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Synthesis, Characterization, Theoretical Study And Effect Of Creatinine Derivatives On The Activity Of GOT And GPT Enzymes.

Zahraa T Khudhair*, and Entesar O Al-Tamimi.

Department of chemistry, College of Science, University of Baghdad, Jadiriya, Baghdad, Iraq.

ABSTRACT

The present report describes the synthesis of heterocyclic compounds on creatinine ring, the Synthetic route started from reaction creatinine with chloroacetyl chloride to give compound(1). Compound (1) react with urea to give compound (2),Shiff bases were synthesized by the reaction compound (2) with *m*-nitrobenzaldehyde, and acetophenone respectively to give compounds (3-4). Esterification of compounds (3-4) with α- chloroethylacetate to give compounds (5-6). Hydrazide derivatives were synthesized by the reaction compounds (5-6) with hydrazine hydrate to give compounds (7-8). The compounds (7-8) reacts with acetonitrile and benzonitrile respectively to give compounds (9-10). The synthesized compounds characterized by FT-IR, ^{1}HNMR, and ^{13}CNMR spectroscopy. Beside the experimental work, we worked theoretical study involving calculated the spectra, total energy, dipole moment etc.. Also this study was designed to show the effects of creatinine derivatives on the activities of some transferase enzymes such as: GOT, and GPT enzymes in sera. This compounds demonstrated activation effects on GOT and GPT activities. These effects increased with increasing the concentration of the compounds. The causes of the increases in the enzymes activities are discussed.

Keywords: creatinine; GOT; GPT; transferase enzymes; dipole moment; spectra;

Corresponding author

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INTRODUCTION

Creatinine is mainly produced from creatineine in skeletal muscle, then transported into blood stream, and finally excreted unaffected by the kidney [1, 2]. In medical practices, Serum creatinine level and creatinine clearance are routinely used as the primary procedure for estimating glomerular function and making dosage changes for drugs excreted by the kidney principally [3]. However, increasing evidences suggested that there could be an undesirable correlation between estimated creatinine clearance and renal clearance of drugs in several clinical settings and creatinine clearance couldn't provide a correct indication for some drugs in renal clearance [4–9]. For example, enal elimination of fluconazole was markedly decreased in people with HIV infection, despite "normal" calculated creatinine clearance [6, 8]. Previous studies suggested that creatinine was mostly excreted by glomerular filtration apart from negligible tubular secretion. However, in recent years, it was reported that renal elimination of creatinine also underwent active tubular secretion, which could account for 10%–40% of total clearance [3, 10]. Shen et al. advised that the clearance of creatinine also could include reabsorption process [10]. Therefore, creatinine clearance could depend on not only glomerular purification, but also tubular secretion. As described above, researches further found that people with HIV infection could alter renal tubular transport independent of the loss of glomerular filtration, and thus the creatinine clearance of HIV patient was within the 'normal' range. Based on this, we have reasons to assume that there are different pathways in the renal tubular clearance of creatinine and fluconazole.

EXPERIMENTAL

Materials and physical measurements

All starting materials and solvents were purchased from Sigma-Aldrich and Fluka and used without further purification. Melting points were measured on Gallen Kamp capillary melting point apparatus and were uncorrected, FT-IR measurements were recorded on Shimadzu model FTIR-8400S. ^{1}HNMR, and ^{13}CNMR spectra were obtained with Bruker spectrophotometer model ultra-shield at 400 MHz in D_2O solution with the TMS as internal standard. Note: in some ^{1}HNMR and ^{13}CNMR spectra, the peaks at δ4.66 and 39.65 ppm are for the solvent (H_2O-D_2) respectively.

Synthesis of the organic compounds

Synthesis of compound (1) [11]

Literature procedure was used with modifications. In 100 mL Round-bottom flask (R.B.F) the creatinine (0.02 mole) was dissolved in DMF (20mL) and then cooled at(0-5˚C) and 2-3 drops of trimethylamine (TEA) were added. Chloroacetyl chloride (0.02 mole) in DMF (20 mL) was slowly added to R.B.F with vigorous stirring for 3 hours at room temperature. The obtained product was filtered, washed with ether and recrystallized from ethanol. The physical properties of synthesized compound (1) is given in Table 1.

Synthesis of compound (2) [12]

Literature procedure was used with modifications. In 100 mL R.B.F (0.02 mole) of compound (1) and (0.02 mole) of urea were dissolved in 1,4-dioxane (20mL) and the blend were refluxed for 18 hours. The obtained product was filtered, and recrystallized from ethanol. The physical properties of synthesized compound (2) is given in Table 1.

Synthesis of compounds (3-4) [13]

To 20 mL of hot ethanol, (0.005 mole) of *m*-nitrobenzalehde/ acetophenone and (0.0025 mole) of compound (2) were dissolved. To this mixture 1.0 mL of glacial acetic acid was added. The reaction mixture was then refluxed on a water bath in a 250 mL R.B.F for 12 hours. Completion of the reaction was monitored by TLC. The mixture was allowed to stand for 24 hours at room temperature. The product was collected and recrystallized with ethanol. The Physical properties of synthesized compounds (3-4) are given in Table1

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Synthesis of compounds (5-6) [14]

Compounds (3-4) respectively (0.01mole) were dissolved in absolute ethanol (20 mL), then NaOH (1M, 10 mL) were added at (0°C). α-chloroethyl acetate (0.01) was added to the mixture. This mixture was stirred at room temperature overnight. The precipitate was filtered and then dried. The product was collected and recrystallized with ethanol. The Physical properties of synthesized compounds (5-6) are given in Table1.

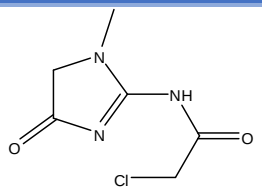
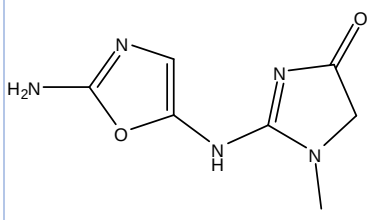
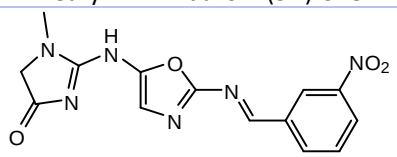
Synthesis of compounds (7-8) [15]

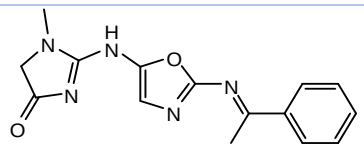
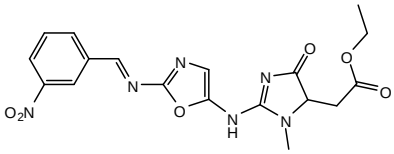
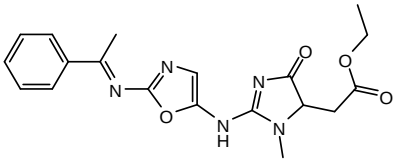
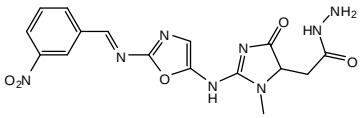
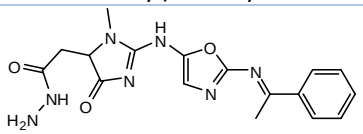
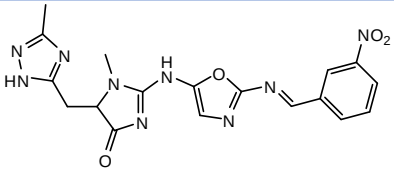
Generally, a solution of compounds (5-6) (0.01mole), hydrazine hydrate (0.01 mole,85%)in absolute ethanol(50mL) were prepared. The reaction mixture was refluxed for 24hr. The obtained product was filtered, and recrystallized from ethanol. The physical properties of synthesized compounds (7-8) are given in Table 1.

Synthesis of compounds (9-10) [16]

A mixture of (0.01 mole) of compounds (7-8), acetonitrile and benzonitrile respectively (0.01) mole in presence of DMF as a solvent were refluxed for 17 hrs. The separated precipitate was cooled, filtered and purified by dissolved in DMF and reprecipitate from acetone. The physical properties of synthesized compounds (9-10) are given in Table 1.

