

# Synthesis, Characterization and Effect of bis-1,3,4-Oxadiazole Containing Glycine Moiety on the Activity of Some Transferase Enzymes.

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## Abstract

We described herein the synthesis of novel bis-1,3,4-oxadiazole containing glycine moiety. N-{5-[5-(4-methoxyphenyl)-1,3,4-oxadiazole-2-yl-sulfanyl]-1,3,4-oxadiazole-2-yl-methyl}-4-methoxybenzamide was fully characterized by elemental analysis, FT-IR and <sup>1</sup>H NMR spectroscopy. Also this study was designed to show the effects of bis-oxadiazole compound on the activities of some transferase enzymes such as: GOT, GPT and  $\gamma$ -GT in sera. This compound demonstrated activation on GOT and GPT activities, inhibitory effects on the  $\gamma$ -GT activity. These effects increased with the increasing of the concentration of the compound. The causes of the increases and decreases in the enzymes activities are discussed.

## Introduction

Derivatives of 1,3,4-oxadiazole constitute an important family of heterocyclic compounds [1]. Substituted 1,3,4-oxadiazole are considerable pharmaceutical and material interest, which are documented by a steadily increasing number of publications and patents, reported among these activities were: nervous system depressing [2], muscle relaxants [3], analgesic[4], herbicidal [5], hypoglycemic [6], antifungal [7], anti-inflammatory [8] and antibacterial [9] activities. In addition to that, derivatives of 1,3,4-oxadiazole are used in agriculture [10], photosensitizer [11] and liquid crystals [12].

Also, the derivatives of heterocyclic compounds were studied to show their effects on activities of some transferase enzymes. Dere et al [13] studied the biochemical analysis of (PQ) (1,1-dimethyl-4,4'-bipyridinium) on some transferase enzymes and they found some changes (increases or decreases) in these enzymes activities.

Glutamate oxaloacetate transaminase (GOT) Enzyme EC 2.6.1.1: it is called also aspartate amino transfer (AST), it is one of the most important of transferase enzymes, catalyzes the transfer of the amino group of aspartate to  $\alpha$ -ketoglutarate. GOT is widely distributed in human tissues; heart, liver, skeletal muscle and kidney. The optimum conditions of maximum enzyme activity are pH= 7.4 and temp. = 37°C. The clinical usefulness of the enzyme is largely restricted to the diagnosis of heart and liver diseases. Large amount of GOT may be released in to the blood. Very high levels are observed in acute liver disease while lesser elevation is seen in chronic liver disease.

Glutamate pyruvate transaminase (GPT) Enzyme EC 2.6.1.2: it is also called alanine amino transferase (ALT) which is prevalent in mammalian tissue catalyzes the transfer of the amino group of alanine to  $\alpha$ -ketoglutarate. GPT is found in a highest concentration in livers in spite of its active occurrence in skeletal muscles, heart and kidneys. The GPT activity in tissues is generally less than GOT. GPT level found to increase in the following diseases; infection hepatitis, liver cirrhosis and biliary cirrhosis, obstructive jaundice and liver cancer.

Gamma glutamate transferase ( $\gamma$ -GT) Enzyme EC 2.3.2.2: it is also called gamma glutamate transpeptidase ( $\gamma$ -GT) or (GGT), it is found in kidneys and liver and catalyzes the transfer of gamma-glutamyl group from glutathione to an amino acid. GGT levels are increased in most forms of liver disease, especially cholestasis. GGT, a plasma membrane-

bound enzyme, provides the only activity capable to effect the hydrolysis of extra cellular glutathione (GSH), thus favoring the cellular utilization of its constituent amino acids.

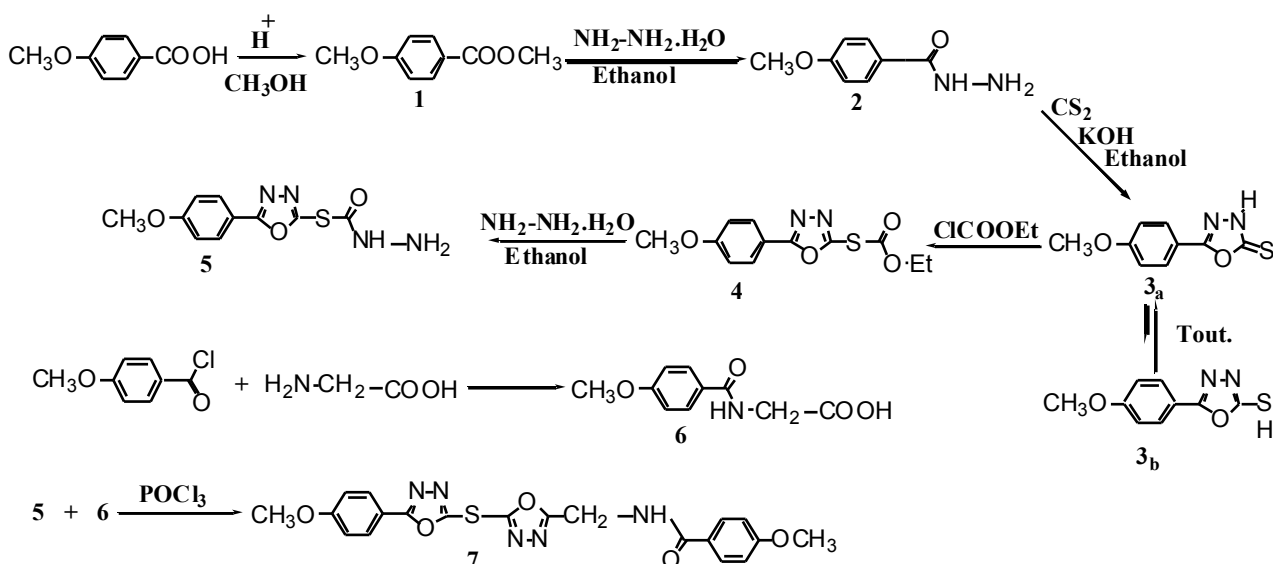
The common procedures of cyclization of oxadiazole ring is promoted by heat and anhydrous reagents like: thionyl chloride [14], phosphorous oxychloride [15], phosphorous pentoxide [16], triphenylphosphine [17] and triflic anhydride [18]. Also, Feray et al [19] showed the synthesis of the oxadiazole derivatives using the synthetic procedure based on the ring closure reactions of appropriate acid hydrazides with carbon disulfide in alkali media [20].

