



Synthesis and biological evaluation of new quinazolinone derivatives

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ABSTRACT

New *N*-substituted-2-methyl quinazolin-4(3*H*)-ones derivatives comprising 1,3,4-thiadiazole, Schiff bases and 2-azetidinone moieties are reported. The structures of the newly prepared compounds were confirmed by FT-IR and ¹H NMR spectra. The compounds were also evaluated for their antimicrobial, antioxidant and study of inhibition on some enzymes activities. The results suggest that the compounds possess broad spectrum of *in-vitro* antimicrobial activity. Antioxidant results obtained into the present study indicate that compound **5** show moderate better scavenging activity. Compound **5** demonstrated inhibitory effects on GOT, GPT, GGT and ALP activities, and these effects increase with increasing the concentration of the compound.

1. Introduction

Quinazolinone is an interesting and important aromatic heterocyclic system. Substituted 4(3*H*) quinazolinones possess a wide range of pharmacological activities such as antimicrobial [1,2], anticancer [3], antiviral [4], anti-inflammatory [5], antitubercular [6], antitumor [7], antioxidant [8] and anticonvulsant [9].

1,3,4-Thiadiazole derivatives play role owing to their wide range of biological activity [10] and industrial applications [11]. It is also well established that various derivatives of 1,3,4-thiadiazole exhibit broad spectrum of pharmacological properties such as anticonvulsant [12] and liver enzymes inhibition [13].

Furthermore, the 2-azetidinone skeleton, otherwise known as the β -lactam ring, has recognized as a useful building block in synthesis of biologically important compounds [14]. In recent years, significant attention has been drawn to 2-azetidinone derivatives due to their diversified therapeutic activities like antioxidant and cholesterol absorption inhibition [15].

1.1. Glutamate oxaloacetate transaminase (GOT) (EC 2.6.1.1)

It's also called aspartate aminotransferase (AST), one of the most important of transaminases enzyme, and catalyzes the transfer of the amino group of aspartate to α -ketoglutarate. GOT

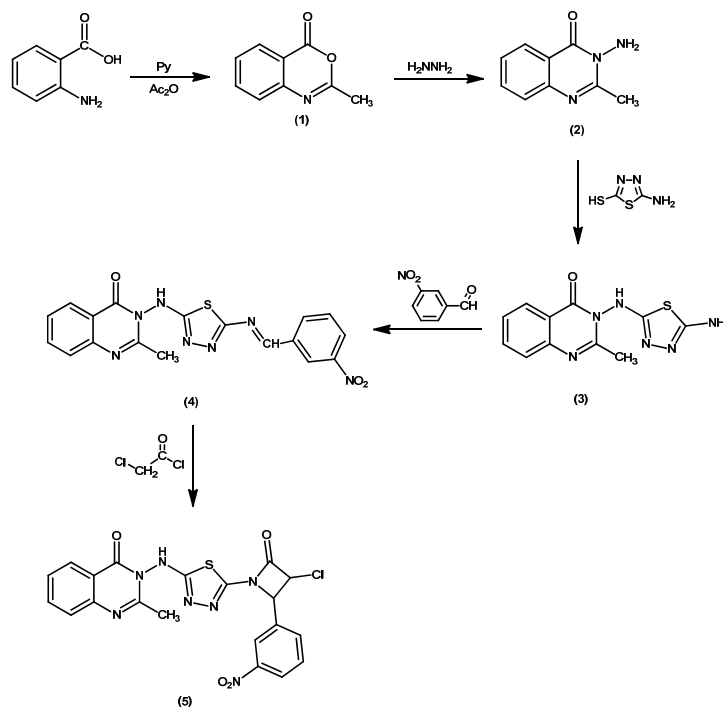
is widely distributed in human tissues; heart, liver, skeletal muscle and kidney. The clinical usefulness of the enzyme is largely restricted to the diagnosis of heart and liver diseases. Large amount of GOT may be released into the blood. Very high levels are observed in acute liver disease while lesser elevation is seen in chronic liver disease [16-18].

1.2. Glutamate pyruvate transaminase (GPT) (EC 2.6.1.2)

Also referred to as alanine amino transferase (ALT), which is prevalent in mammalian tissue catalyzes the transfer of the amino group of alanine to α -ketoglutarate. GPT found in a highest concentration in liver in spite of its active occurrence in skeletal muscles, heart and kidney. The GPT activity in tissues is generally less than GOT. GPT level is found to increase in the following diseases; infection hepatitis, liver cirrhosis and biliary cirrhosis, obstructive jaundice, liver cancer [16,18,19].

