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Synthesis , Characterization and Study the Effect of Benzothiazol-pyrazole Derivatives on the Activity of AST, ALT Enzymes

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Abstract

This work includes synthesis some of 2-(4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-benzothiazole derivatives which synthesized from 2-hydrazino-1,3-benzothiazole with dicarbonyl compounds. The prepared compounds were characterized by FT-IR, ^{1}HNMR and also studied the physical properties. The effect of the biological activity for these compounds were studied on the activity of AST and ALT enzymes in human serum of myocardial infarction patients. The studies show the compounds (A2 and A5) caused inhibition while the compound (A3 and A4) caused activation for the activity for AST and ALT enzymes.

Key words: Benzothiazole, Pyrazole, AST, ALT, Biological Activity

1. Introduction

Nitrogen-containing heterocyclics are abundant in natural products and also in synthetic drug molecules, because of a variety of applications and superior pharmacological profile action. Pyrazole is the 5-membered simple aromaticheterocyclic moiety, characterized by the two adjacent

nitrogen atoms in the ring and they belong to the alkaloids groups. Pyrazoles are the integral architects of many of the heterocyclic compounds with superior biological activity. Pyrazofurin is the well-known antibiotic and it contains the pyrazole moiety in their structure. Pyrazole linked with the other heterocyclic analogs creates endeavor in higher pharmaceutical activity with lower toxic effects [1,2]. Benzothiazole is one of the prestigious scaffolds and plays a major role in designing the new pharmacological action of drug molecules because of the diversified activity [3]. In addition to this, pyrazole derivatives exhibit the variety of biological activities like anti-inflammatory[4], antitumour, antihypotensive [5] etc. Benzothiazole amides derivatives were found to exhibit the anti-mitotic activity and they inhibit the percentage of cell division up to 80 % compared to the water[6]. The synthesis of pyrazole moiety linked with the benzothiazole scaffold may help to increase the biological activities. Some of the pyrazole-based drugs are shown in Fig. (1).

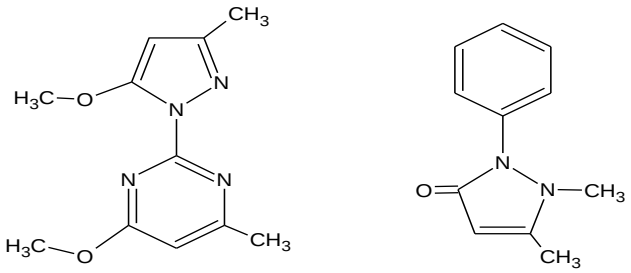


Fig. (1). Pyrazole-based drug molecules in medicine.

Aspartate amino transferase (AST) is an enzyme belonging to the class of transferases. It is commonly reffered to as a transaminases and is involved in the transfer of an amino group between aspartate and α-keto acids. The older terminology, serum glutamic oxaloacetic transaminases (SGOT or GOT), may also be used. The transamination reaction is important in intermediary metabolism because of its function

in the synthesis and degredation of amino acids. The Keto acids formed by the reaction are altimatly oxidized by the tricarboxylic acid cycle to provide a source of energy[7,8]. Aspartate amino transferase is widely distributed in human tissue. The highest concentrations are found in cardiac tissue, liver and skeletal muscle, with smaller amounts found in the kidney, pancrease and erythrocytes.The clinical use of AST is limited mainly to the evaluation of hepato cellular disorders and skeletal muscle involvement[9]. Alanin amino transferase (ALT) is a transferase with enzymatic activity similar to AST. Specifically, it catalyzes the transfer of an amino group from alanin to α- Keto glutarate with the formation of glutamate and pyruvate. The older terminology was serum glutamic pyruvic transaminase (SGPT or GPT).It is distributed in many tissues , with comparatively high concentrations in the liver. It is considered more liver specific enzyme of the transferases.Clinical applications of ALT assays are confined mainly in evaluation of hepatic disorders. Higher elevations are found in hepato cellular disorder, then in extra hepatic or intrahepatic obstructions of the liver.The elevation of ALT activity are frequently higher than those of AST and tend to remain elevated longer as a result of the longer half-life of ALT in serum[7,8,10].

2. Experimental

2.1. Chemical materials

All reactants and solvents used in this study were reagents grade and they are available from Sigma- Aldrich and Fluka companies Melting points are determined in open capillary tubes in a Germany, Stuarts, SMP30 Melting points apparatus and are un corrected. Infrared spectra (FT-IR) were recorded using a SHIMADZU FT-IR8400S

spectrophotometer at the Department of Chemistry/Collage of Science/ University of Mustansiriyah.

^{1}HNMR spectra were recorded on a Bruker, in Iran, Ultra Shield 400Mhz, spectrometer (Switzerland) using DMSO-d6 as a solvent with a tetramethylsilane (TMS) as an internal standard, all progress of the reactions and checking the purity were performed with thin layer chromatography (TLC) technique and revealed by mixture of n-hexane and ethyl acetate (3 : 2) as eluent in the staining jar and irradiation with UV. light chromatograms.

2.1.1. Synthesis of 2-hydrazino-1,3-benzothiazole (1)[11]

A mixture of 2-Mercaptobenzothiazole (1.67g, 0.01 mole) with (0.5g, 0.01mole) from hydrazine hydrate in 15 mL ethanol refluxed for 3 hrs . the mixture cooled to precipitate product and then filtered of and washed with cold water to remove hydrazine excess and give compound (1). The physical properties of compound (1) are listed in table (1).

2.1.2. Synthesis of 1-(1,3-benzothiazol-2-yl)pyrazolidine-3,5-dione (2)[12]

A Mixture of compound (1) (1g, 0.006mole) with diethyl malonate (0.96g, 0.006 mole), and absolute ethanol (30mL) were mixed in a closed microwave tube. The mixture was subjected to microwave irradiation in standard mode at (120°C for 20 min,600 watt). Upon completion of the reaction, the mixture filtered and recrystallized from ethanol to give compound (2) The physical properties of compound (2) are listed in table (1).

2.1.3. Synthesis of 5-amino-2-(1,3-benzothiazol-2-yl)-2,4-dihydro-3*H*-pyrazol-3-one (3)[12]

Mixture from compound (1), (0.5g, 0.003mole) and ethyl 2-cyanoacetate (0.34g, 0.003mole) was taken 20.0 mL of ethanol and the mixture was refluxed for 10-11 h. The reaction mixture was allowed to attain the room temperature. The mixture was then poured into the ice-cold water. The resulting solid product was filtered, dried, and recrystallized from Ethanol to give compound [3]. The physical properties of compound [3] are listed in table (1).