In the literature, we did not found any studies of the effect of oxadiazole derivatives on the activity of transferase enzymes. So, we studied here the effective of bis-1,3,4-oxadiazole compound containing sulfur atom between the two oxadiazole rings derived from N-protected glycine attached at C-5 of the oxadiazole ring, on the activities of some tranferase enzymes in sera. This product might be potential compound for biological activity tests on transferase enzymes. A literature search revealed that no such oxadiazoles derived from amino acids have yet been prepared except Sebastiao et al [21] who synthesized some 3-aryl-1,2,4-oxadiazoles carrying protected L-alanine side chain.

## Result and Discussion

### Synthesis

The characterization data of all compounds **1-7** are given in the experimental section. All the newly synthesized compounds gave satisfactory analysis for the proposed structures, which were confirmed on the basis of their elemental analysis, FT-IR and  $^1\text{H}$  NMR data. Our synthetic strategy is show in scheme 1.

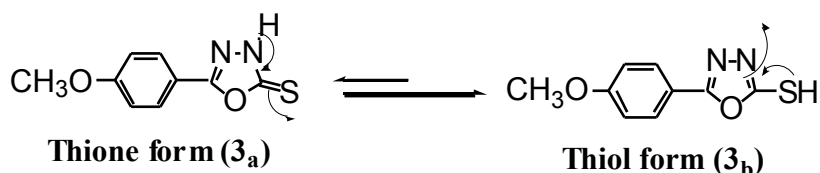


**Scheme 1. Synthesis of compounds (1-7)**

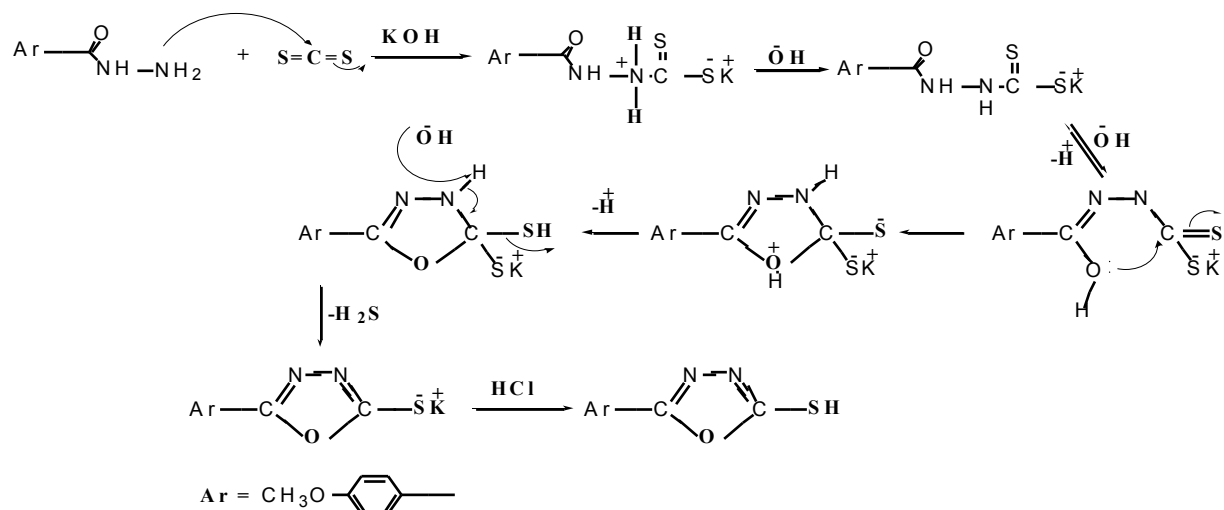
The first step was began to convert 4-methoxybenzoic acid (Anisic acid) to 4-methoxymethyl benzoate **1** by the standard esterification method [22]. 4-Methoxybenzoyl hydrazine **2** was synthesized in good yield from the reaction of ester **1** with excess of hydrazine hydrate in ethanol. Compound **3** was synthesized by the ring closure reaction of acid hydrazide **2** with carbon disulfide in ethanolic KOH.

The tautomeric equilibrium in compound **3** (**3<sub>a</sub>** and **3<sub>b</sub>**) was investigated both for isolated and for solvated species considering the solution medium. Tsoleridis [23] studied the same equilibrium and he found that the thiol form (**3<sub>b</sub>**) is more stable in the gas phase. However,

this is contrary to the known experimental finding. On the basis of FT-IR and  $^1\text{H}$  NMR data, it is known that 5-(4-methoxyphenyl)-1,3,4-oxadiazole-2-thione (**3<sub>a</sub>**) exist in solution in the thione rather than the thiol form [24]. Parallel to experimental data, the thione tautomer is more stable than thiol in the solution. The equilibrium is even more favored towards the thione because the thione is better solvated than thiol. In the  $^1\text{H}$  NMR spectrum of compound **3** (Fig. 1.), the broad peak at  $\delta$  14.38-14.78 of the NH proton was recorded, although it was very weak. It has been reported that the crystal structure of compound **3** corresponds to the thione form [25], but the reaction conditions for the synthesis of compound **4** prove that compound **3** can be in the thiol form too. Finally, the crystal structure of compound **3** corresponded to the thione form, but they showed thiol – thione tautomerism in solution.