1.3. Gama glutamate transferase (GGT) (EC 2.3.2.2)

Also known as Gama Glutamate Transpeptidase (γ -GT) or (GGT) and is found in kidneys and liver and catalyzes the transfer of a gamma-glutamyl group from glutathione (GSH) to an amino acid. GGT levels are increased in most forms of liver disease, especially cholestasis, a plasma membrane-bound enzyme, provides the only activity capable to affect the hydrolysis of extracellular glutathione, thus favoring the cellular utilization of its constituent amino acids [16,20].



Scheme 1

1.4. Alkaline phosphatase (ALP) (EC 3.1.3.1)

Alkaline phosphatase is present in high concentration in the liver, bone and intestinal epithelium. These tissues each contain specific isoenzymes of ALP. Phosphatase transfers a phosphate moiety from one group to another, forming an alcohol and a second phosphate compound at high pH. The activity of ALP has been elevated in the bone and liver disease such as malignant disease and carcinoma with osteoblastic metastasis [18,19].

2. Experimental

2.1. Materials and measurements

All reagents were commercially available at (Aldrich Chemical Co.) and were used without further purification. Melting points were determined with an electrical thermal melting point apparatus and are uncorrected. Purity of the compounds was checked on silica coated Merck-TLC plates using water, chloroform benzene and acetone as mobile phase. FT-IR measurements were recorded on Shimadzu model FT-IR-8400S. ^1H NMR spectra were obtained with a Bruker spectrophotometer model Ultra Shield at 300 MHz in DMSO- d_6 solution with the TMS as internal standard.

2.1.1. Synthesis of 2-methyl-4H-benzo[d][1,3]oxazin-4-one (1)

This compound has been synthesized by following the procedure mentioned in reference [21], by refluxing a mixture of anthranilic acid (0.01 mol) and 10 mL of acetic anhydride for 4 h, then the reaction mixture was cooled and the product obtained was recrystallized from water (Scheme 1). Yield: 92%. M.p.: 180-182 °C. FT-IR (KBr, ν , cm^{-1}): 1693 (C=O), 1651 (C=N). ^1H NMR (300 MHz, DMSO- d_6 , δ , ppm): 8.3-7.5 (m, 4H, Ar-H), 2.4 (s, 3H, CH_3).

2.1.2. Synthesis of 3-amino-2-methyl quinazolin-4(3H)-one (2)

A mixture of 2-methyl quinazolin-4(3H)-one (1) (0.01 mol) and hydrazine hydrate (99%) (10 mL) in absolute ethanol (25 mL) was refluxed for 7 h. The excess of solvent was distilled off then the product was left to cool, filtered and then recrystallized from ethanol (Scheme 1). Yield: 78%. M.p.: 150-151 °C. FT-IR (KBr, ν , cm^{-1}): 3370, 3300 (NH_2), 1686 (C=O). ^1H NMR (300 MHz, DMSO- d_6 , δ , ppm): 8.4-6.8 (4H, m, Ar-H), 5.6 (2H, s, NH_2), 2.4 (3H, s, CH_3).

2.1.3. Synthesis of 3-[(5-amino-1,3,4-thiadiazol-2-yl) amino]-2-methyl quinazolin-4(3H)-one (3)

A mixture of 3-amino-3-methyl-quinazolin-4(3H)-one (2) (0.02 mol) and 2-amino-5-mercapto-1,3,4-thiadiazole (0.02 mol) in absolute ethanol (30 mL) was refluxed for 14 h till the evolution of H_2S finished. The excess solvent was distilled off and the product recrystallized for chloroform (Scheme 1). Yield: 60%. M.p.: 113-115 °C. FT-IR (KBr, ν , cm^{-1}): 3377, 3279 (NH_2), 1670 (C=O). ^1H NMR (300 MHz, DMSO- d_6 , δ , ppm): 8.61-7.56 (m, 4H, Ar-H), 6.1 (s, 2H, NH_2), 4.6 (s, 1H, NH), 2.4 (s, 3H, CH_3).