2.1.4. Synthesis of 1-acetyl-2-(1,3-benzothiazol-2-yl)pyrazolidine-3,5-dione (4)[12]

A mixture of compound (1) (1.65g, 0.01 moles) and diethyl malonate (1.6g, 0.01mole) were taken in a round bottom flask and dissolved in (30mL) glacial acetic acid. Then the well-stirred mixture was refluxed for 24 hr. The solvent was evaporated to precipitate product and then filtered and recrystallized from Ethanol to give compound [4]. The physical properties of compound [4] are listed in table (1).

2.1.5. Synthesis of (4*Z*)-1-(1,3-benzothiazol-2-yl)-4-(4-hydroxy benzylidene) pyrazolidine-3,5-dione (5)[13]

A mixture of compound (2) (0.23g, 0.001mol) , p-hydroxy benzaldehyde (0.13g, 0.001mol) , 3 drops of piperidine, and ethanol (20 mL). The reaction mixture was heated under reflux and continuously stirred for a period of 24h. The course of the reaction was monitored by TLC. The reaction mixture was poured into water and acidified with acetic acid. The resulting precipitate was filtered off and recrystallized from acetic acid to give compound [5]. The physical properties of compound [5] are listed in table (1).

Table (1): The physical properties of compounds (1-5)

Comp. symbol	M.F	M.W *gm/mole*	Rec. solvent	R_f	Yield (%*t*)	Color	m.p/°C
1	C7H7N3S	165	ethanol	0.30	92	pale yellow	199-201
2	$C_{10}H_7N_3O_2S$	233	ethanol	0.34	85	yellow	168-170
3	$C_{10}H_8ON_4S$	232	ethanol	0.41	80	light yellow	187-189
4	$C_{12}H_9O_3N_3S$	275	glacial acetic acid	0.89	57	dark brown	232-234
5	$C_{17}H_{11}O_3N_3S$	337	ethanol	0.42	70	light brown	207-209

2.2. Effect of compounds (2-5) on AST, and ALT Activities colorimetric determination of AST or ALT activity according to the following reactions:

L- Aspartate + oxoglutarate —AST→ Oxalacetate+ L- Glutamate

L- Alanine + oxoglutarate —ALT→ Pyruvate + L- Glutamate

The pyruvate or oxaloacetate formed was measured in its derived from 2,4-dinitrophenylhydrazine, which was absorbed at wave length 546 nm (SYRBIO kit) (measured by UV.-Vis. Spectrophotometer)

2.3. A stock solution (0.01 M) of compound (2-5):

A stock solution (0.01 M) of compounds (2-5) were prepared by dissolving it in distilled water, and the following concentrations (10^{-2} , 10^{-3} M) were prepared by diluting with distilled water. The enzymes AST, and ALT activities were measured in human serum by using the same methods of these enzymes with replace 20 µl of buffer with 20 µl of compounds (2-5). The activation percentage was calculated by comparing the activity with and without compounds (2-5) and under the same conditions, according to the equation:

% Activation =100×The activity in the presence of activator/The activity in the absence of activator–100

The activation constant (K_i) was calculated according to the following equation:

$V_{max} + A = V_{max} - A / (1 + [A] / K_i)$ Where A is activation constant.

+A is with activator , -A is without activator, [A] is activator concentration

3. Results and Discussion

3.1. Characterization of benzothiazol-pyrazole compounds

The compound 2-hydrazino-1,3-benzothiazole (1) was selected as key to prepare all derivatives in this work. The compounds (2,4) include synthesis pyrazole ring from cyclization of hydrazine compound (1) with

diketone, while compound (3) from compound (1) and ethylcyano acetate, scheme (2). The FT-IR of compound (3), fig. (3), shows stretching bands symmetrical and asymmetrical at 3319, 3200 cm^{-1} for (NH_2) group, 3126 cm^{-1} for (CH arom.), 2983-2868 cm^{-1} for (CH aliph.), 1743 cm^{-1} for (C=O), 1651 cm^{-1} for (C=N endocyclic for thiazole ring) and 1597 cm^{-1} for (C=C). The ^{1}HNMR of compound (3), figure (6) show signals at δ=3.30 ppm (s, 2H, CH_2 overlapping with protons of DMSO-d_6 solvent), δ=5.01 ppm (s, 2H, CH_2), δ=6.88-7.78 ppm (m, 4H, Ar-H) and δ=8.96 ppm (s, 2H, NH_2). The FT-IR of compound (4), fig. (4), shows stretching bands at 3109 cm-1 for (CH arom.), 2983-2897 cm-1 for (CH aliph.), 1747 cm-1 1734 cm-1 for (two C=O of pyrazol), 1641 cm-1 for (C=O of amid) ,1593 cm-1 for (C=N) and 1558 cm^{-1} for (C=C).. The ^{1}HNMR of compound (4), fig. (7), shows signals at δ=1.98 ppm (s, 3H, CH_3), δ=3.31 ppm (s, 2H, CH_2) overlapping with protons of solvent, δ=7.02-7.91 ppm (m, 4H, Ar-H). The FT-IR of compound (2), figure (2), shows stretching bands at 3188 cm^{-1} for (NH endo cyclic.) 3036 cm^{-1} for (CH arom.), 2935-2978 cm^{-1} for (CH aliph.), 1737 and 1732 cm^{-1} for (two C=O), , 1614 cm^{-1} for (C=N) and 1583 cm^{-1} for (C=C). The ^{1}HNMR of compound (2), fig. (5), show signals at δ=4.14 ppm (s, 2H, CH_2), δ=6.93-8.08 ppm (m, 4H, Ar-H), and δ=10.52 ppm (s, 1H, NH). The compound (5) was synthesized by reaction compound (2) with p-hydroxy benzaldehyde in presence of pipredine as base. The FT-IR of compounds (5) shows disappearance of (CH aliph.), and stretching bands at 3353 cm^{-1} for (OH), 3180 cm^{-1} for (NH) 3056 cm^{-1} for (CH arom.), 1737 and 1730 cm^{-1} for (two C=O), 1614 cm^{-1} for (C=N) and 1600 cm^{-1} for (C=C). The ^{1}HNMR of compound (5), fig. (8), show signals at δ=7.14-7.89 ppm (m, 9H, Ar-H and C=CH) and δ=9.68 ppm (s, 2H, NH overlapping with OH).