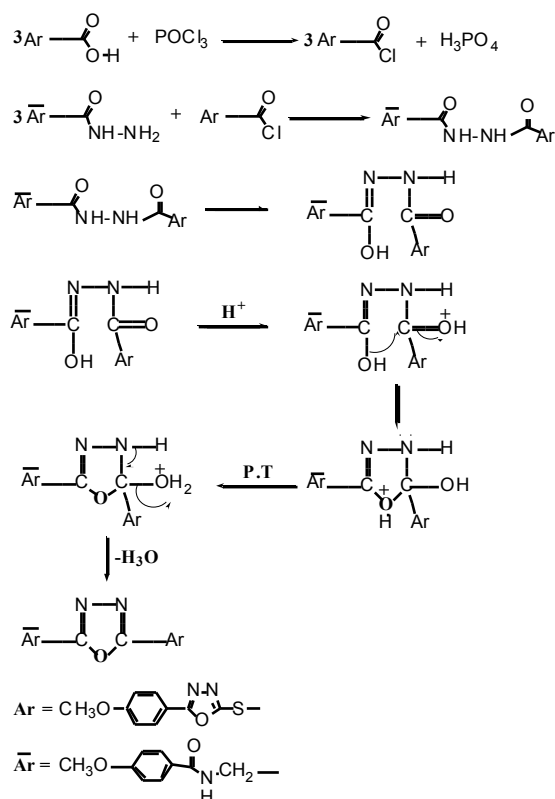
The mechanism [26] of ring closure of hydrazide by carbon disulfide may be outlined as follows in scheme 2.



**Scheme 2. The mechanism steps of formation of compound (3)**

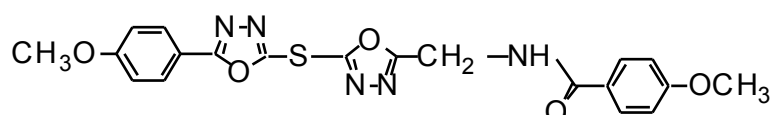
An attempt to prepare the thio ester of oxadiazole ring **4** by the treatment of compound **3** with an excess of ethyl chloroformate was successful and the product obtained was identified by elemental analysis, FT-IR and  $^1\text{H}$  NMR, Figure 2. shows the  $^1\text{H}$  NMR spectrum of compound **4**. The hydrazide of oxadiazole ring **5** was prepared in a good yield by the reaction of compound **4** with an excess of 80% hydrazine hydrate in ethanol. The peaks at  $\delta$  5.75 and 13.75 in  $^1\text{H}$  NMR spectrum (Fig. 3.) are the good evidence for the formation of oxadiazole's hydrazide **5**.

The compound **6** was synthesized by the reaction of 4-methoxybenzoyl chloride with glycine according to Steiger's procedure [27], to afford the corresponding hippuric acid **6**. The  $^1\text{H}$  NMR spectrum of compound **6** was shown in figure 4. The hydrazide of oxadiazole ring **5** and compound **6** could be smoothly cyclodehydrated by boiling in phosphorus oxychloride affording the bis oxadiazole **7** in good yield. The  $^1\text{H}$  NMR spectra of this compound was shown in Figure 5. The mechanism of dehydration in the presence of  $\text{POCl}_3$  is depicted in the following steps: (Scheme 3).



**Scheme 3.** The mechanism steps of formation of compound (7)

### Biological activity of Transferase enzymes (GOT, GPT and GGT)



This research addresses investigation of the effects of compound (7) of GOT, GPT and  $\gamma$ -GT enzymes. The biochemical tests revealed that this compound caused inhibitory effects on  $\gamma$ -GT enzyme activity and activatory effects on GOT and GPT enzymes activities. Table 1. shows the effect of different concentrations of compound (7) on the activity of GOT, GPT and  $\gamma$ -GT enzymes in human serum.

The normal value of the GOT and GPT enzyme activities were (17 and 16 U/L) respectively. The relationship between compound (7) concentrations versus and the activity of enzymes were shown in figure 6. These results observed that any increase in compound concentrations caused an increase in the percentage of activation of enzymes. The greater activation of compound (7) was demonstrated at concentration  $10^{-2}$  M (364.705%) for GOT and  $10^{-2}$  M (300%) for GPT as shown in figure 7.

The normal value of the  $\gamma$ -GT enzyme activity was (6.545 U/L). The relationship between compound (7) concentration versus and the activity of enzyme was shown in figure 8(a). These results observed that any increase in compound concentrations caused an increase in the percentage of inhibition of enzyme. The greater inhibition of compound (7) was demonstrated at concentration  $10^{-5}$  M (55%) as shown in figure 8(b).

Competitive, noncompetitive and uncompetitive inhibition can be easily distinguished with the use of double reciprocal plot of the Lineweaver-Burk plot. Two sets of rate determination

in which enzyme concentration was held constant, were carried out. In the first experiment the velocity of uninhibited enzyme was established, in the second experimental constant amount of inhibitor is included in each enzyme assay. Varieties of substances have the ability to reduce or eliminate the catalytic activity of specific enzyme [28].

Table 2. and figure 9(a). showed that the type of enzyme activation using Lineweaver-Burk plot for compound (7) on serum GOT activity. The  $V_{max}$  and  $K_m$  values determined with  $10^{-2}$  M of compound (7) and without it.  $V_{max}$  and  $K_m$  values without compound (7) were 67 U/L, 200 M respectively. A liquate  $10^{-2}$  M of compound (7) was noncompetitive activation for enzyme activity. Noncompetitive activation changed the  $V_{max}$  of the enzyme but not the  $K_m$ . When concentration of compound was  $10^{-2}$  M, the  $V_{max}$  was 50 M. By using Lineweaver-Burk equation, the  $K_i$  values of enzyme for compound which was studied in different concentrations. The  $K_i$  of compound (7) in  $10^{-2}$  M was 0.0129 M.