2.1.4. Synthesis of (E)-2-methyl-3-((5-((3-nitrobenzylidene)amino)-1,3,4-thiadiazole-2-yl)amino)quinazolin-4(3H)-one (4)

A mixture of compound 3 (0.02 mol) and 3-nitrobenzaldehyde (0.02 mol) was refluxed in absolute ethanol (25 mL) for 7 h. The reaction mixture was cooled and the product obtained recrystallized from ethanol (Scheme 1). Yield: 75%. M.p.: 156-157 °C. FT-IR (KBr, ν , cm^{-1}): 1690 (C=O), 1617 (C=N), 1539, 1324 (NO_2). ^1H NMR (300 MHz, DMSO- d_6 , δ , ppm): 8.5 (s, 1H, N=CH), 8.27-7.63 (m, 8H, Ar-H), 4.7 (s, 1H, NH), 2.3 (s, 3H, CH_3).

2.1.5. Synthesis of 3-((5-(3-chloro-2-(3-nitrobenzyl)-4-oxoazetidin-1-yl)-1,3,4-thiadiazol-2-yl)amino)-2-methylquinazolin-4(3H)-one (5)

To a stirred solution of compound **M4** (0.01 mol) and triethyl amine (0.02 mol) in dry dioxin (15 mL), chloroacetyl chloride (0.02 mol) was added dropwise at (0-5 °C), the reaction mixture was then stirred for 6 h, then poured into ice-water. The solid was filtered and recrystallized from benzene (Scheme 1). Yield: 66%. M.p.: 175-177 °C. FT-IR (KBr, ν , cm^{-1}): 1755 (C=O, β -Lactom), 1653 (C=O). ^1H NMR (300 MHz, DMSO- d_6 , δ , ppm): 7.37-6.81 (m, 8H, Ar-H), 4.6 (s, 1H, NH), 4.9 (d, 1H, N-CH), 5.0 (d, 1H, CH-Cl), 2.4 (s, 3H, CH_3).

2.2. Biological activities

2.2.1. Antimicrobial activity

The in vitro antimicrobial screening effects of the synthesized compounds evaluated against two Gram positive *Staphylococcus aureus* and *Streptococcus pyogenes* and two gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and two fungal strains namely *Candida albicans* and *Aspergillus niger* using well diffusion method [22].

DMSO was run as a control and the test was performed at 10 mg/mL concentration using DMSO as solvent. The bacteria and fungi were sub-cultured in agar and potato dextrose agar medium and were incubated for 24 h for bacteria and 48 h for fungi at 37 °C.

2.2.2. Antioxidant activity

The free radical scavenging activity of compound **5** towards the radical (DPPH) 1,1-diphenyl-2-picryl hydrazyl was measured as described by reference [23]. The 2-azetidinone stock solution (1 mg/mL) was diluted to final concentration of 20-100 $\mu\text{g/mL}$. Methanolic DPPH solution (1 mL, 0.3 mmol) was added to sample solution in DMSO (3 mL) at different concentration.

The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. the absorbance was then measured at 517 nm (As), using "Shimadzu175 spectrophotometer". The methanol solution of DPPH was used as control sample Ac. The ability of scavenge the DPPH radical was calculated using the following formula:

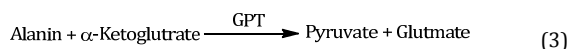
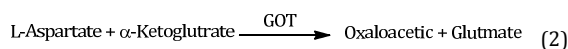
$$\% \text{ Radical scavenging activity} = 100 \times (\text{Ac-As})/\text{Ac} \quad (1)$$

Methanol was used as the solvent and ascorbic acid as the standard.

2.2.3. Effect of compound 5 on some enzymes activities

2.2.3.1. Effect of compound 5 on GOT and GPT activities

Colorimetric determination of GOT or GPT activity according to the following reactions:

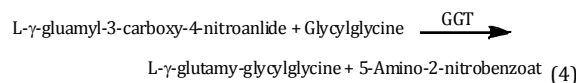


The pyruvate or oxaloacetat formed was measured in its derivated form 2,4-dinitrophenylhydraone, which was absorbed at wave length 505 nm [16,24].

2.2.3.2. Effect of compound 5 on GGT activity

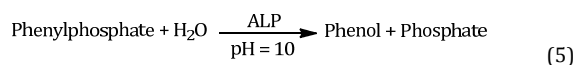
Kinetic colorimetric method for the determination of GGT activity was assayed by Persijn and Van Der Slik [25]. The principle of the method was the measurement of the 5-amino-

2-nitro-benzoate form from reaction, which was absorbed at wave length 405 nm [16].



