count of WBCs, PCV and Hemoglobin percentage. The compound (2b)

was found to be the most active against GOT, GPT and urea while compound (2a) showed moderate activity against these tests.

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