Table1: The physical properties of synthesized compounds (1- 10)

No. of compd.	Structure and name of compounds	Chemical formula	Color	Molecular weight	M. P. °C Dec.	Yield%
1	 2-chloro-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)acetamide	$C_6H_8ClN_3O_2$	Pale yellow	189.60	142-144	89.28
2	 2-(2-aminooxazol-5-ylamino)-1-methyl-1H-imidazol-4(5H)-one	$C_7H_9N_5O_2$	Yellow	195.18	172-174	82.40
3	 1-methyl-2-(2-(3-nitrobenzylideneamino)oxazol-5-ylamino)-1H-imidazol-4(5H)-one	$C_{14}H_{12}N_6O_4$	Deep yellow	328.28	232-235	83.30

4	 1-methyl-2-(2-(1-phenylethylideneamino)oxazol-5-ylamino)-1H-imidazol-4(5H)-one	$C_{15}H_{15}N_5O_2$	Yellow	297.31	157-159	85.44
5	 ethyl 2-(1-methyl-2-(2-(3-nitrobenzylideneamino)oxazol-5-ylamino)-4-oxo-4,5-dihydro-1H-imidazol-5-yl)acetate	$C_{18}H_{18}N_6O_6$	Yellow	414.37	163-165	82.00
6	 ethyl 2-(1-methyl-4-oxo-2-(2-(1-phenylethylideneamino)oxazol-5-ylamino)-4,5-dihydro-1H-imidazol-5-yl)acetate	$C_{15}H_{15}N_5O_2$	Yellow	383.40	152-155	79.40
7	 2-(1-methyl-2-(2-(3-nitrobenzylideneamino)oxazol-5-ylamino)-4-oxo-4,5-dihydro-1H-imidazol-5-yl)acetohydrazide	$C_{17}H_{19}N_7O_3$	Pale yellow	400.35	195-197	72.00
8	 2-(1-methyl-4-oxo-2-(2-(1-phenylethylideneamino)oxazol-5-ylamino)-4,5-dihydro-1H-imidazol-5-yl)acetohydrazide	$C_{16}H_{16}N_8O_5$	White	383.40	209-210	79.40
9	 1-methyl-5-((3-methyl-1H-1,2,4-triazol-5-yl)methyl)-2-(2-(3-nitrobenzylideneamino)oxazol-5-ylamino)-1H-imidazol-4(5H)-one	$C_{18}H_{17}N_9O_4$	Off white	423.39	238-240	78.11

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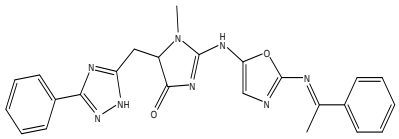
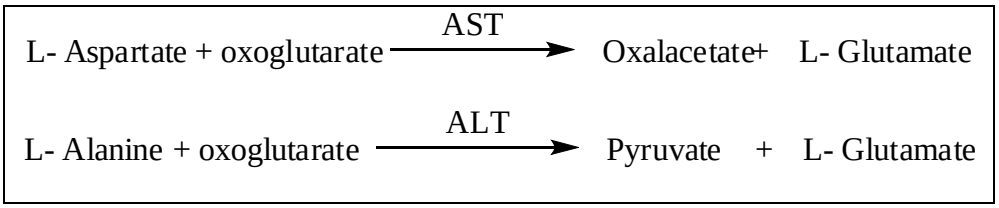
10	 1-methyl-5-((3-phenyl-1H-1,2,4-triazol-5-yl)methyl)-2-(2-(1-phenylethylideneamino)oxazol-5-ylamino)-1H-imidazol-4(5H)-one	$C_{24}H_{22}N_8O_2$	White	454.48	245-247	77.00

MATERIALS AND METHODS

Effect of compounds (9-10) 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 546 nm (SYRBIO kit).

A stock solution (0.01 M) of compounds (9-10)

A stock solution (0.01 M) of compounds (9-10) were prepared by dissolving it in distilled water, and the following concentrations (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} M) were prepared by diluting with distilled water. The enzymes GOT, and GPT activities were measured in human serum by using the same methods of these enzymes with replace 100 µl of buffer with 100 µl of compounds (9-10). The activation percentage was calculated by comparing the activity with and without the activator 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

A constant concentration of compounds (9-10) (10^{-2} M)

A constant concentration of compounds (9-10) (10^{-2} M) were used with different substrate concentrations of (40, 80, 120, 160, 200) mmol/L for GOT and GPT to study the type of activation. Buffers were used to prepared different substrates concentrations of these enzymes, GOT, GPT (phosphate buffer pH = 7.40, 100 mmol/L). The enzymes velocity was determined with and without compounds (9-10), by using the Linweaver and Burke equation and plotting 1/v against 1/[s] were evaluated values; Ki, apparent V_{max} (V_{mapp}), apparent Km (K_{mapp}), type of inhibition or activation [17].

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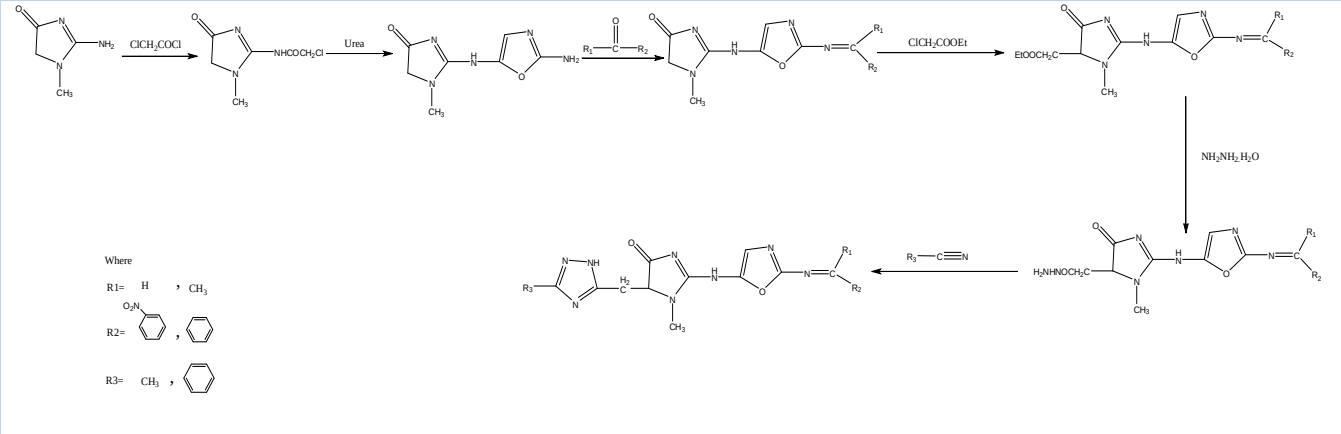
RESULT AND DISCUSSION

Synthesis

Scheme 1 included synthesis creatinine derivatives. Thecharacterization data of all compounds 1–10 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, FTIR, ^{1}HNMR, and ^{13}CNMR data.



Scheme 1: The chemical steps for the synthesis of compounds (1-10)

FT-IR spectra

The FTIR spectrum of compound (1) revealed a medium stretching vibration band at (1625cm^{-1}) that corresponds to (C=O) amide band (see Table2), while(C=O) amide band in compound (2) which are disappearedThe FT-IR spectrum of compounds (1-2) arelisted in Table 2 [18].

Table 2: FT-IR Spectral data of synthesized compounds (1-2) in cm^{-1}

Compd. No.	C=N	ν C-H aliphatic	νC=C Olefinic	νC=O aliph.amide	ν=CH	ν NH$_2$	ν N-H
1	1670	2894	-	1625	-	-	3249
2	1650	2864	1633	-	3141	Asy.= 3460 Sy.= 3409	3259

The FTIR spectrum of compounds (3-4) have two new important characteristic stretching vibration bands that corresponds to (C=N) shiff base band and (C-H) aromatic band and (NH2) band which are disappeared [18]. show Table 3.

Table 3: FT-IR Spectral data of synthesized compounds (3-4) in cm^{-1}

Comp. No.	C=N Shiff base	ν C-H Aromatic	νC=C Aromatic	ν NH$_2$	ν N-H
3	1637	3022	1650	---	3328
4	1631	3016	1662	---	3257

The FTIR spectra of compounds (5-6) have important characteristic stretching vibration bands that corresponds to (C=O) ester band. Show Table 4.

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Table 4: FT-IR Spectral data of synthesized compounds (5-6) in cm^{-1}

Comp. No.	ν C-H Aromatic	ν C=O Ester	νC=C Aromatic	ν C=O Cycl. amide	ν N-H
5	3078	1745	1650	1701	3298
6	3072	1749	1670	1699	3269

The FTIR spectra of compounds (7-8) have important characteristic stretching vibration bands that corresponds to (C=O) ester band which are disappeared and stretching vibration bands that corresponds to (C=O) amide band which are appeared Show Table 5.