2.2.3.3. Effect of compound 5 on ALP activity

Colorimetric determination of ALP activity according to the following reaction.



The phenyl phosphate was hydrolyzed to phenol, which was measured in the presence of amino-4-antipyrine and potassium ferricyanide, which was yellow in alkaline solution, and absorbed at wave length 520 nm [26]. The enzymes GOT, GPT, GGT and ALP activities were measured in human serum using above methods with added 100 μL of DMSO to calculate the activities of these enzymes without inhibitors. A stock solution (0.1 M) of compound **5** were prepared and the following concentration of 1×10^{-2} , 1×10^{-3} , 1×10^{-4} , 1×10^{-5} , and 1×10^{-6} M were prepared, diluting with dimethyl sulfoxide (DMSO). The enzymes GOT, GPT, GGT and ALP activities were measured in human serum using the same methods of these enzymes with replaces 100 μL of DMSO with 100 μL of compound **5** [16].

The inhibition percentages were calculated by comparing the activities with and without the compound and under the same conditions, according to the equation [16].

$$\% \text{Inhibition} = \frac{(\text{Enzyme activity without inhibitor}) - (\text{Enzyme activity with inhibitor})}{\text{Enzyme activity without inhibitor}} \times 100 \quad (6)$$

A constant concentrations of compound **5** 1×10^{-2} and 1×10^{-4} M were used with different substrate concentrations of 40, 80, 120, 160 and 200 mmol/L for GOT, GPT 0.4, 0.8, 1.2, 1.6 and 2.0 mmol/L for γ -GT and 1, 2, 3, 4 and 5 mmol/L for ALP to study the types of inhibition. Buffers were used to prepare different substrates concentrations of these enzymes, GOT, GPT (phosphate buffer pH = 7.4, 100 mmol/L), γ -GT (TRIS buffer pH = 8.25, 100 mmol/L) and ALP (carbonate buffer pH = 10, 100 mmol/L).

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.

When the enzymes activity is determined with and without compound **5**, it can be calculate the $[k_i, \text{Apparent } v_{\text{max}} (v_{\text{mapp}}), \text{Apparent } k_m (k_{\text{mapp}}), \text{type of inhibition}]$ by using Lineweaver-Burk equation and plotting $1/v$ against $1/[s]$ [19].

3. Results and discussion

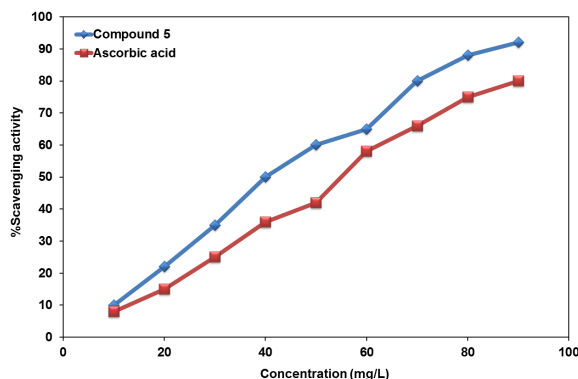
3.1. Synthesis

The new quinazolinone derivatives were prepared following the reaction sequences depicted in Scheme 1.

The 3-amino-2-methylquinazolin-4(3H)-one (**2**) was prepared by refluxing of compound **1** with hydrazine hydrate in absolute ethanol for 7 h.

Table 1. Antimicrobial evaluation of compounds 3-5.

Compound	Antibacterial Activity				Antifungal Activity	
	Zone of inhibition in (mm)					
	Gram positive		Gram negative		Fungi	
	<i>S. aureus</i>	<i>S. pyogenes</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>A. niger</i>
3	112	100	120	118	70	65
4	130	110	130	140	75	78
5	155	140	150	155	100	80
Ampicillin	100	100	100	100	-	-
Fluconazole	-	-	-	-	100	100

**Figure 1.** % Scavenging activity of the compound 5 using DPPH.

IR spectrum of compound 2 showed the appearance of new characteristic bands at 3370, 3300, 1686 and 1629 cm^{-1} , which belonged to NH_2 , C=O, C=N, respectively. While the ^1H NMR showed singlet signal at 2.4 ppm due to methyl proton and a singlet at 6.1 ppm related to NH_2 , while a multiplet signal at 7.61-8.05 ppm due to four aromatic protons.