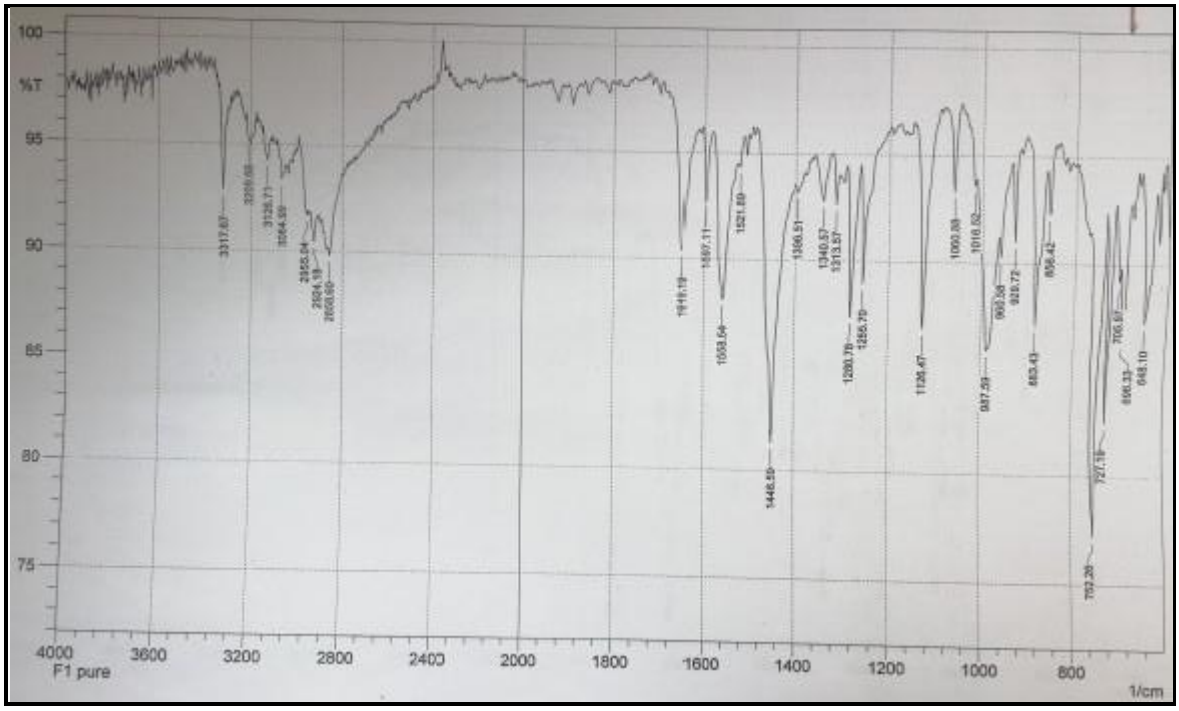


Figure (1): FT-IR spectrum of compound (1)

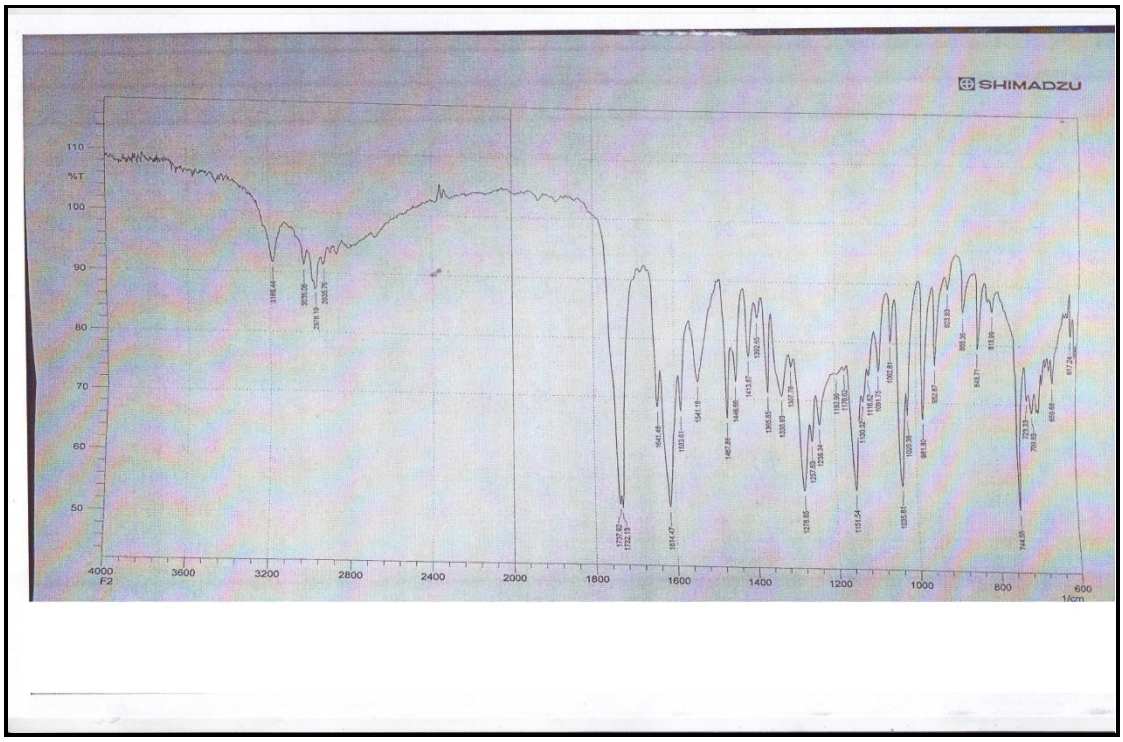


Figure (2): FT-IR spectrum of compound (2)