Table 5: FT-IR Spectral data of synthesized compounds (7-8) in cm^{-1}

Comp. No.	ν C-H Aromatic	ν C-H Aliphatic	ν C=O Amide	ν NH_2	ν N-H
7	3035	2947	1668	Asy.= 3562 Sy.=3429	3296
8	3002	2964	1668	Asy.= 3454 Sy.=3413	3250

The FTIR spectra of compounds (9-10)

The FTIR spectra of compounds have important characteristic stretching vibration bands that corresponds to (NH) of triazole ring band which are appeared, also stretching vibration bands that corresponds to (NH_2) and (C=O) amide band which are disappeared. Table 6 [18].

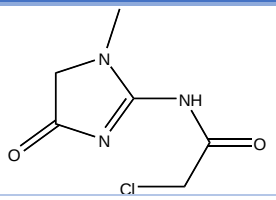
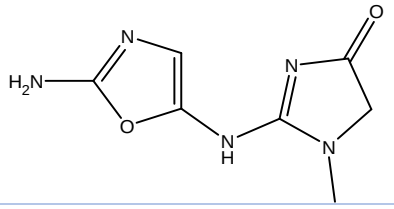
Table 6: FT-IR Spectral data of synthesized compounds (9-10) in cm^{-1}

Comp. No.	ν C-H Aliphatic	ν C=O Amide	ν NH_2	ν C=N (Triazole ring)	ν N-H (Triazole ring)
9	2950	-----	-----	1602	3390
10	2947	-----	-----	1618	3305

^{1}HNMR and ^{13}CNMR Spectra

The ^{1}HNMR and^{13}CNMR spectra of compounds (1,2,9and 10) are listed in Table 7 and 8 and show in figures respectively (1-8) [19].

Table 7: ^{1}HNMR data of compounds (1,2, 9 and 10) in ppm

Compd. No.	Compound structure	^{1}HNMR data of (δ-H) in ppm
1		Singlet 1H of –NH group (7.78); Singlet 2H of CH_2-Cl (4.01); Singlet 3H of N-CH_3 (2.92); Singlet 2H of CH_2-CO (2.50).
2		Singlet 2H of –NH_2 group (4.95); Singlet 1H of =CH (4.69); Singlet 1H of -NH (4.00); Singlet 3H of N-CH_3 (2.90); Singlet 2H of CH_2-CO (2.78).

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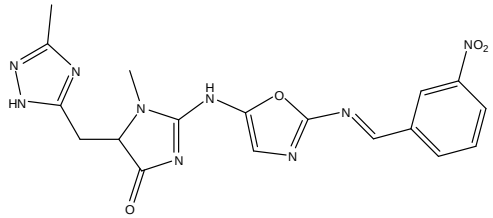
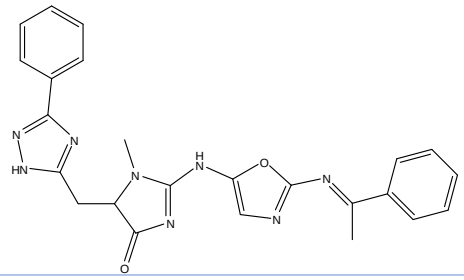
9		Singlet 3H of –CH_3 group (1.75); Singlet 1H of -NH in triazole ring (8.42); Singlet 2H of -CH_2 group (2.16); Singlet 1H of –CO-CH (2.69); Singlet 3H of -N-CH_3 (2.87); Singlet 1H of -NH (3.97); Singlet 1H of -CH= (6.65); Singlet 1H of -N=CH (3.02); multiplet 3H of aromatic ring(6.65-7.78).
10		Singlet 3H of –CH_3 group (1.77); Singlet 1H of -NH in triazole ring (8.30); Singlet 2H of -CH_2 group (2.66); Singlet 1H of –CO-CH (2.71); Singlet 3H of -N-CH_3 (2.81); Singlet 1H of -NH (3.90); Singlet 1H of -CH= (6.66); Singlet 1H of -N=CH (3.05); multiplet 6H of aromatic ring (6.66-8.05).

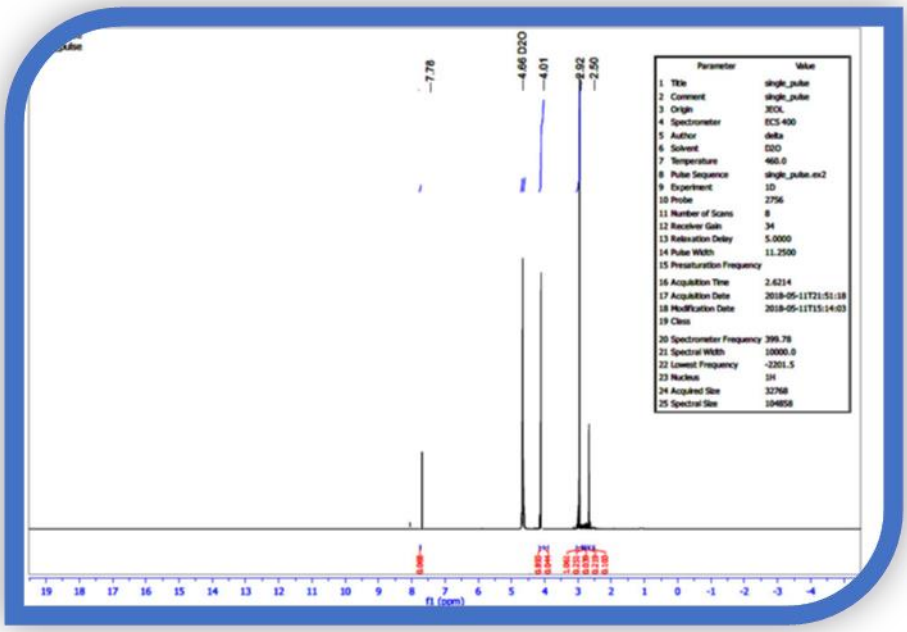


Figure 1: ^{1}HNMR spectrum of compound (1)

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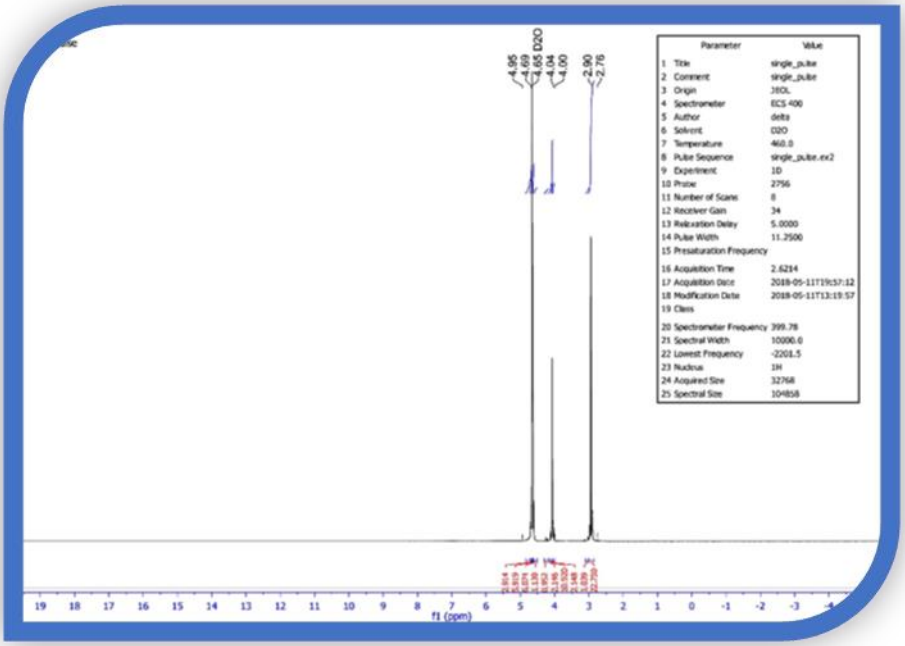

Figure 2: ^{1}HNMR spectrum of compound (2)