Table 2. and figure 9(b). showed that the type of enzyme activation using Lineweaver-Burk plot for compound (7) on serum GPT activity. The  $V_{max}$  and  $K_m$  values determined with  $10^{-2}$  M of compound (7) and without it.  $V_{max}$  and  $K_m$  values without compound (7) were 100 U/L, 400 M respectively. A liquate  $10^{-2}$  M of compound (7) was noncompetitive activation for enzyme activity. Noncompetitive activation changed the  $V_{max}$  of the enzyme but not the  $K_m$ . When concentration of compound was  $10^{-2}$  M, the  $V_{max}$  was 50 M. By using Lineweaver-Burk equation, the  $K_i$  values of enzyme for compound which was studied in different concentrations. The  $K_i$  of compound (7) in  $10^{-2}$  M was 0.02 M.

The enzymes play important role in amino acid metabolism and in urea and tricarboxylic acid cycles. We suggested that compound (7) molecule has (N- and O=) groups by which, it activates the active sides of amino acids of GOT and GPT enzymes by increasing affinity of active sides of enzymes to react with the substrates.

Table 2. and figure 9(c). showed that the type of enzyme inhibitor using Lineweaver-Burk plot for compound (7) on serum  $\gamma$ -GT activity. The  $V_{max}$  and  $K_m$  values determined with  $10^{-2}$  M of compound (7) and without it.  $V_{max}$  and  $K_m$  values without compound (7) were 37.037 U/L, 0.4 M respectively. A liquate  $10^{-2}$  M of compound (7) was noncompetitive inhibition for enzyme activity. Competitive inhibition changed the  $K_m$  of the enzyme but not the  $V_{max}$ . When concentration of compound was  $10^{-2}$  M, the  $K_m$  was 1 M. By using Lineweaver-Burk equation, the  $K_i$  values of enzyme for compound which was studied in different concentrations. The  $K_i$  of compound (7) in  $10^{-2}$  M was 0.000263 M. Molecule of compound (7) have an interaction between the groups (N- and O=) with active sides of amino acids of  $\gamma$ -GT enzyme.

## Conclusion

Novel bis-1,3,4-oxadiazole compound containing glycine moiety was prepared and structurally characterized using spectroscopic techniques. The synthetic route started from esterification of anisic acid followed by reaction the ester with hydrazine hydrate. The acid hydrazied was converted to thione-thiol oxadiazole tautomer (3) by ring closure mechanism. The thio ester of oxadiazole (4) was prepared by the reaction of tautomer (3) with ethyl chloroformate. The acid hydrazide of oxadiazole (5) was synthesized by the same conditions for prepared compound (2). Compound (7) have been synthesized by dehydration mechanism in presence of  $POCl_3$ . The biochemical studies revealed that the bis-oxadiazole caused activatory effects on GOT and GPT enzymes activities, and inhibitory effects on  $\gamma$ -GT enzyme activity.

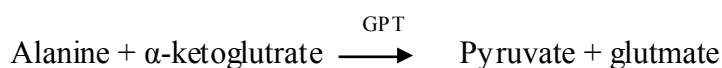
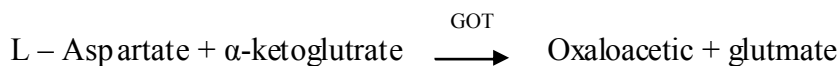
## Experimental

### Materials and physical measurements.

All starting materials and solvents were purchased from Aldrich and Fluka and used without further purification. Melting points were determined on Electrothermal capillary apparatus and are uncorrected, Elemental analysis (C, H, N) were carried out using a Perkin-Elmer model 2400 instrument in university of Al-albait, Amman, Jordan, FT-IR measurements were recorded on Shimadzu model FTIR-8400S in university of Al-Mustanseriya, department of chemistry, college of science, Baghdad, Iraq.  $^1\text{H}$  NMR spectra were obtained with Bruker spectrophotometer model ultra shield at 300 MHz. in university of Al-albait, Amman, Jordan. The compounds dissolved in  $\text{DMSO-d}_6$  solution with the TMS as internal standard. Note: in some  $^1\text{H}$  NMR spectra, the peaks at  $\delta$  2.5 and 3.35 are for the solvent ( $\text{DMSO-d}_6$ ) and dissolved water in ( $\text{DMSO-d}_6$ ) respectively.

### Materials and methods of biological activity section.

Effect of compound (7) on GOT and GPT activities: Colorimetric determination of GOT or GPT activity according to the following reactions:



The pyruvate or oxaloacetate formed was measured in its derived from 2,4-dinitrophenylhydrazine, which was absorbed at wave length 505 nm [29].

Effect of compound (7) on  $\gamma$ -GT activity: Kinetic colorimetric method for the determination of  $\gamma$ -GT activity was assayed by Persijn and Van Der Slik [30]. The principle of the method was measurement of the 5-amino-2-nitro benzoate form from reaction, which was absorbed at wave length 405 nm.