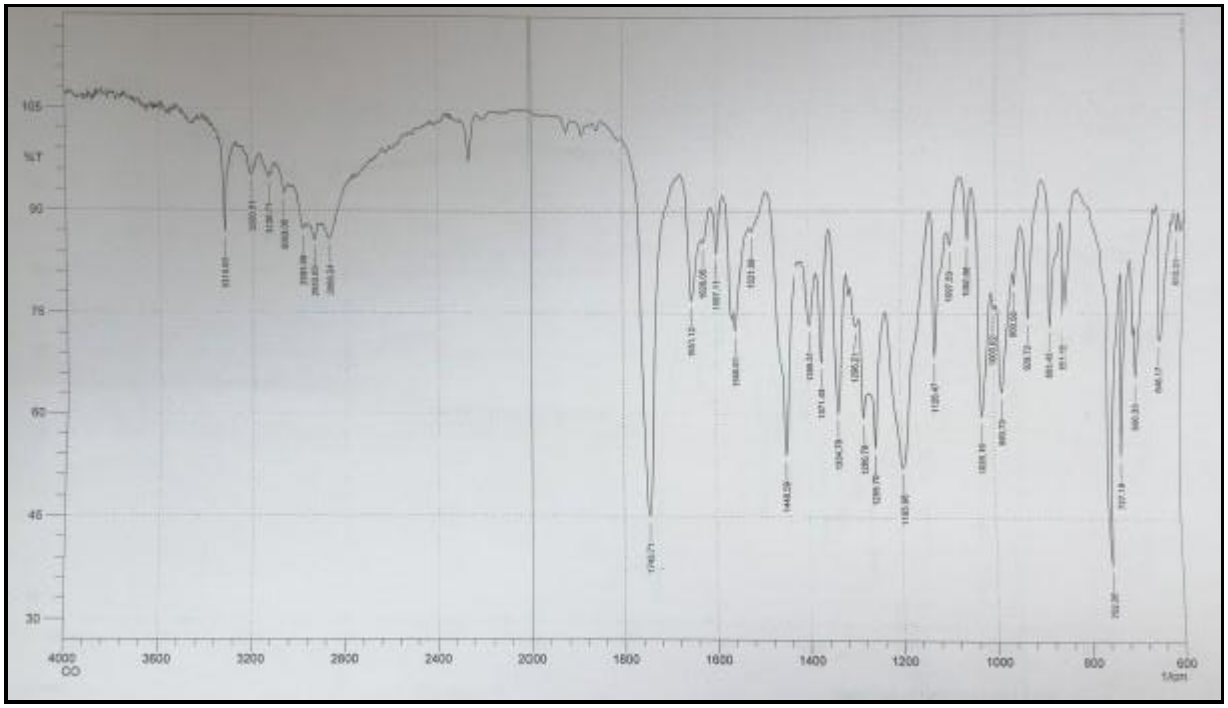


Figure (3): FT-IR spectrum of compound (3)

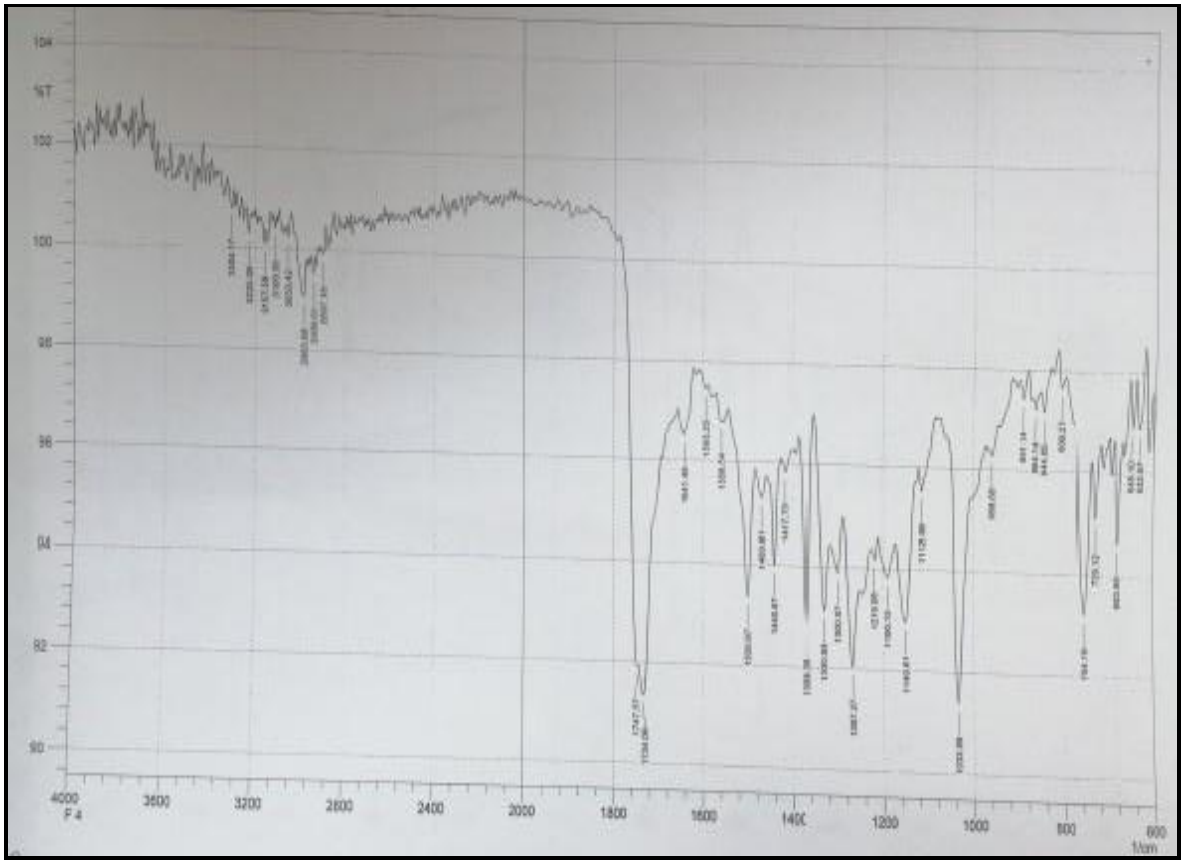


Figure (4): FT-IR spectrum of compound (4)