Reaction of compound 2 with 2-amino-5-mercapto-1,3,4-thiadiazole afforded compound 3. The reaction was followed using lead acetate paper till the end of liberation of H_2S . This reaction occurs via nucleophilic replacement of SH by amino group of compound 2. The IR spectrum showed absorption bands due to NH_2 at 3336 and 3254 cm^{-1} and NH band at 3134 cm^{-1} for NH.

Condensation of compound 3 with 3-nitrobenzaldehyde in absolute ethanol gave Schiff base (4). The formation of Schiff base was indicated by the presence in their IR spectra of the azomethine (CH=N) stretching band at 1617 cm^{-1} , combined with the disappearance of NH_2 stretching band.

On the other hand, the cyclo addition of Schiff base (4) with chloroacetyl chloride in triethylamine and dioxane yielded azetidiny derivative compound 5. The IR spectrum of compound 5 showed the disappearance band of (CH=N) from azomethine at 1617 cm^{-1} , combined with the appearance of absorption band at 1755 cm^{-1} (C=O) for β -lactam ring. The ^1H NMR spectrum of compound 5 showed singlet signal at 4.6 ppm due to NH and two doublets one at 4.9 ppm relate to N-CH another at 5.0 ppm due to CH-Cl, while a multiplet signal at 7.37-6.81 ppm due to aromatic protons.

3.2. Biological activities

3.2.1. Antimicrobial activity

Standard antibacterial drug (Ampicillin) and antifungal drug (Fluconazole) were used for comparison. The experiments were performed in triplicate in order to minimize errors.

Zone of inhibition produced by each compound was measured in mm and the results of antimicrobial studies are given in Table 1. Compound 3, 4, and 5 are potential antimicrobial. It seems that the compound 5 is very significant

for activity against both bacterial and fungal species due to the presence of β -lactam ring.

3.2.2. Antioxidant activity

Hydrogen peroxide is generated in vivo by several oxidase enzymes and which product hydroxyl radical causes severe damage to biological systems. The antioxidant potential of compound 5 was determined on the basis of its scavenging of the stable (DPPH) free radical. Figure 1 showed that the compound 5 exhibit excellent antioxidant properties, the potential is comparable with antioxidant activity of ascorbic acid activity. This may be due to the presence of -NH group.

3.2.3. Effect of compound 5 on some enzymes activities

The biochemical tests revealed that compound 5 caused inhibitory effects on all enzymes activity. Figure 2 showed that the different concentration of compound 5 caused inhibitory effects on GOT, GPT, GGT and ALP enzyme, the value of the GOT, GPT, GGT and ALP enzymes activities without compound 5 were 18, 16, 6.545 and 30 U/L, respectively. The relationship between compound 5 concentration versus and the activities of enzyme as shown in Figure 3, these results observed that any increase in compound concentrations caused increase in percentage of inhibition of enzymes. The greater inhibition of compound 5 was demonstrated at concentration, 1×10^{-2} M [16].

Figure 4 showed that the inhibitory effect by compound 5 was higher inhibitory on the ALP enzyme (%33.3) and the lower inhibitory effect was on the GOT enzyme (%25.5).

By using double reciprocal plot of the Lineweaver-Burk plot can be easily distinguished type of inhibition of enzyme activity, when was established the velocity of enzyme with and without inhibitor.

Table 2 and Figure 5 showed that the type of enzyme inhibition using Lineweaver-Burk plot for compound 5 on serum GOT, GPT, GGT and ALP activities. A lique 10^{-2} and 10^{-4} M of compound 5 were non-competitive inhibition for all enzymes activities. Non-competitive inhibition changed the V_{max} of the enzyme but not the K_m . K_m with 10^{-2} and 10^{-4} M of compound 5 on GOT, GPT, GGT and ALP enzymes were 200, 500, 1, 5 M, respectively.