A stock solution (0.01 M) of compound (7) was prepared by dissolving it in ethanol/DMSO (10/1), and the following concentrations ( $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  M) were prepared by diluting with absolute ethanol. The enzymes GOT, GPT and GGT activities were measured in human serum by using the same methods of these enzymes with replacing 100 $\mu\text{l}$  of buffer with 100 $\mu\text{l}$  of compound (7). The inhibition percentage was calculated by comparing the activity with and without the compound (7) and under the same conditions, according to the equation:

$$\% \text{ Inhibition} = 100 - 100 \times \left( \frac{\text{The activity in the presence of inhibitor}}{\text{The activity in the absence of inhibitor}} \right)$$

The activation percentage was calculated by comparing the activity with and without the activator and under the same conditions, according to the equation:

$$\% \text{ Activation} = 100 \times \left( \frac{\text{The activity in the presence of activator}}{\text{The activity in the absence of activator}} - 100 \right)$$

A constant concentration of compound (7) ( $10^{-2}$  M) was used with different substrate concentrations of (40, 80, 120, 160, 200) mmol/L for GOT and GPT, and (0.4, 0.8, 1.2, 1.62) mmol/L for  $\gamma$ -GT, to study the type of inhibition or activation. Buffers were used to prepare different substrates concentrations of these enzymes, GOT, GPT (phosphate buffer pH= 7.40, 100 mmol/L) and  $\gamma$ -GT (TRIS buffer pH= 8.25, 100 mmol/L). The enzymes activities were determined with and without compound (7), by using the Lineweaver-Burk equation and

plotting  $1/v$  against  $1/[s]$  were evaluated values [31];  $k_i$ , Apparent  $v_{max}$  ( $v_{mapp}$ ), Apparent  $k_m$  ( $k_{mapp}$ ), Type of inhibition or activation.

### Preparation methods and physical data of synthesized compounds (1-7)

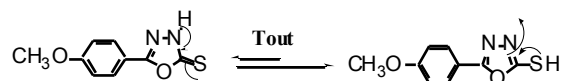
#### 4-Methoxymethyl benzoate (1)

This compound was prepared by following the procedure described by Vogel [22]. Yield (95%); mp: 49-51 °C.

#### 4-Methoxybenzoyl hydrazine (2)

This compound was prepared by following the procedure by described by Smith [32]. Yield (91%); mp: 135-137 °C.

#### 5-(4-Methoxyphenyl)-1,3,4-oxadiazole-2-thione (3)



To a solution of hydrazide **2** (1.66 g, 0.01 mol) in ethanol (20 mL), potassium hydroxide (0.56 g, 0.01 mol) in water (5 mL) and carbon disulfide (2 mL, 0.03 mol) were added. The reaction mixture was heated under reflux till the evolution of hydrogen sulfide ceased (approximately 5 h.), therefore, it was cooled, diluted with cold water (30 mL) and acidified with 10% hydrochloric acid. The solid that separated was collected by filtration, washed with water and recrystallized from ethanol. Yield (84%); mp: 199-201 °C; FT-IR (KBr disk,  $\text{cm}^{-1}$ ) 3217 (N-H), 1616 (C=N), 1253, 1080 (C-O-C);  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz,  $\delta$ ) 14.38-14.78 (s, br. 1H, NH), 7.79-7.85 (dd, 2H, Ar.H), 7.11-7.16 (dd, 2H, Ar.H), 3.86 (s, 3H, OCH<sub>3</sub>). Anal. Calcd. For C<sub>9</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>S (208 g/mol): C, 51.92; H, 3.85; N, 13.46. Found: C, 51.88; H, 3.80; N, 13.48.

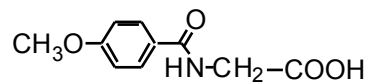
#### 5-(4-Methoxyphenyl)-1,3,4-oxadiazole-2-ethoxycarbonylsulfanyl (4)

Compound **3** (2.08 g, 0.01 mol) in an excess of ethyl chloroformate (3mL) was heated under reflux for 5 h. After cooling the residue of ethyl chloroformate was removed under reduced pressure and the solid residue obtained was recrystallized from ethanol. Yield (79%); mp: 124-125 °C; FT-IR (KBr disk,  $\text{cm}^{-1}$ ) 2980, 2937, 2841 (C-H aliph.), 1773 (C=O), 1626 (C=N), 1259, 1078 (C-O-C);  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz,  $\delta$ ) 7.88-7.85 (dd, 2H, Ar.H), 7.17-7.14 (dd, 2H, Ar.H), 4.48-4.41 (q, 2H, OCH<sub>2</sub>-), 3.86 (s, 3H, OCH<sub>3</sub>), 1.38-1.33 (t, 3H, CH<sub>3</sub>). Anal. Calcd. For C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>S (280 g/mol): C, 51.43; H, 4.29; N, 10.00. Found: C, 51.44; H, 4.32; N, 10.03.

#### 5-(4-Methoxyphenyl)-1,3,4-oxadiazole-2-hydrazine carbonylsulfanyl (5)

This compound was prepared by the same method described for compound **2**. Yield (87%); mp: 222-224 °C; FT-IR (KBr disk,  $\text{cm}^{-1}$ ) 3316, 3146 (N-H), 2935, 2920, 2851 (C-H aliph.) 1640 (C=O), 1610 (C=N), 1255, 1074 (C-O-C);  $^1\text{H}$  NMR (DMSO- $d_6$ , 300 MHz,  $\delta$ ) 13.85-13.75 (s, br. 1H, NH), 7.95-7.92 (dd, 2H, Ar.H), 7.05-7.02 (dd, 2H, Ar.H), 5.72 (s, 2H, NH<sub>2</sub>), 3.78 (s, 3H, OCH<sub>3</sub>). Anal. Calcd. For C<sub>10</sub>H<sub>10</sub>N<sub>4</sub>O<sub>3</sub>S (266 g/mol): C, 45.11; H, 3.76; N, 21.05. Found: C, 45.14; H, 3.77; N, 21.08.