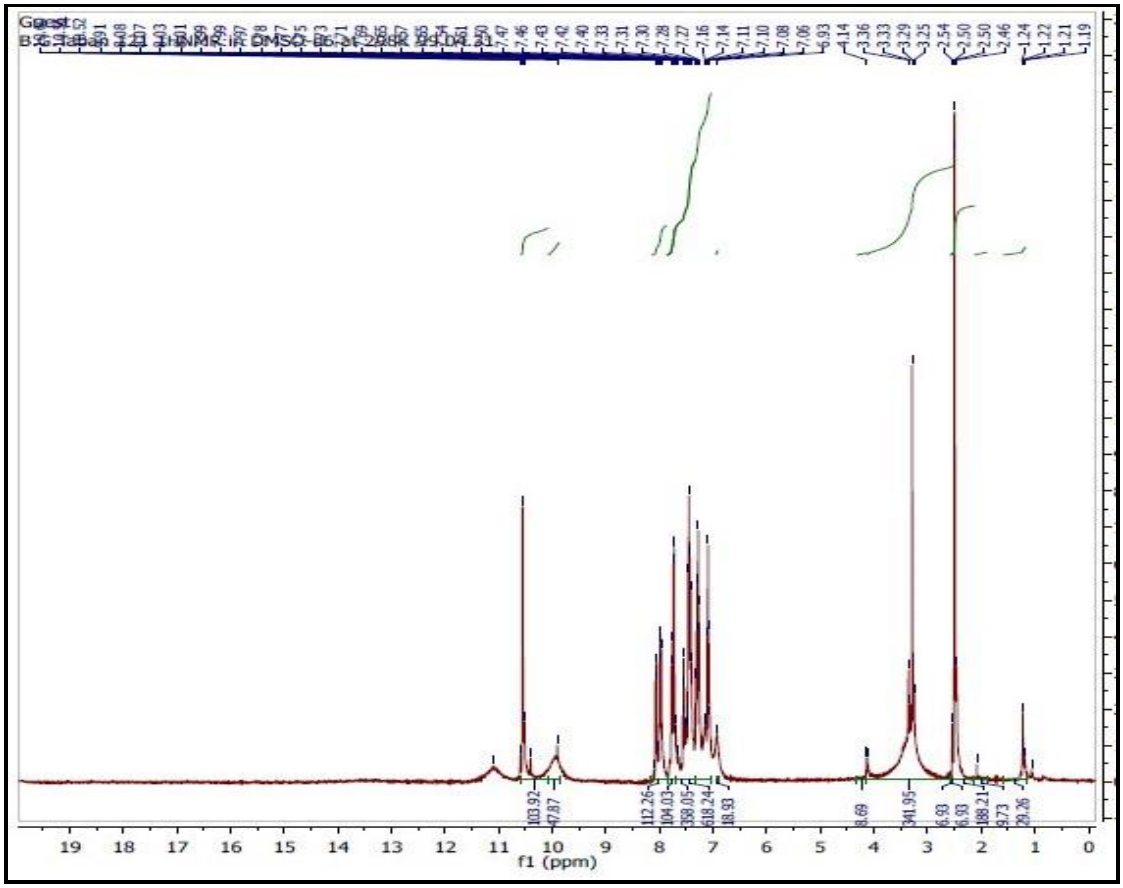


Figure (5): ^{1}HNMR spectrum of compound (2)

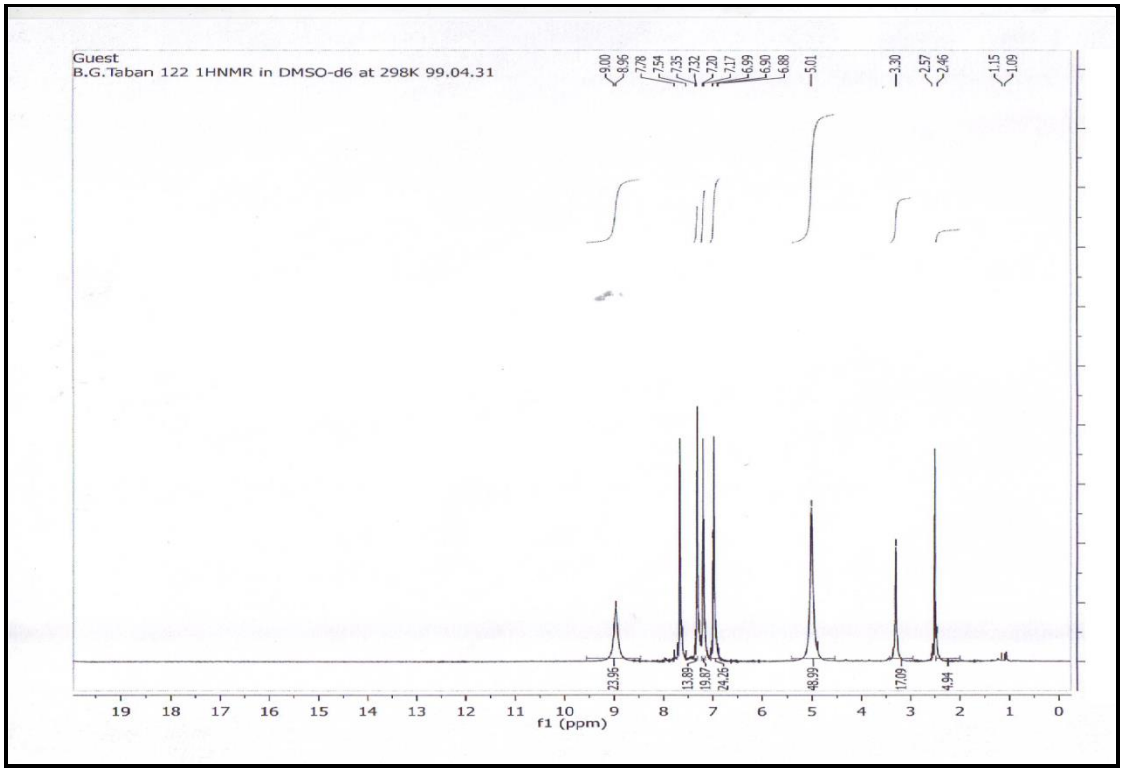


Figure (6): ^{1}HNMR spectrum of compound (3)

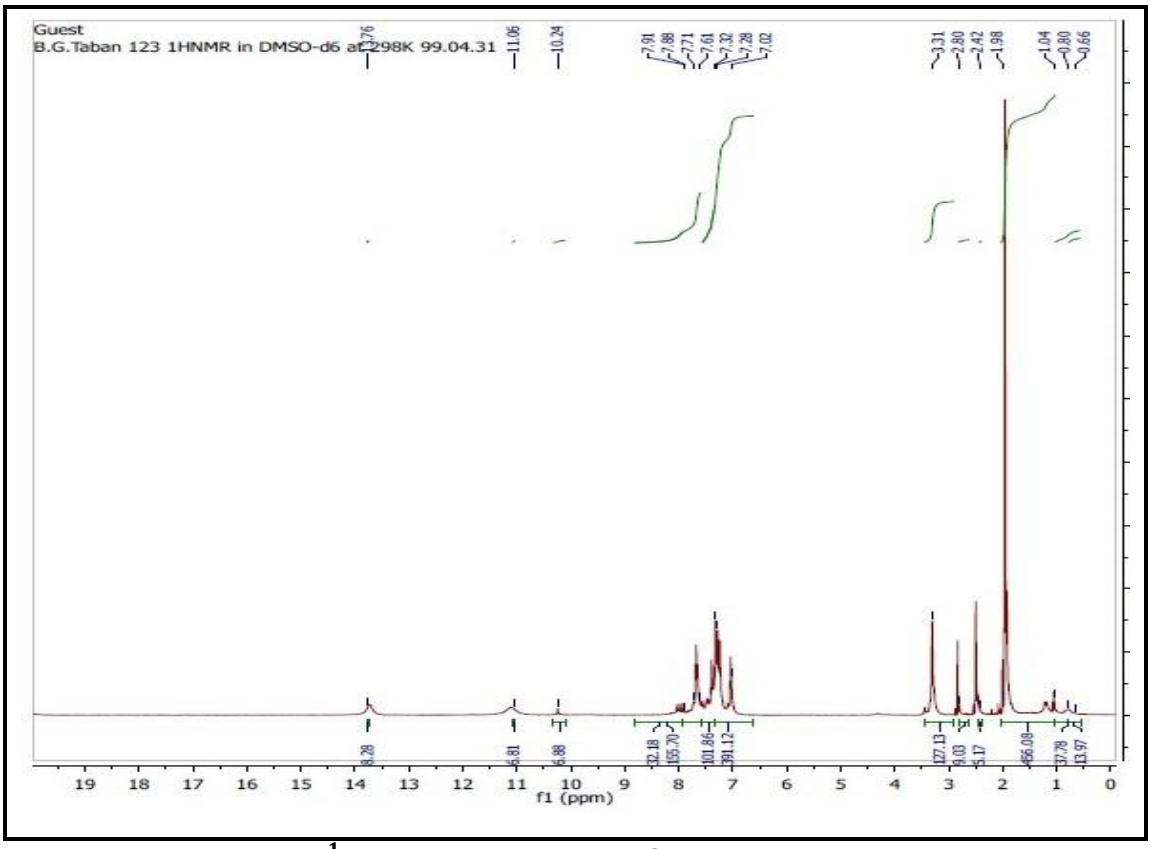


Figure (7): ^{1}HNMR spectrum of compound (4)