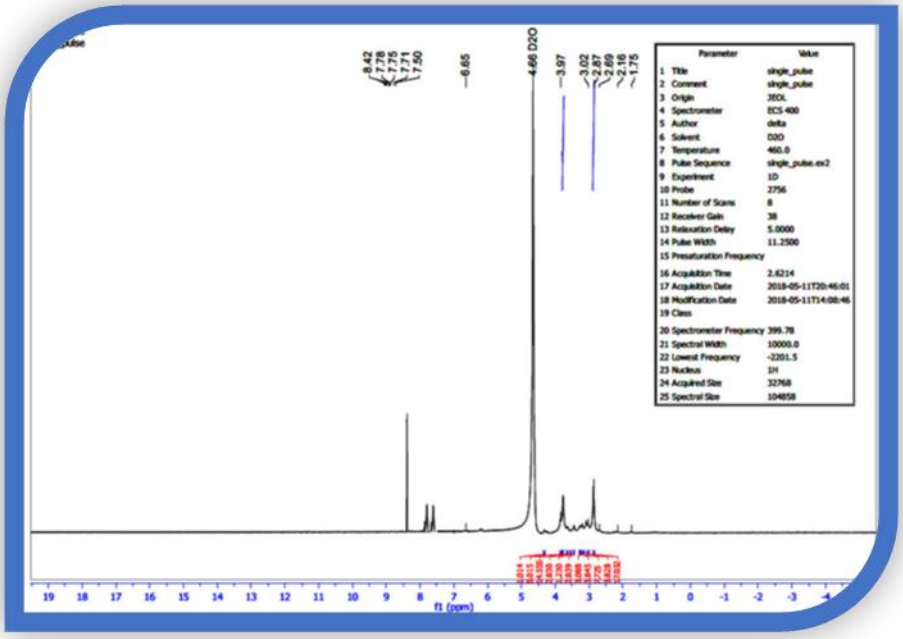

Figure 3: ^{1}HNMR spectrum of compound (9)

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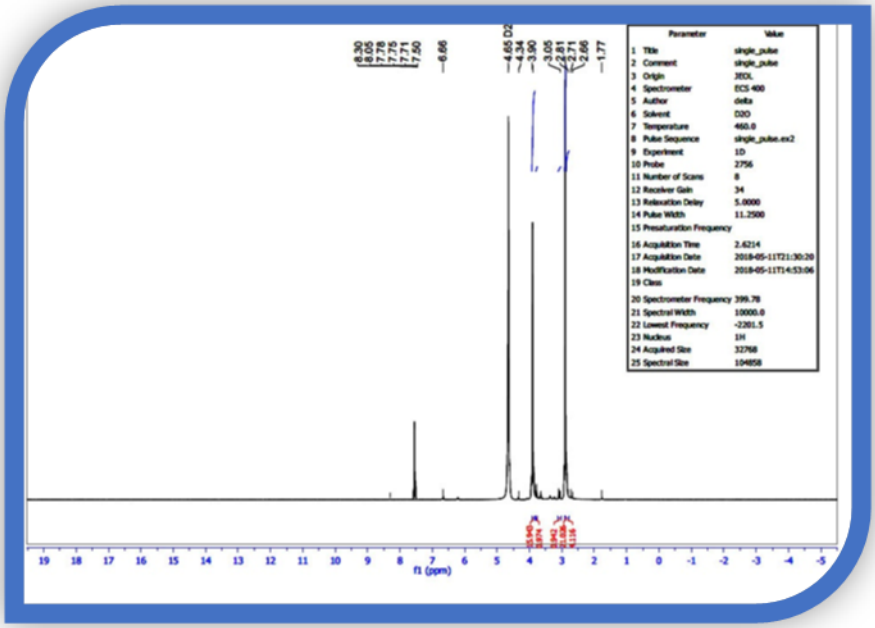
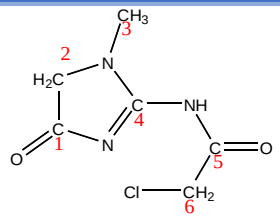
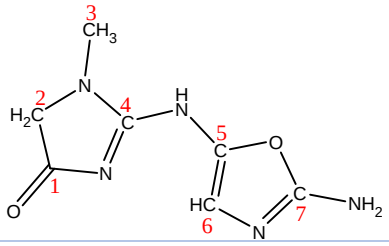
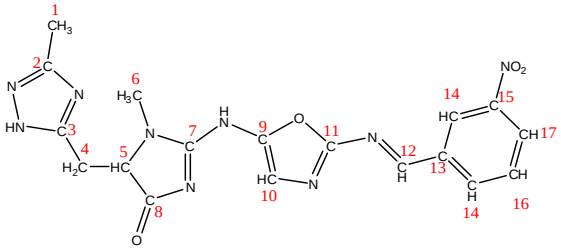
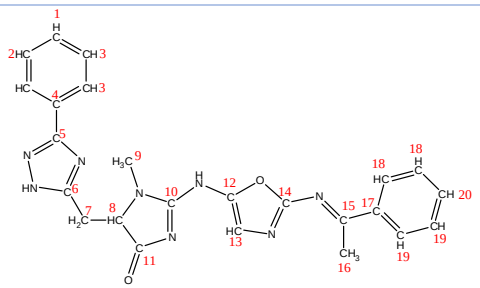


Figure 4: ^{1}HNMR spectrum of compound (10)

Table 8: ^{13}CNMR data of compounds (1,2,9 and 10) in ppm

Comp. No.	Compounds structure	^{13}CNMR data of (δ-H) in ppm
1		C1= 172.34; C2=36.92; C3= 54.13; C4= 164.91; C5= 41.62; C6= 31.37.
2		C1= 175.84; C2=36.92; C3= 54.56; C4= 157.06; C5=129.11; C6=130.50; C7= 159.31.
9		C1= 31.09; C2= 153.14; C3= 157.06 C4= 41.74; C5= 54.22; C6= 37.21 C7=150.75; C8= 172.55; C9= 122.87 C10= 131.60; C11= 152.53; C12= 164.91 C13= 131.18; C14= 122.40; C15=149.68; C16= 124.35; C17= 123.98.

10		C1= 125.63; C2= 126.52; C3= 128.64 C4= 129.14; C5= 150.61; C6= 152.36 C7= 39.21; C8= 56.49; C9= 38.65; C10= 152.41; C11= 189.17; C12= 129.33 C14= 152.85; C15= 169.28; C16= 30.31 C17= 142.53; C18= 134.40; C19=130.50; C20= 129.33

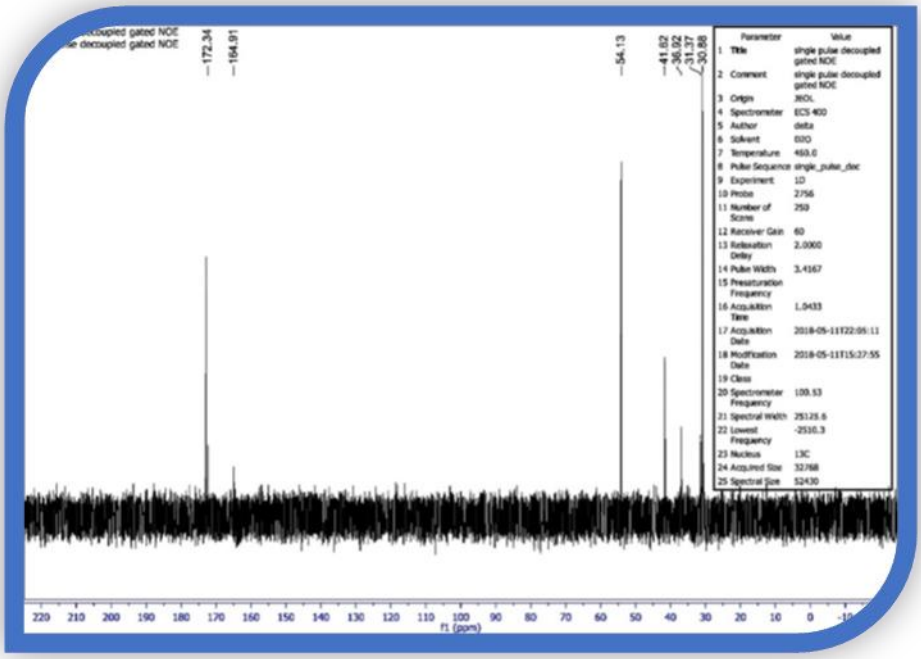


Figure 5: ^{13}CNMR spectrum of compound (1)