#### 4-(4-Methoxybenzenesulfonyl)-hippuric acid (6)



Glycine (1.5g, 0.02 mol) in 1N sodium hydroxide solution (20 mL) was cooled at 0-5 °C and the cold solution was added dropwise to a solution of 4-methoxybenzoyl chloride (3.41g, 0.02 mol) in chloroform (30mL). The reaction mixture was continued under stirring for an additional 1 h. The aqueous layer was separated and acidified with 2N hydrochloric acid. The product **6** was collected by filtration and recrystallized from ethanol as colorless needles. Yield (83%); mp: 178-180 °C; FT-IR (KBr disk,  $\text{cm}^{-1}$ ) 3366 (N-H), 3190-2534 (O-H, carboxylic), 2933, 2847 (C-H aliph.) 1743 (C=O, acid), 1625 (C=O, amide);  $^1\text{H}$  NMR

(DMSO-d<sub>6</sub>, 300 MHz,  $\delta$ ) 8.65-8.63 (t, 1H, NH), 7.86-7.79 (dd, 2H, Ar.H), 6.98-6.95 (dd, 2H, Ar.H), 3.86-3.84 (d, 2H, CH<sub>2</sub>), 3.77 (s, 3H, OCH<sub>3</sub>). Anal. Calcd. For C<sub>10</sub>H<sub>11</sub>N<sub>1</sub>O<sub>4</sub> (209 g/mol): C, 57.41; H, 5.26; N, 6.69. Found: C, 57.44; H, 5.23; N, 6.72.

**N-{5-[5-(4-methoxyphenyl)-1,3,4-oxadiazole-2-yl-sulfanyl]-1,3,4-oxadiazole-2-yl-methyl}-4-methoxybenzamide (7)**

Compound **5** (2.66 g, 0.01 mol) and Compound **6** (2.09 g, 0.01 mol) were refluxed with phosphorous oxychloride (5mL) for 24 h. and the reaction mixture was then treated with ice water carefully and made basic by adding concentrated sodium bicarbonate solution. The resulting solid was filtered, dried and recrystallized by ethanol-DMSO (10/1). Yield (69%); mp: 193-195 °C; FT-IR (KBr disk, cm<sup>-1</sup>) 3362 (N-H), 2920, 2839 (C-H aliph.), 1620 (C=O, amide), 1610 (C=N), 1255, 1026 (C-O-C); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300 MHz,  $\delta$ ) 8.29-8.22 (t, 1H, NH), 8.17-8.11 (dd, 2H, Ar.H), 7.88-7.81 (dd, 2H, Ar.H), 7.23-7.18 (dd, 2H, Ar.H), 7.09-7.02 (dd, 2H, Ar.H) 3.87 (s, 3H, OCH<sub>3</sub> attached to phenyl-oxadiazole), 3.81 (s, 3H, OCH<sub>3</sub> attached to benzamide), 3.79-3.82 (d, 2H, CH<sub>2</sub>). Anal. Calcd. For C<sub>20</sub>H<sub>17</sub>N<sub>5</sub>O<sub>5</sub>S (439 g/mol): C, 54.67; H, 3.87; N, 15.94. Found: C, 54.71; H, 3.89; N, 15.98.

### Acknowledgment

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**Table (1): The effect of different concentrations of comp. (7) on the activity of GOT, GPT and  $\gamma$ -GT enzymes in human serum.**

No.	Conc. [M]	GOT activity U/L	Activation %	GPT activity U/L	Activation %	$\gamma$ -GT Activity U/L	Inhibition %
1	0	17	0.000	16	0.000	6.545	0.000
2	$10^{-2}$	79	364.705	64	300.000	1.303	0.000
3	$10^{-3}$	30	76.470	20	25.000	2.618	20.000
4	$10^{-4}$	29	70.580	18	12.500	5.236	60.000
5	$10^{-5}$	24	41.176	17	6.250	6.545	80.000

**Table (2): The kinetic properties of GOT, GPT and  $\gamma$ -GT with comp. (7)**

Enzymes	$K_{man}$ (M)	$V_{man}$ (U/L)	$K_i$ (M)	Type of effect
GOT	100	50	0.0129	Noncompetitive
GPT	400	50	0.02	Noncompetitive
$\gamma$ -GT	1	0.256	0.000263	Noncompetitive