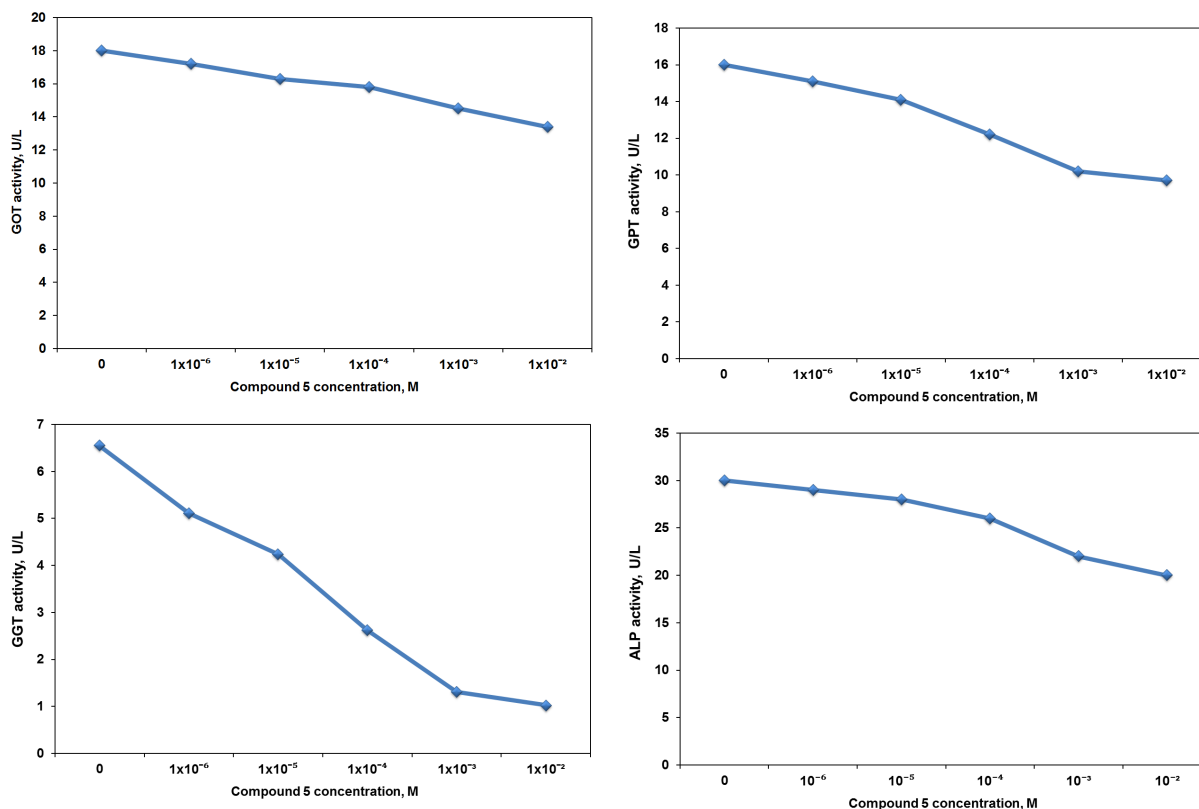


Figure 2. The relationship between concentration of compound 5 and GOT, GPT, GGT and ALP enzymes activities.