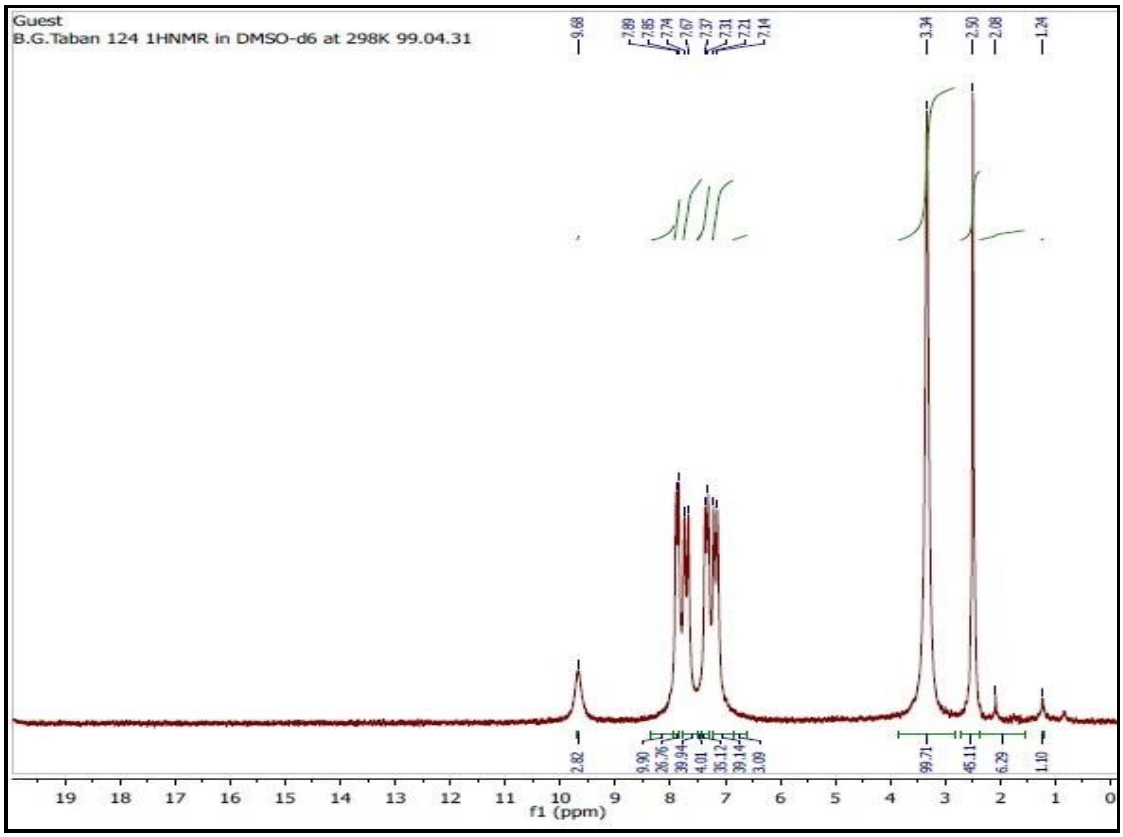
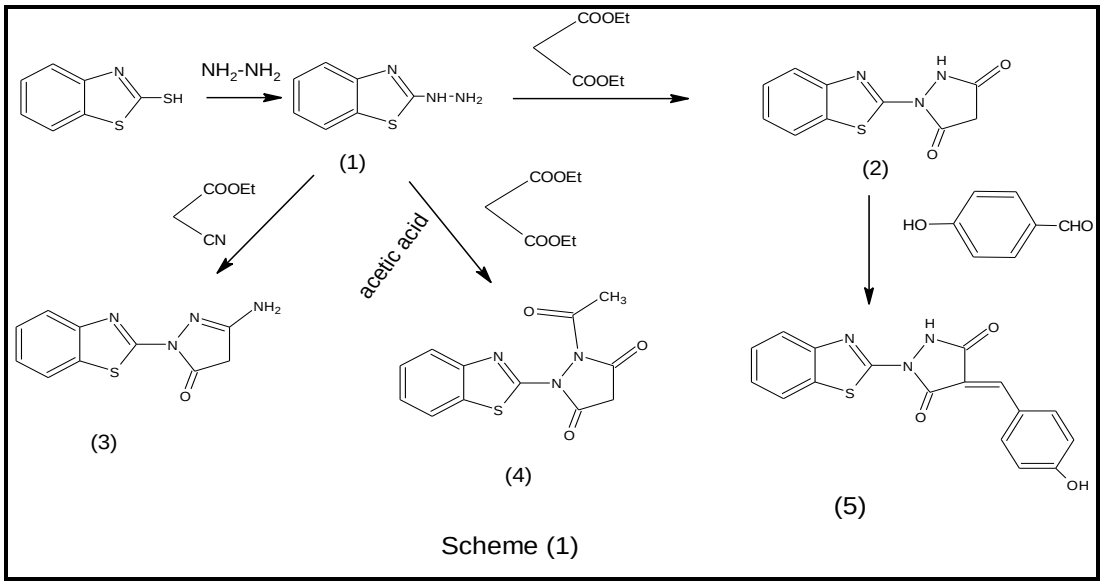
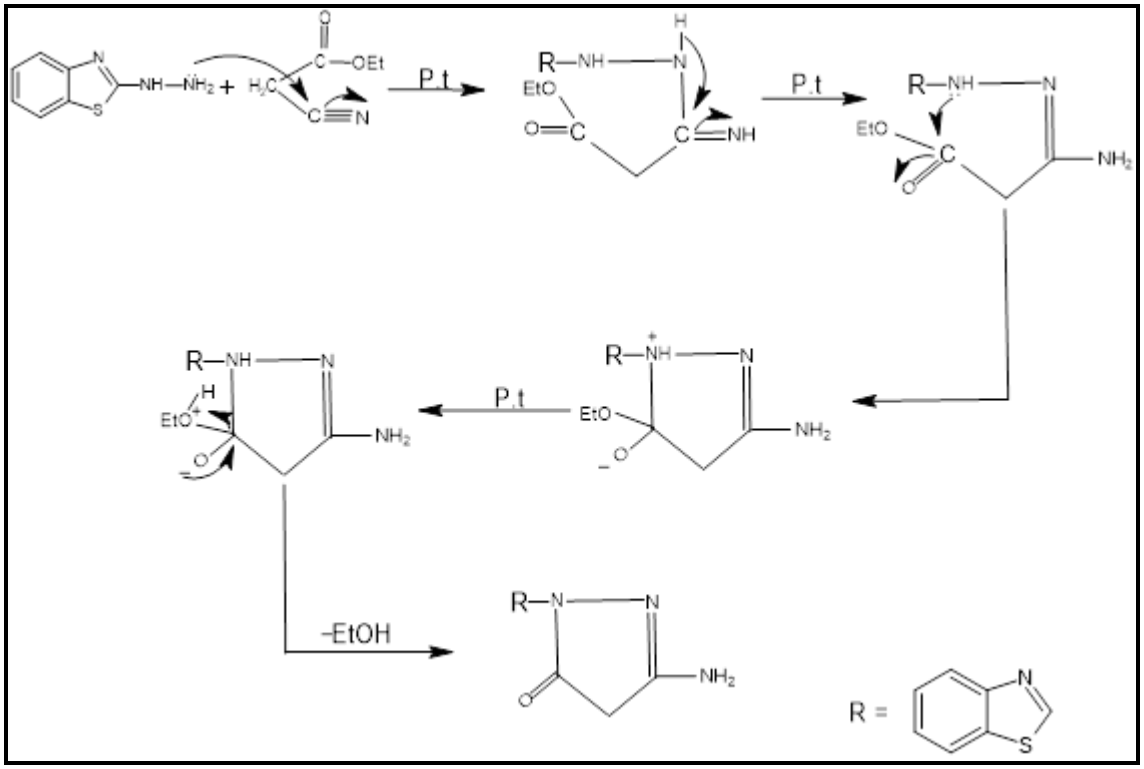