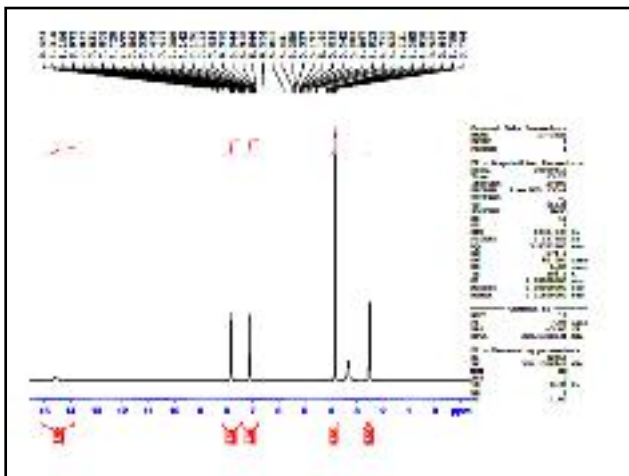


Figure 1. <sup>1</sup>H NMR spectrum of compound 3

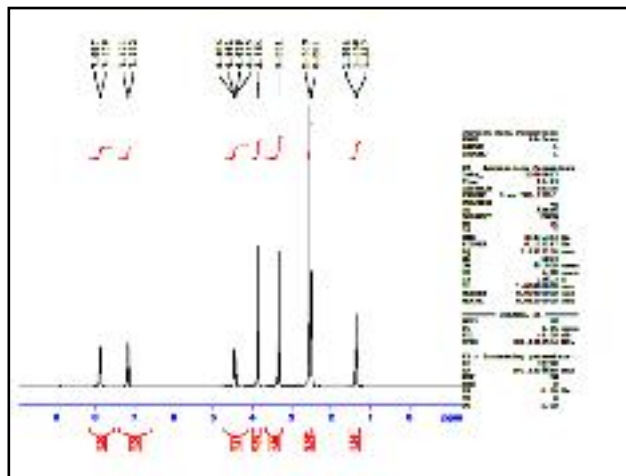


Figure 2. <sup>1</sup>H NMR spectrum of compound 4

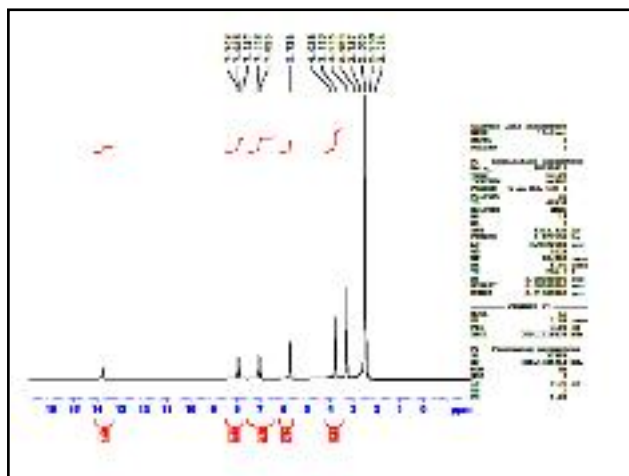


Figure 3. <sup>1</sup>H NMR spectrum of compound 5

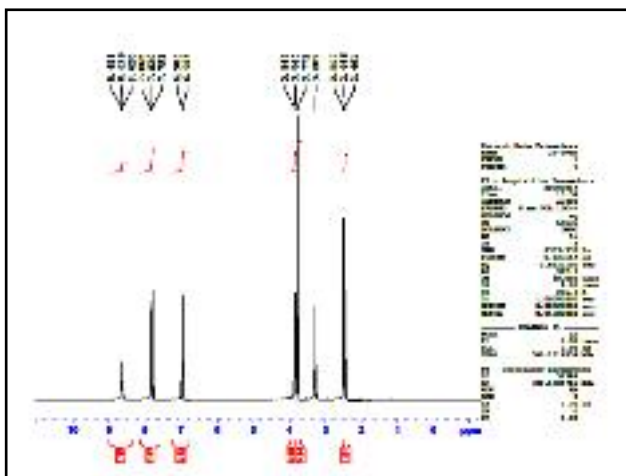


Figure 4. <sup>1</sup>H NMR spectrum of compound 6

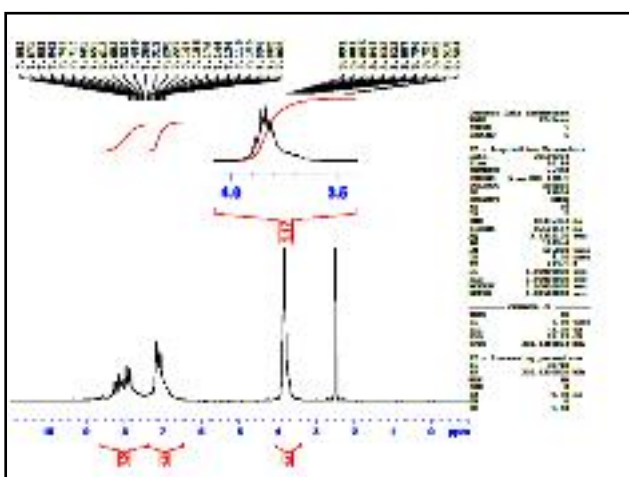


Figure 5. <sup>1</sup>H NMR spectrum of compound 7

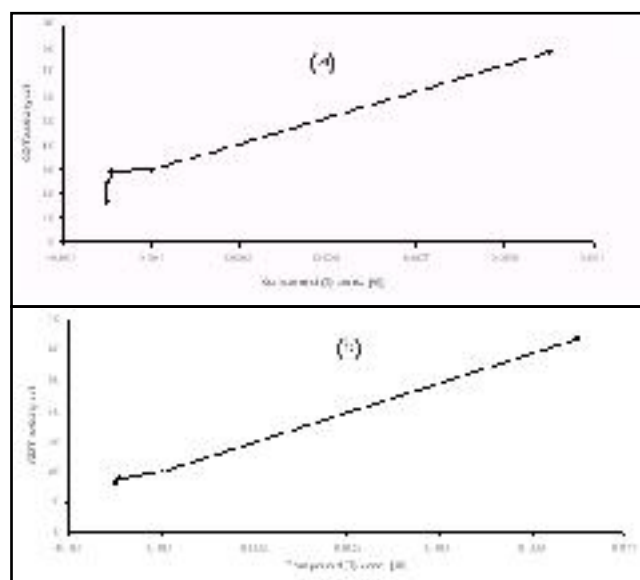
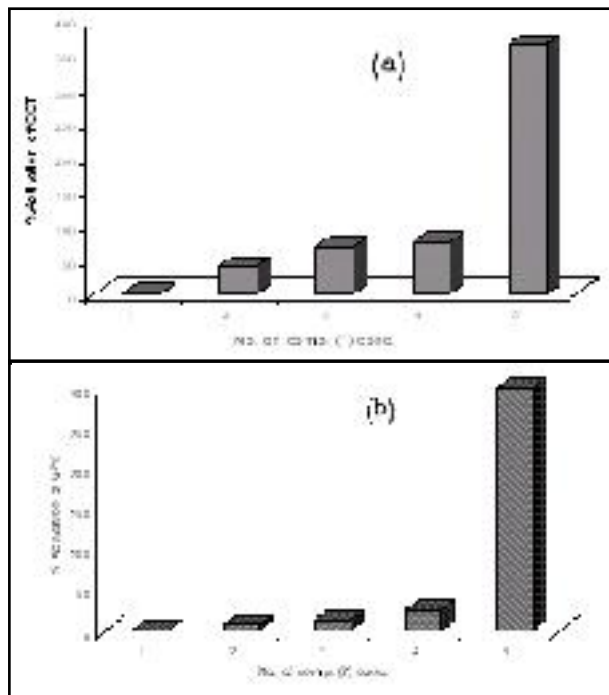
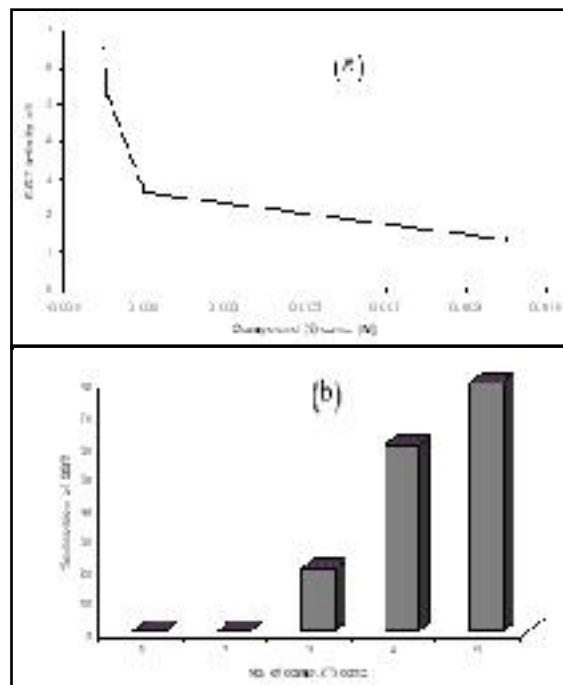