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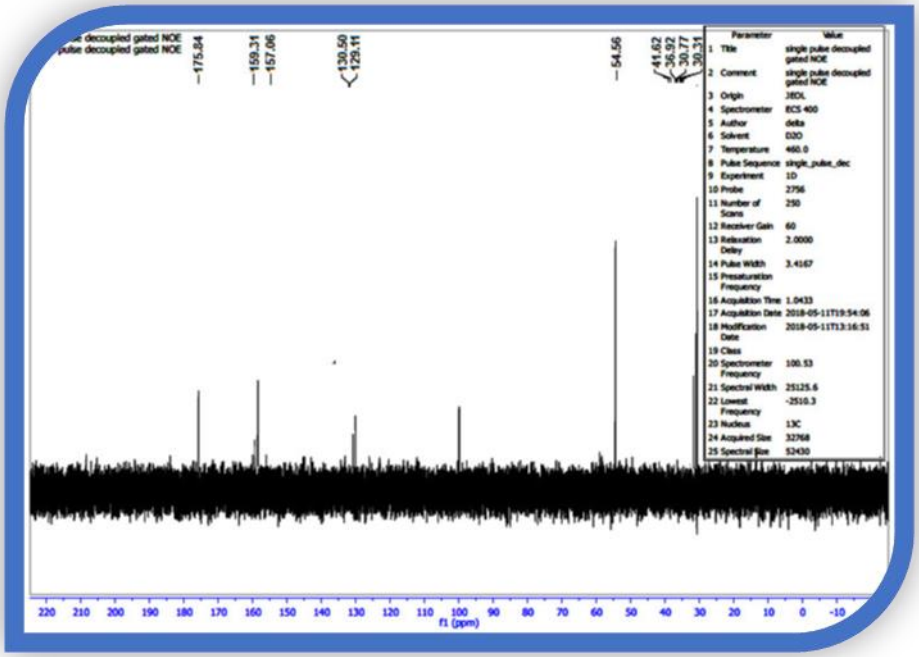

Figure 6: ^{13}CNMR spectrum of compound (2)

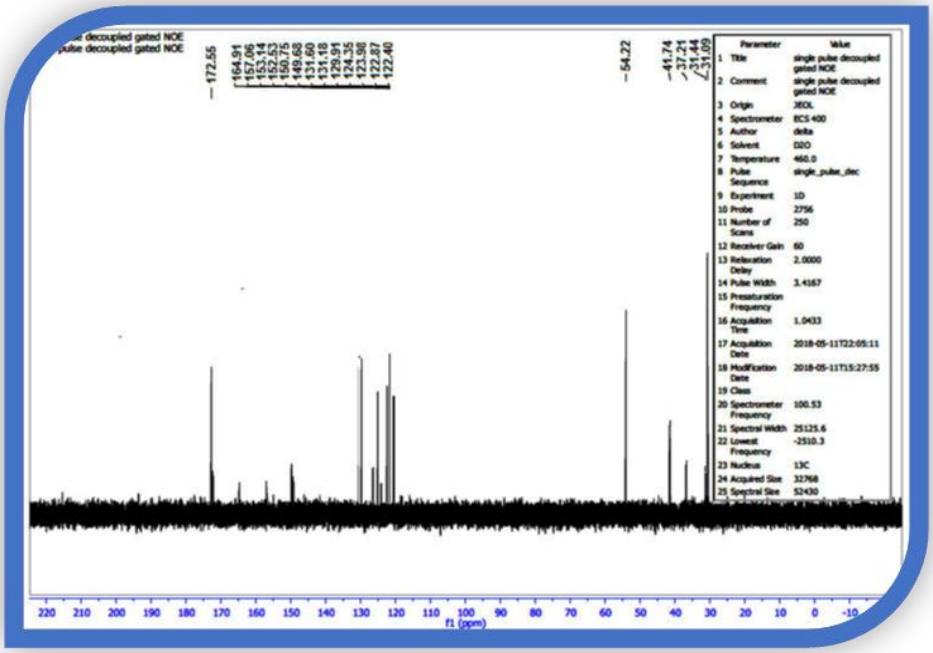

Figure 7: ^{13}CNMR spectrum of compound (9)

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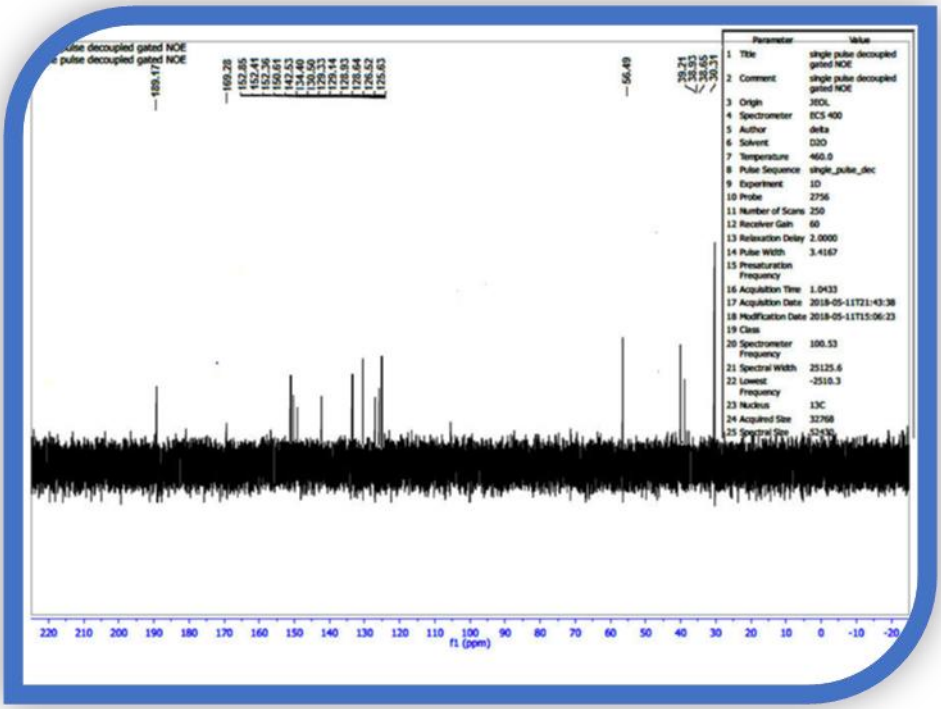

Figure 8: ^{13}CNMR spectrum of compound (10)

Biological activity of transferase enzymes (GOT and GPT)

This research addresses investigation of the effects of compounds (9-10) of GOT and GPT enzymes. The biochemical tests revealed that these compounds caused stimulation effects on GOT and GPT enzymes activities. Table (9) is listed below shows the effect of different concentration of compounds (9-10) on the activity of GOT and GPT enzymes in human serum. This research addresses investigation of the effects of compounds (9-10) of GOT and GPT enzymes. The biochemical tests revealed that these compounds caused activatory effects on GOT and GPT enzymes activities. The normal value of the GOT and GPT enzyme activities were (16 and 17 U/L) respectively. The relationship between compounds (9-10) concentrations versus and the activity of enzymes were shown in Figures (9-10). These results observed that any increase in compound concentrations caused increase in percentage of activation of enzymes.

Table 9: The effect of different concentration of compounds (9-10) on the activity of GOT and GPT enzymes in human serum

Concentration (M)	GOT activity (U/L)	Activation (%)	GPT activity (U/L)	Activation (%)
Sample				
0	16	0.000	17	0.000
Compound (9)				
10^{-2}	100	525.000	92	441.176
10^{-3}	71	341.750	63	270.588
10^{-4}	34	112.500	29	70.588
10^{-5}	22	37.500	20	17.647
Compound (10)				
10^{-2}	83	418.750	90	429.411
10^{-3}	48	200.000	73	329.411
10^{-4}	29	81.250	35	105.882
10^{-5}	21	31.250	24	41.176

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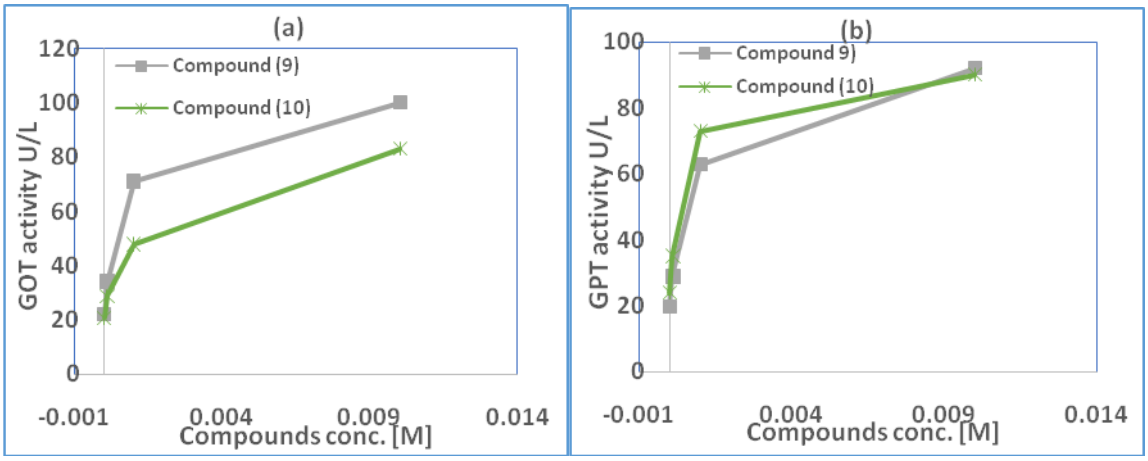

Figure 9: (a) The relationship between concentration of compounds (9-10) and GOT enzyme activity. (b) The relationship between concentration of compounds (9-10) and GPT enzyme activity.