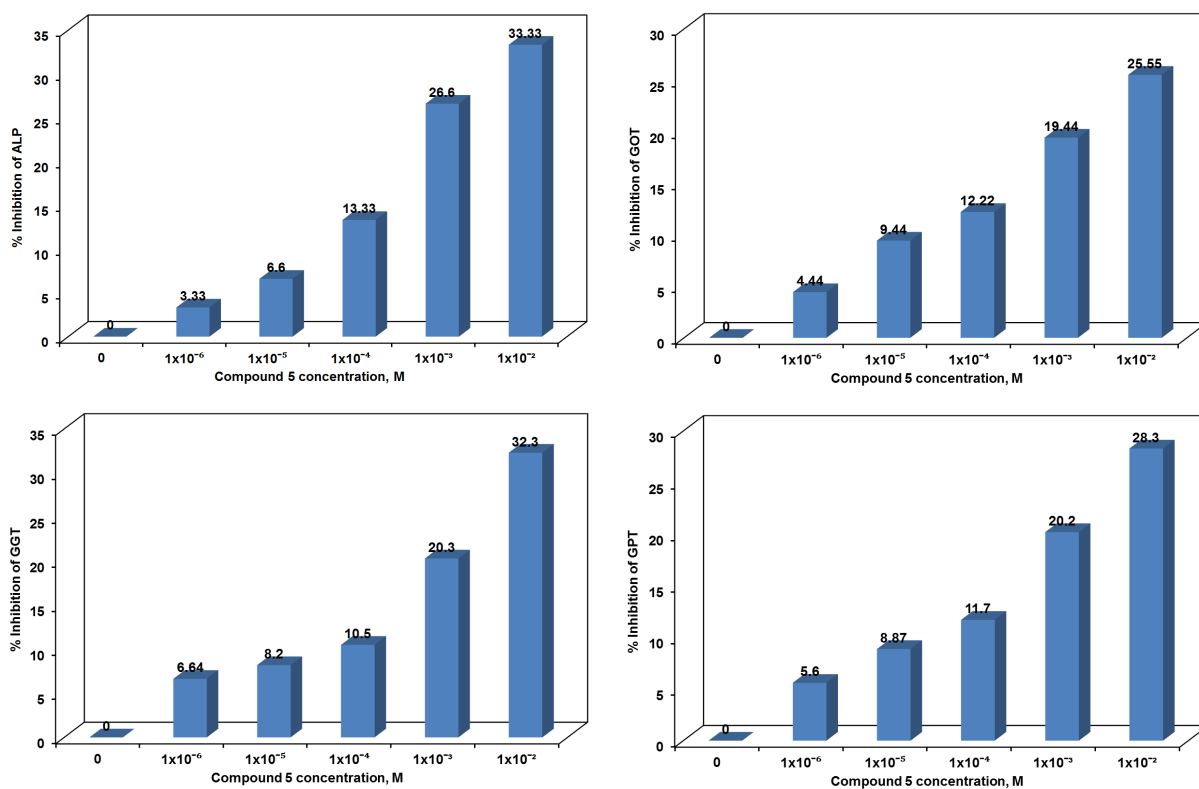
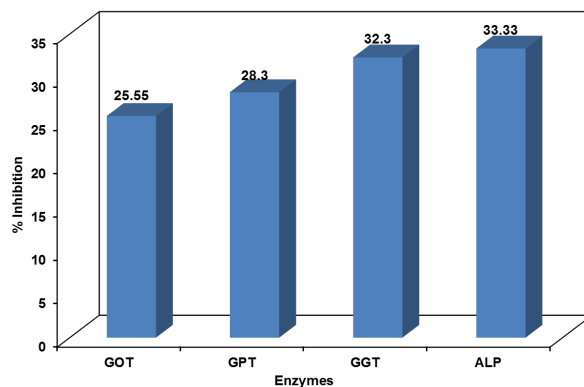
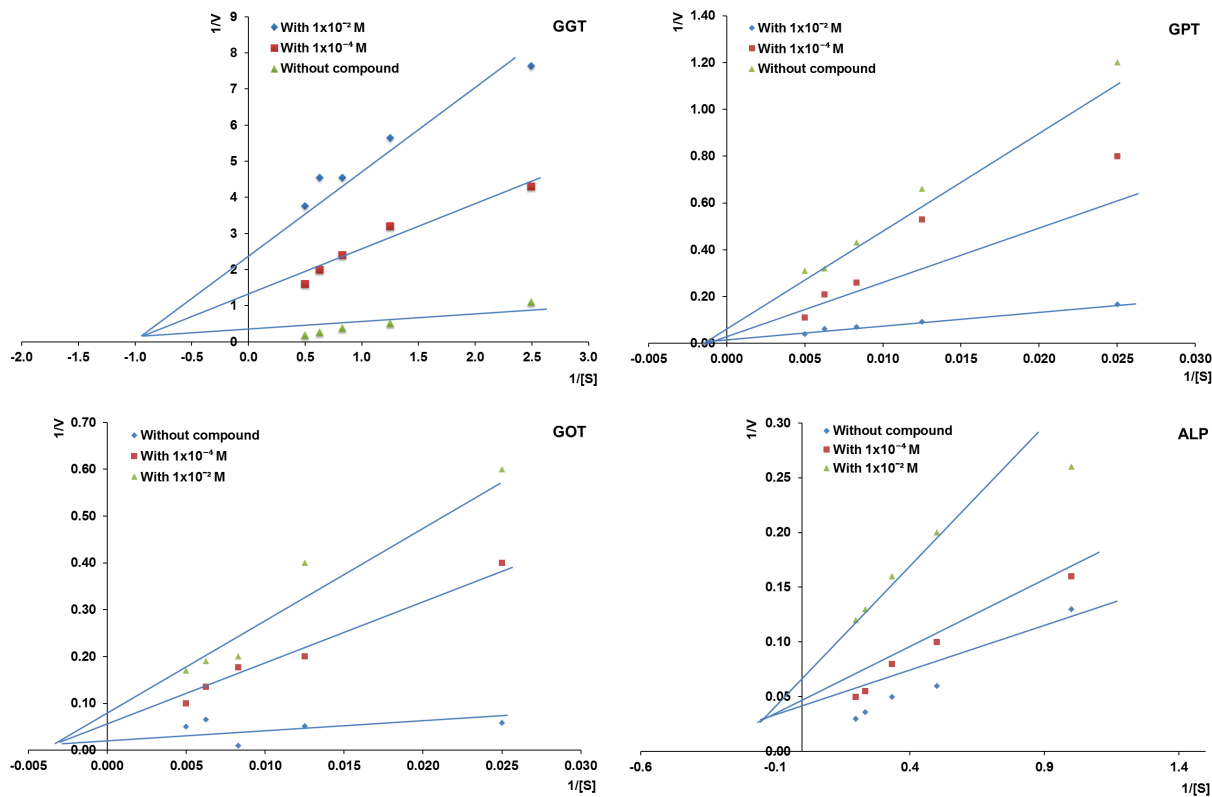