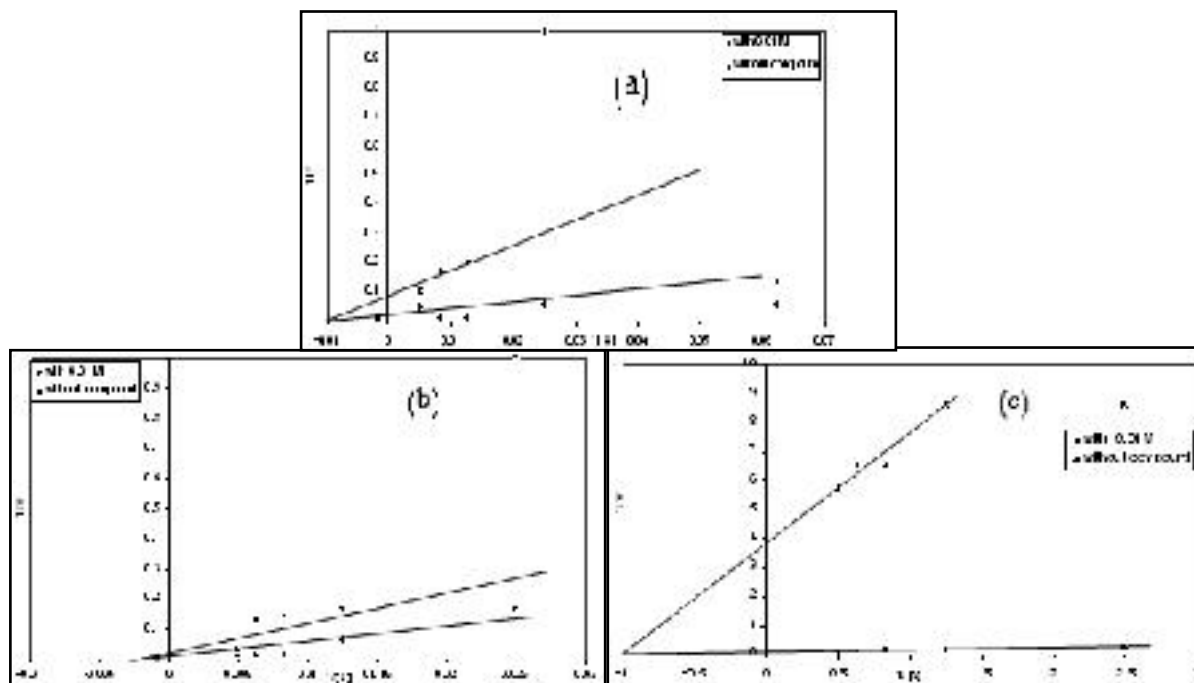
Figure 6. (a) The relationship between conc. of compound (7) and GOT enzyme activity. (b) The relationship between conc. of compound (7) and GPT enzyme activity.



**Figure 8.** (a) The relationship between conc. of and compound (7) conc. (b) The percentage of activation GPT enzyme and compound (7) conc.



**Figure 7.** (a) The percentage of activation GOT enzyme compound (7) and GGT enzyme activity. (b) The percentage of activation GGT enzyme and compound (7) conc.



**Figure 9.** Lineweaver-Burk plots for comp. (7) effects on (a) GOT, (b) GPT and (c) GGT

## تحضير تأثير حلقتي الاوكساديازول الحاوية على الكلايسين في فعالية بعض الانزيمات الناقلة وتشخيصها ودراستها

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### الخلاصة

في هذا البحث حضر مركب جديد يحتوي على حلقتين من 1,3,4-oxadiazole ومركب الكلايسين [5-5]-N-(4-methoxyphenyl)-1,3,4-oxadiazole-2-yl-methyl-4- methoxybenzamide . شخص هذا المركب بوساطة التحليل الدقيق للعناصر ، وطيف الاشعة تحت الحمراء وطيف الرنين النووي المغناطيسي . كذلك صممت هذه الدراسة لبيان تاثير حلقتي الاوكساديازول الملتصقة في فعالية بعض الانزيمات الناقلة، مثل: GOT ، GPT ، GGT في مصل الدم. ووجد ان هذا المركب قام بتنشيط فعالية الانزيمات GOT و GPT بينما كان تأثيره مثبطا لفعالية الانزيم GGT . ووجد ان هذه التأثيرات في فعالية الانزيمات تزداد بزيادة تركيز مركب الاوكساديازول. ان الزيادة والنقصان في الفعالية الحياتية لهذه الانزيمات بوجود هذا المركب نوقشت بالتفصيل في هذا البحث .