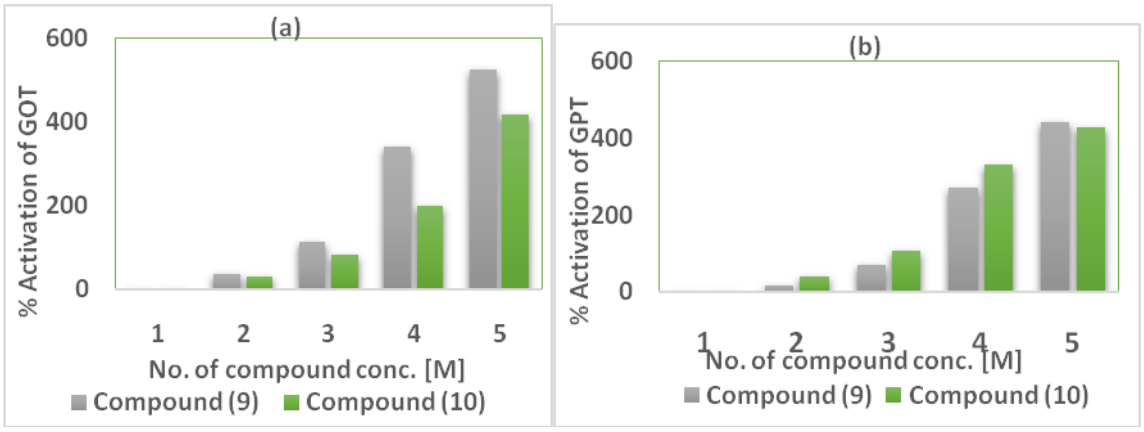

Figure 10: (a) The percentage of activation GOT enzyme and compounds (9-10) concentration. (b) The percentage of activation GPT enzyme and compounds (9-10) concentration.

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 [20].

Table 10 and Figure (11) showed that the type of enzyme activation using Lineweaver–Burk plot for compounds (9-10) on serum GOT and GPT activity. The Vmax and Km values determined with 10^{-2} M of compounds (9-10) and without it. Vmax without compounds (9-10) were greater than Vmax in the presence compounds (9-10). A liquate 10^{-2} M of compounds (9-10) were noncompetitive activation for enzymes activity. Noncompetitive activation changed the Vmax of the enzyme but not the Km. By using Lineweaver– Burk equation, the Ki values of enzyme for compounds were studied in different concentrations.

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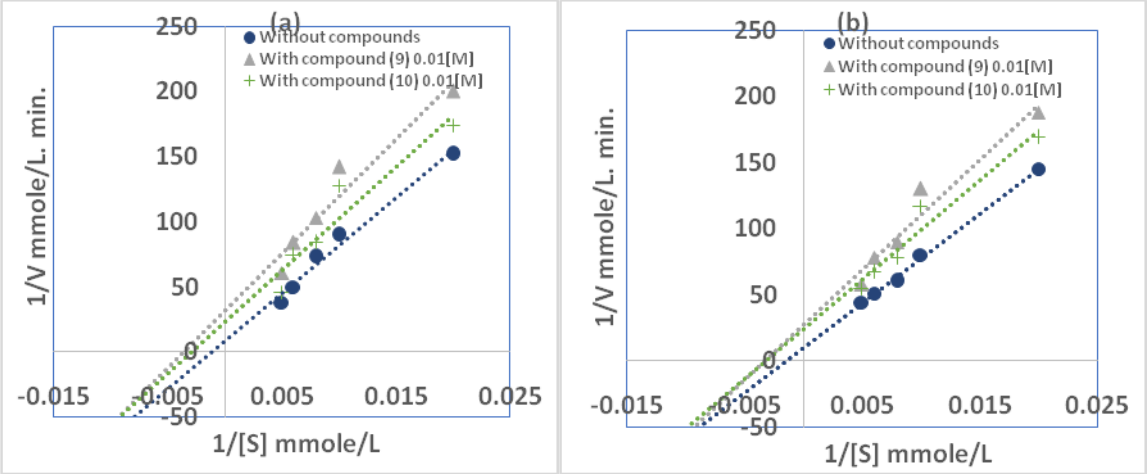

Figure 11: Lineweaver-Burk plots for compounds (9-10) effects on (a) GOT, (b) GPT

Table 10: The kinetic properties of GOT and GPT with compounds (9-10)

Enzymes	K_m (mmole/L)	V_{max} (mmole/ L). min.	K_i (mmole/L)	Type of effect
GOT				
Without compounds	200	0.116		------
Compound (9)	200	0.031	0.0036	Noncompetitive
Compound (10)	200	0.042	0.0057	Noncompetitive
GPT				
Without compounds	200	0.104		-------
Compound (9)	200	0.036	0.0052	Noncompetitive
Compound (10)	200	0.041	0.0065	Noncompetitive

The enzymes play important role in amino acid metabolism and in urea and tricarboxylic acid cycles. We suggested that compounds (9-10) have (N– and O=) groups by which, it activities the active sides of amino acids of GOT and GPT enzymes by increasing affinity of active sides of enzymes to react with the substrates.

Optimize geometry of compound (9).

The Optimize geometry of atoms for the compound (9) calculating by using semi-empirical (PM3) method was depicted in figure 12

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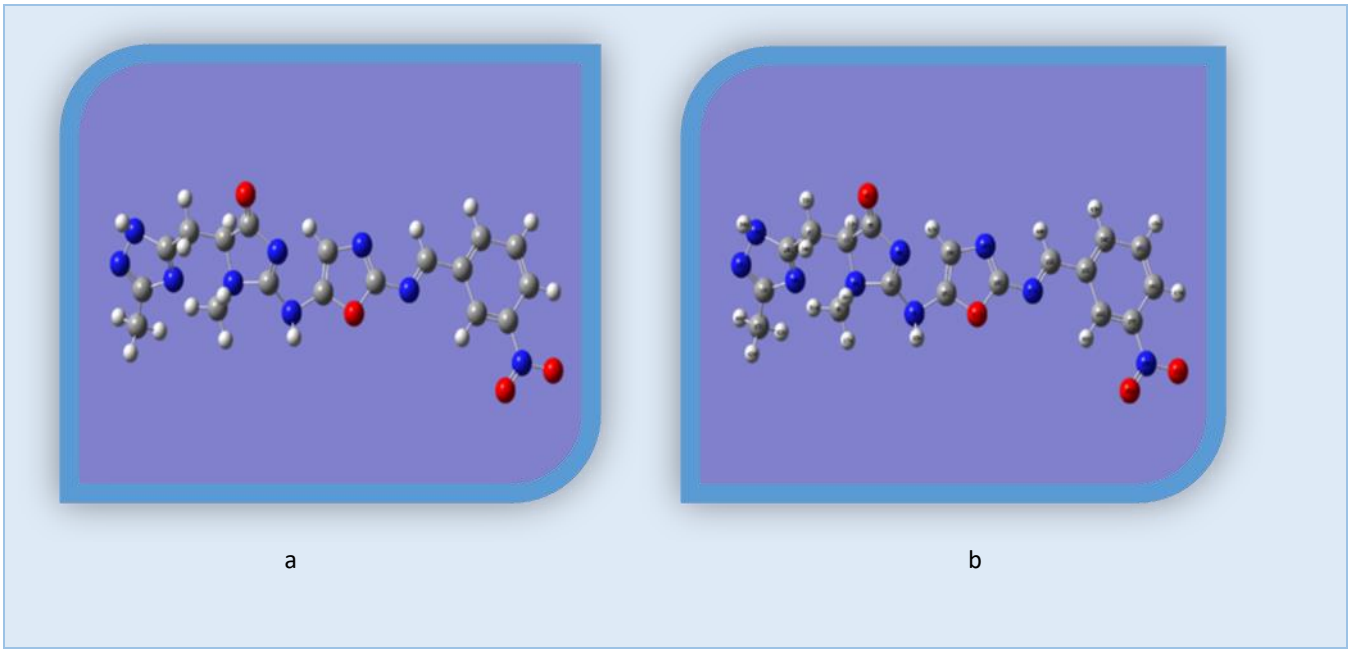