Figure 3. The percentages of inhibition of GOT, GPT, GGT and ALP enzymes and compound 5 concentration.

Table 2. The kinetic properties of GOT, GPT, GGT and ALP with compound 5.

Enzymes	Concentration of compound 5	k_{map}, k_m (M)	v_{map} U/L	k_i (M)	Type of inhibition
GOT	10^{-2}	200	20	25×10^{-4}	Non-competitive
	10^{-4}	200	11.12	125×10^{-7}	
GPT	10^{-2}	500	10	25×10^{-4}	Non-competitive
	10^{-4}	500	20	66×10^{-6}	
GGT	10^{-2}	1	0.4	25×10^{-4}	Non-competitive
	10^{-4}	1	0.72	55×10^{-6}	
ALP	10^{-2}	5.00	20	66×10^{-4}	Non-competitive
	10^{-4}	5.00	33.3	2×10^{-4}	

**Figure 4.** % Inhibition of GOT, GPT, GGT and ALP enzymes activities with 1×10^{-2} M of compound 5.**Figure 5.** Lineweaver-Burk plots for compound 5 effects on GOT, GPT, GGT and ALP.

V_{max} and V_{mapp} with 10^{-2} and 10^{-4} M of compound 5 on GOT, GPT, GGT and ALP enzymes were (100, 20, 11.12), (50, 10, 20), (2, 0.4, 0.72), (50, 20, 33.3), respectively. By using Lineweaver-Burk equation was calculated the K_i values of enzymes for compound which was studied in different concentration. The K_i of compound 5 in 10^{-2} and 10^{-4} M on GOT, GPT, GGT and ALP

enzymes were (25×10^{-4} , 125×10^{-7}), (25×10^{-4} , 66×10^{-6}), (25×10^{-4} , 55×10^{-6}), (66×10^{-4} , 2×10^{-4}) M, respectively.

The enzymes play important role in amino acid metabolism and in the urea and tricarboxylic acid cycles. We suggested that compound 5 molecule has (N-, O=, Cl, S and NO_2) groups by which, molecular of compound 5 interaction between the

groups with GOT, GPT, GGT and ALP enzymes, it inhibits the active sites of enzymes by decreasing the affinity of active sites of enzymes to react with the substrates. The results of our study are in agreement with previous studies of the same enzyme [17-19,23].

The tripeptide glutathione (GSH) is used by cells to detoxify hydroperoxides, produced during oxidative stress, and is consumed in the process. Previous studies have indicated that cells can be protected against oxidative stress by extracellular GSH through its degradation catalyzed by the exoenzyme gamma-glutamyl transpeptidase (GGT) and its de novo synthesis within the cytosol [24]. Because of this, induction of GSH depletion has been proposed as a good strategy for sensitizing tumor cells to anti-tumor agents [25]. We hypothesized that (GGT) would be increased as part of the adaptation of cells to oxidative stress.

4. Conclusions

New derivatives of 2-methyl quinazolin-4(3H)-one were synthesized and characterized using spectroscopic techniques. We studied the antimicrobial activities of these derivatives against some gram positive and negative species as well as fungal species. It seems that the compound **5** is very significant for activity against both bacterial and fungal species and it is an excellent antioxidant when comparable with the antioxidant activity of ascorbic acid. Compound **5** shows weak inhibition on GOT, GPT, GGT and ALP enzyme activities. So, compound **5** can be used as an easily accessible source of antioxidant in the pharmaceutical industry.

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