Figure (8): ^{1}HNMR spectrum of compound (5)



Scheme (1): Synthesis of Compounds (1-5) from 2-mercaptobenzothiazole



Scheme (2): mechanism synthesis compound (3)

3.2. Effect benzothiazol-pyrazole compounds on AST

Compounds (2 and 5) have shown good inhibitory effect on the activity of AST enzyme in patients and the inhibition was sufficient to reduce the activity to lower values than that of controls, all results are shown in a table (2)

Table (2) : Effect of different concentration of compounds (2 and 5) on activity of serum AST And a measure of the percentage of inhibition

novel comp.	conc.[M]	controls	Percentage%	patients	Percentage%
without novel comp.		44	—	74	—
2	$1*10^{-3}$	35	20	54	27
	$1*10^{-2}$	27	39	43	42
	$1*10^{-1}$	19	56	28	62
5	$1*10^{-3}$	36	18	59	20
	$1*10^{-2}$	27	39	42	43
	$1*10^{-1}$	19	56	29	61

The current results are agreed with Jianfei Wang and co-workers [14] he suggests that Pyrazole-Thiadiazole Derivatives especially those with a hydroxyl group showed beneficial effect against cardiac injury as evidenced by improvement in cardiac injury marker Through the decrease in his levels of AST adding compounds from what they were before

adding any compound. Moustafa M. Madkour and co-workers[15] Through the study of many organic compounds, including pyrazole derivatives, which contain carbonyl and carboxyl groups. ,In this study, the percentage of inhibition for controls and patients was studied, and The compound (2) was found to have the highest inhibition rate from (5), The reason may be attributed to the compound (2) being less steric than the compound (5) and therefore more bound to the enzyme as shown in the figure (5).

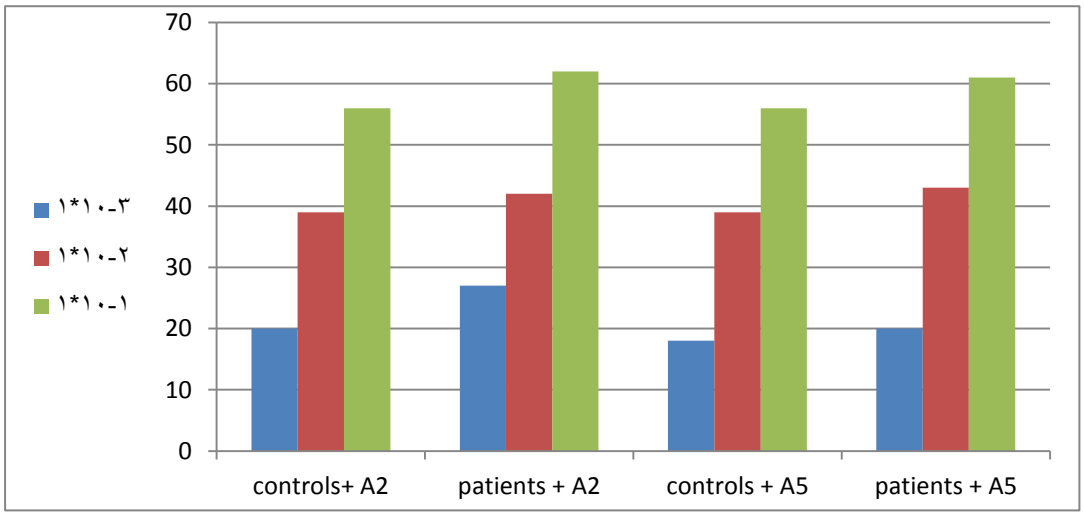


Figure (5) : Percentages of enzyme AST inhibition in controls and patients by compounds (2 and 5)

Compounds A3 and A4 have been shown to be activator by increasing the AST enzyme level in patients and controls , We suggest that these compounds increase the affinity of the enzyme's binding to the substrate through the functional groups it as shown in table (3) and figure (6).