Figure 12: a) The Optimize geometry, b)The numbering Optimize geometry for compound (9)

Table 11: Some calculated bonds length for the compound (9)

Atoms	Actual	Optimal
N(21)-Lp	0.6003	0.6000
N(19)-Lp	0.601	0.6000
O(17)-Lp	0.6005	0.6000
N(11)-Lp	0.6013	0.6000
N(6)-Lp	0.6002	0.6000
N(3)-Lp	0.6008	0.6000
C(28)-H(48)	1.1009	1.1000
C(26)-H(47)	1.1023	1.1000
C(25)-H(46)	1.1040	1.1000
C(24)-H(45)	1.1032	1.1000
C(22)-H(44)	1.1005	1.1000
C(20)-H(43)	1.1003	1.1000
N(14)-H(42)	1.0914	1.0500
C(13)-H(41)	1.1127	1.1130
C(13)-H(40)	1.1128	1.1130
C(13)-H(39)	1.1127	1.1130
C(8)-H(38)	1.1143	1.1130
C(7)-H(37)	1.1141	1.1130

The result of the calculated optimized structural parameters for compound (9) such as bonds length and bonds angle (A°) were calculating by using semi-empirical (PM3) method. The Table 11 and 12 respectively revealed that the results of this work were in good agreement with experimental data.

Table 12: Some calculated bonds angle for the compound (9)

Atoms	Actual	Optimal
O(31)-N(29)-O(30)	76.9170	--------
O(31)-N(29)-C(27)	93.9889	--------
O(30)-N(29)-C(27)	85.7907	-------

H(48)-C(28)-C(27)	119.9765	120.0000
H(48)-C(28)-C(23)	119.1701	120.0000
C(27)-C(28)-C(23)	119.7604	--------
N(29)-C(27)-C(28)	98.1337	120.0000
N(29)-C(27)-C(26)	98.1001	120.0000
C(28)-C(27)-C(26)	96.7236	120.0000
H(47)-C(26)-C(27)	118.1839	120.0000
H(47)-C(26)-C(25)	118.2482	120.0000
C(27)-C(26)-C(25)	121.9722	--------
H(46)-C(25)-C(26)	116.3945	120.0000
H(46)-C(25)-C(24)	114.9810	120.0000
C(26)-C(25)-C(24)	117.2337	--------
H(45)-C(24)-C(25)	115.8530	120.0000
H(45)-C(24)-C(23)	118.1292	120.0000
C(25)-C(24)-C(23)	116.2818	--------
C(28)-C(23)-C(24)	113.1103	120.0000
C(28)-C(23)-C(22)	115.2403	120.0000
C(24)-C(23)-C(22)	116.9454	120.0000
H(44)-C(22)-C(23)	120.5265	120.0000
H(44)-C(22)-N(21)	116.0175	116.5000

Table 13: Some energies and physical properties for compound (9)

Property	PM_3 method
Point group	C_1
Symmetry	A
E_{tot} (kcal/mole)	-115850.3599
E_b (kcal/mole)	-5151.2181
ΔH^o_f (kcal/mole)	65.7718
E_{HOMO} (a.u.)	-0.3541
E_{LOMO} (a.u.)	-0.0546
$\Delta E_{HOMO-LUMO}$(a.u.)	0.2995
μ (debye)	1.6980

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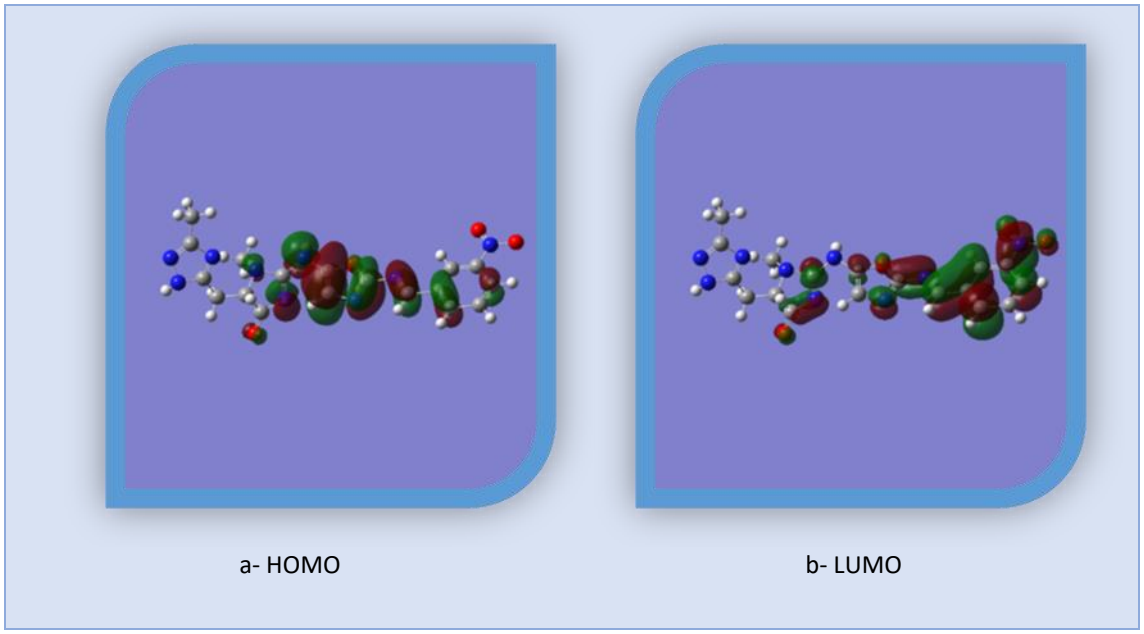

Figure 13: The calculated a- HOMO, b- LUMO for the compound (9)

The following figure14 electron distribution governs the electrostatic potential of the molecules. The electrostatic potential (E.P) describes the interaction of energy of the molecular system with a positive point charge. The E.P is useful for finding sites of reaction in a molecule; positively charged species tends to attack a molecule where the electrostatic potential is strongly negative. [21,22]

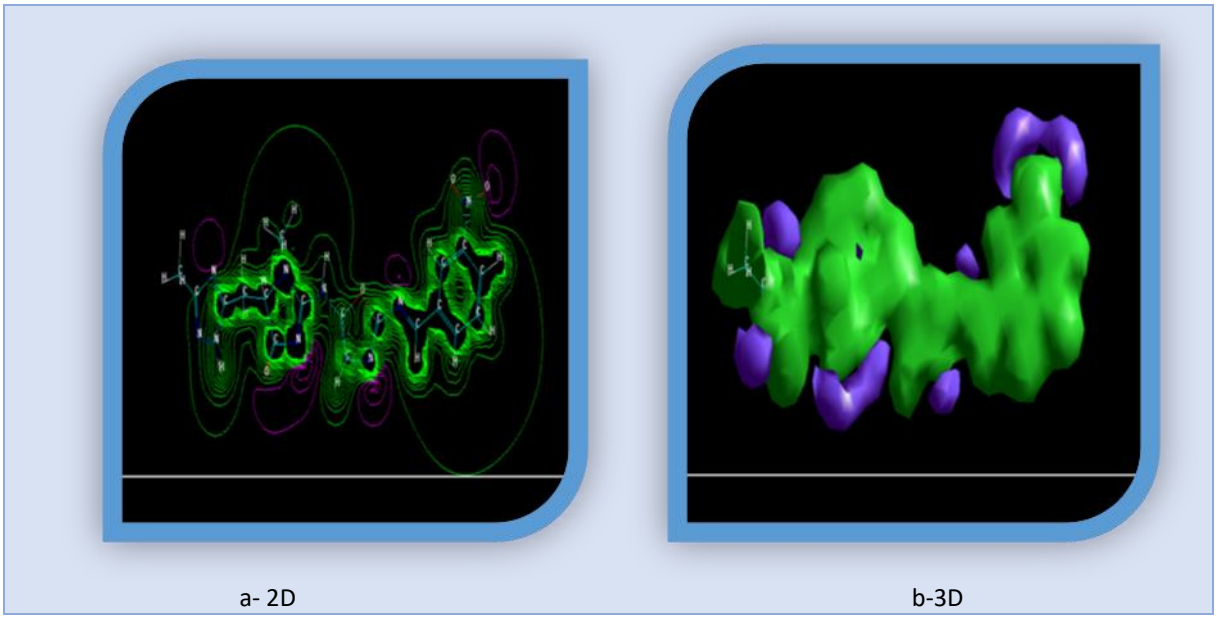

Figure 14: The calculated electrostatic potential a- 2D, b- 3D for the compound (9)