Table (3) : Effect of different concentration of compounds (3 and 4) on activity of serum AST And a measure of the percentage of activation

novel comp.	conc.[M]	controls	Percentage %	patients	Percentage %
without novel comp.		44	—	74	—
3	1*10-3	52	18	89	20
	1*10-2	67	52	116	56
	1*10-1	78	77	132	82
4	1*10-3	58	32	99	22
	1*10-2	69	57	119	30
	1*10-1	80	81	157	42

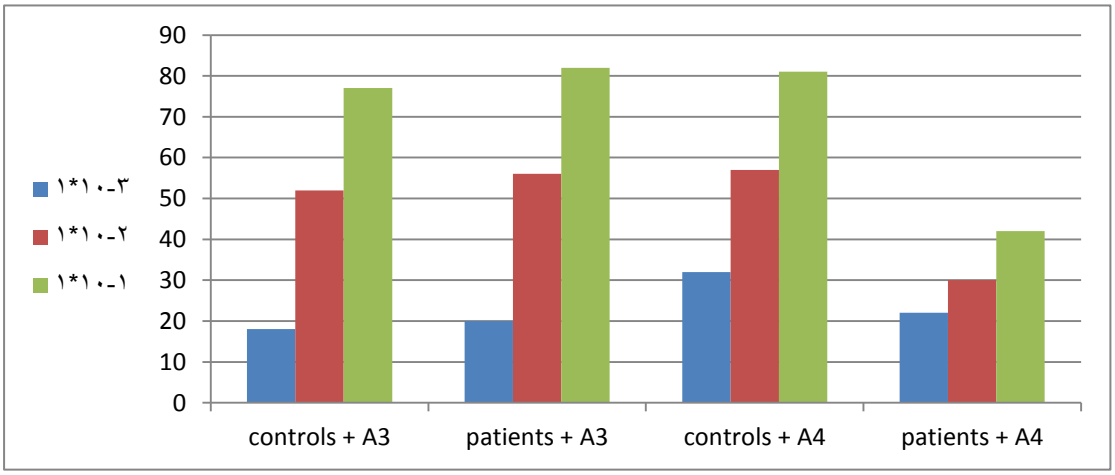


Figure (6) : Percentages of enzyme AST activation in controls and patients by compounds (3 and 4)

3.3. Effect benzothiazol-pyrazole compounds on ALT

Also, the compounds (2 and 5) showed enzyme inhibition, as the results shown in the table (4)

Table (4) : Effect of different concentration of compounds (2 and 5) on activity of serum ALT And a measure of the percentage of inhibition

novel comp.	conc.[M]	controls	Percentage%	patient	Percentage%
without novel comp.		42	–	89	–
2	$1*10^{-3}$	30	28	68	39
	$1*10^{-2}$	21	35	47	52
	$1*10^{-1}$	11	55	25	67
5	$1*10^{-3}$	25	14	51	33
	$1*10^{-2}$	17	20	54	60
	$1*10^{-1}$	10	76	17	81

This study agrees with Mohd. Javed Naim and co-workers[16] Who prepared pyrazole derivatives and studied their effect on AST which contains significantly lowered the AST, ALT, and ALP levels and caused no damage to the heart. Khaled R.A. Abdellatif and co-workers [17]. This study, he is showed the one of pyrazole derivatives response of the heart towards the most active and highest selective; was evaluated through

assessment of the heart function biomarkers in sera including (ALP), the highest selectivity towards ALT enzyme and was the most active anti-inflammatory agent. Interestingly, its cardiovascular profile showed the cardiac biomarkers (ALP, AST, CK-MB, and LDH). In this study, when calculating the percentage of inhibition in the serum of controls and patients, the compound (5) was found to have the highest inhibition rate from (2) as shown in the following figure (7). Because compound (5) contains a hydroxyl group, we suggest that this compound has hydrogen bonds with the amino acid residues present in the enzyme or cause the size of the compound is greater than compound (2), which may hinder the binding of the enzyme to its substrate

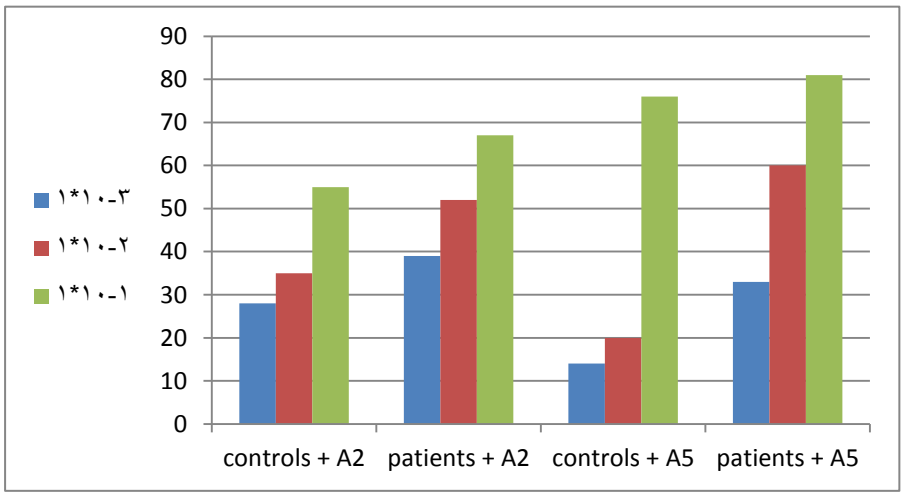


Figure (7) : Percentages of enzyme ALT inhibition in controls and patients by compounds (2 and 5)

The compounds (3 and 4) have been shown to be activator by increasing the AST enzyme level in patients and controls , We suggest that these

compounds increase the affinity of the enzyme's binding to the substrate through the functional groups it shown in table (4) and figure (8).

Table (4) : Effect of different concentration of compounds (3 and 4) on activity of serum ALT And a measure of the percentage of activation

novel comp.	conc.[M]	controls	Percentage%	patients	Percentage%
without novel comp.		42	–	89	–
3	$1*10^{-3}$	54	28	119	33
	$1*10^{-2}$	66	57	143	60
	$1*10^{-1}$	80	90	186	108
4	$1*10^{-3}$	47	11	101	13
	$1*10^{-2}$	58	38	128	43
	$1*10^{-1}$	59	40	136	52

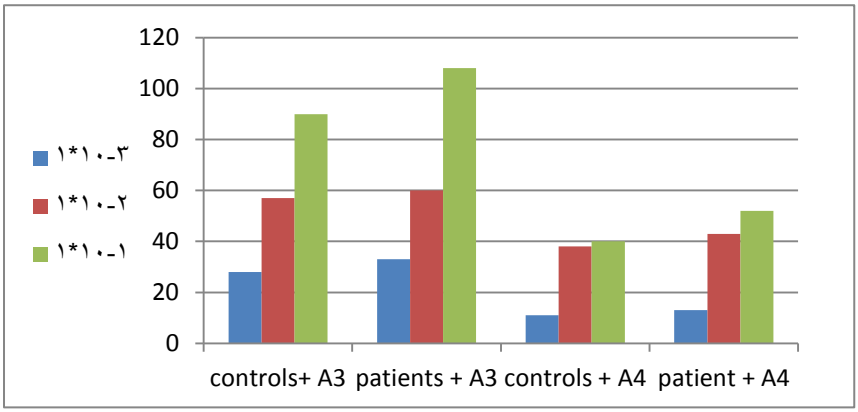


Figure (8) : Percentages of enzyme ALT activation in controls and patients by compounds (3 and 4)

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