Partial atomic charge of compound (9)

The calculated partial atomic charge using the PM3 method for individual atoms were illustrated in figure 15. The PM3 method give more reasonable value, showing that the O, N atoms have negative partial charge and positive in C atoms, figure 15 Since the O, N atom have more electronegativity than C atom.

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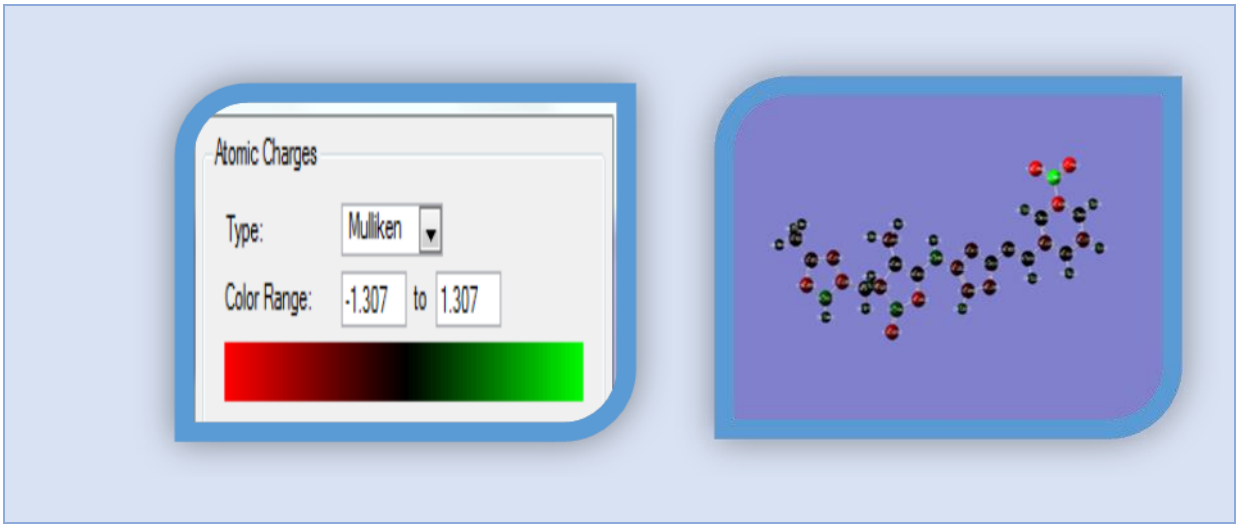


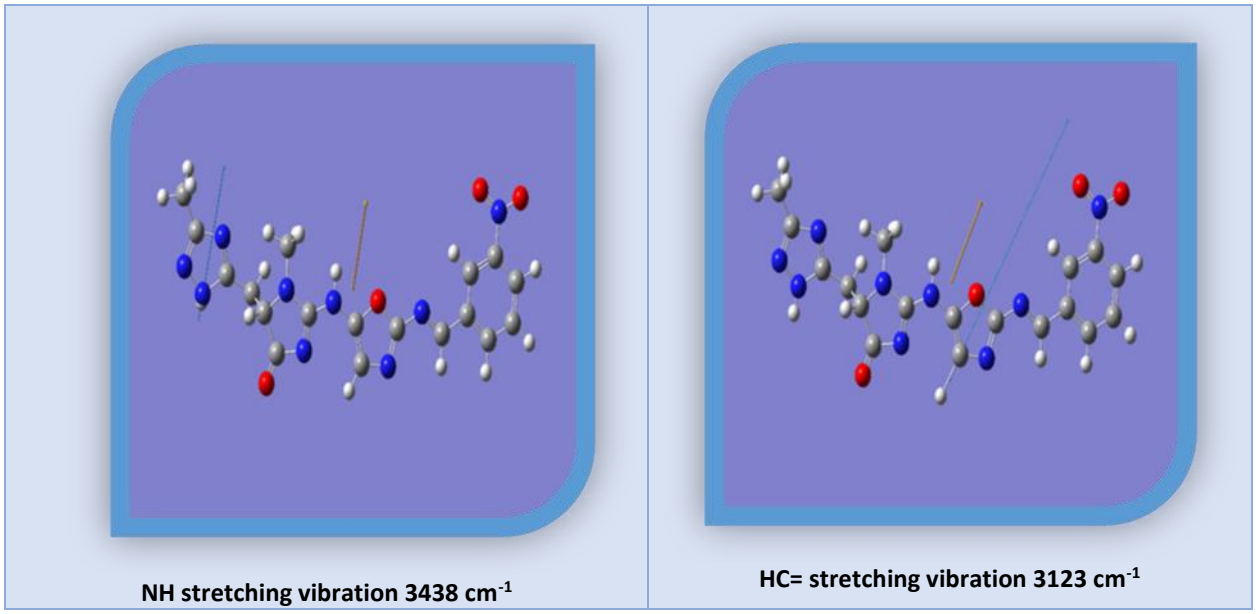
Figure 15: The partial atomic charges for the compound (9)

The vibrational spectra of compound (9)

Compound (9) belongs to (C_1) point group and symmetry (A), The Table are shown below revealed that the theoretical data of this work were in good agreement with experimental data, calculating by using semi-empirical method (PM3).

Table 14: PM3 vibration frequencies and IR absorption intensities for compound (9)

Description	Theoretical		Experimental
	Frequency cm^{-1}	Intensity Km/mole	Frequency
NH str.(ring)	3438	39.96	3431
HC= str. (olifinic)	3123	15.97	3126
C-H str. (aromat.)	3063	58.70	3060
C-H str. (aliph.)	2954	11.45	2950



NH stretching vibration 3438 cm^{-1} **HC= stretching vibration 3123 cm^{-1}**

Figure 16: Some Modes of vibration frequencies for compound (9)

The NMR spectra of compound (9)

The Table are shown below revealed that the theoretical data were in good agreement with experimental data, calculating by using DFT and B_3LYP methods (3-21G).

Table 15: DFT and B3LYP (3-21 G) ^{13}CNMR for compound (9)

Description	Chemical shift ppm	
	Experimental	Theoretical
C1	30.88	30.93
C11	152.53	152.60
C16	124.35	124.40
C17	123.98	124.11

Table 16: DFT and B3LYP (3-21 G) ^{1}HNMR for compound (9)

Description	Chemical shift Ppm	
	Experimental	Theoretical
3H of $-CH_3$ group	1.72	1.75
1H of –CO-CH	2.69	2.67
3H of -N-CH_3	2.87	2.90
1H of -NH in triazole ring	8.42	8.40

CONCLUSION

Creatinine derivatives were synthesized and structually characterized by using spectroscopic techniques. The Synthetic route started fromreaction creatinine with chloroacetyl chloride to give compound(1), then compound (1) converted to compound (2) by the reaction it with urea. Shiff bases were synthesized by the reaction compound(2) with *m*- nitrobenzaldehyde, and acetophenone respectively to givecompounds (3-4). Esterification of compounds (3-4) with α- chloroethylacetate to give compounds (5-6). Hydrazide derivatives were synthesized by the reaction compounds (5-6) with hydrazine hydrate to give compounds (7-8). The compounds (7-8) reacts with acetonitrile and benzonitrile respectively to give compounds (9-10).The biochemical studies revealed that the creatinine derivatives caused activatory effects on GOT and GPT enzymes activities. Finally, we we worked theoretical study For the purpose of comparison with the experimental results.

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