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Uracil Compounds As Inhibitors For Glutamate Oxaloactate Tansaminase (GOT) In Serum Of Some Leukemia Patients

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ABSTRACT

Effect of Uracil's derivatives on enzyme glutamate oxaloacetate transaminase (GOT) for sample of normal human and some types of leukemia patients, acute lymphatic(ALL) (11 cases), acute mylosyticl (AML) (4 cases), acute mylomonocyticl (AMOL) (2cases), acute pro- mylocyticl (APL) (3 cases), chronic mylobid (CM) (4 cases) and chronic lymphatic (CLL) (4 cases) is investigated. The ages of patients are between (10-69 years). Some synthesized derivatives of uracil were used as an inhibitors and a comparison between the activity of (GOT) in the presence and absence of inhibitors for two-types of serums under the same conditions is studied. The percentage of inhibition and type of inhibition is also included in this study, which was showed degree of inhibitions in normal individuals between (31% - 73%) and degree of inhibitions in patient's individuals between (25% - 80%) which showed that inhibition was significantly higher in the sera of patient with leukemia. The activity was determined colorimetrically by Retiman and Frankel method.

Keyword: *GOT, uracil, leukemia, pyrimidine*

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مركبات اليوراسيل كمثبطات على الانزيم الناقل لمجموعة الامين (GOT) لمصول المرضى المصابين ببعض انواع سرطان الدم

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الملخص

تضمن البحث دراسة تأثير بعض مشتقات اليوراسيل المحضرة على انزيم الناقل لمجموعة الامين glutamate oxaloacetate transaminase (GOT) لنماذج من مصل الدم البشري الطبيعي ونماذج من مصل دم المصابين بسرطان الدم (المزمن والحادة) 28 حالة مقارنة مع مصول الاشخاص الاصحاء حيث تروح اعمار المصابين بين 10-69 عاما . في هذه الدراسة تم قياس فعالية الانزيم بوجود مشتقات اليوراسيل كمثبطات وبعدم وجودها لكلا النوعين من المصول تحت نفس الظروف وحساب نسب التثبيط ومعرفة اي من المثبطات لها اعلى نسبة تثبيط وكذلك معرفة نوع التثبيط . حيث اظهرت الدراسة بان هذه المركبات لها نسبة تثبيط في مصول الاشخاص الاصحاء تتراوح بين (31% - 73%) بينما في في مصول الاشخاص المصابين تتراوح بين (25%-80%) .وقد استخدمت طريقة االكلرمتري لرتمان وفرانكل في حساب فعالية الانزيم .وقد اظهر البحث ان لمركبات اليوراسيل تاثير كبير كمثبط لفعالية الانزيم (GOT) في مصل دم المصابين بسرطان الدم (المزمن والحادة).

الكلمات الدالة: انزيم الناقل لمجموعة الامين؛ اليوراسيل؛ سرطان الدم؛ البريمدين.

1.INTRODUCTION

The glutamate oxaloacetate transaminase (GOT) which is identical to aspartate amino transfers (AAT, E.C.2.6.1.1) is one of the enzyme systems often used in isozyme studies on plant and animal genetics. It catalyzes the reversible reaction of glutamate and oxaloacetate to 2- oxoglutarate and aspartate, thus having an important role in nitrogen metabolism by distributing this nutrient originally assimilated into glutamate to other compounds [1]. GOT

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(EC. 2. 6. 1. 1) is present in high concentrations in the heart, liver, skeletal, muscle, kidney and erythrocytes. The causes of raised plasma GOT levels are malignant infiltration "may be normal, but may rise to twice the upper reference limit", Skeletal muscle diseases, after trauma or surgery and severe hemolytic anemia [2] Miekl concluded that there is an increase in GOT enzyme in the case of Acute lymphatic and this is because the enzyme is liberated from the carcinogenic cell as result of their destroy [3]. Inhibitor are different in their chemical nature and its biological activity their the response of the enzyme type differ in their activity toward inhibitors, many organic compound were used as inhibitors for GOT enzyme for exampel (Glutaric acid, Maliec acid, Acetate and Benzoate) [4] .An increase in the SGPT, SGOT and SALP activities was recorded in diabetic rats in comparison with non-diabetic rats, indicating an altered liver function in diabetic condition [5]. SGOT is found nearly in every tissue of the body including RBC. It is present in high concentration in muscle, myocardium, and liver. The serum SGOT concentrations increase shortly after the myocardial infraction and hepatic parenchymal injury. The measurement of the serum SGOT is therefore helpful for the diagnosis of myocardial infraction, hepatocellular diseases and skeletal muscles disorders [6].The aim of the present study is to investigate the effect of Uracil's derivatives on GOT enzyme.

2.MATERIALS AND APPARATUS

Aspartic acid, Na_2HPO_4, KH_2PO_4, NaCl was purchased from, BDH CO., α- ketoglutrate and 2, 4- dinitrophenyl hydrazine was purchased from Hopkins and Williams CO., All chemicals used were of analytical grad. Uracil's derivatives were prepared according of the literature [7] Table (1). Activity measurements of GOT were performed by (UV-Visible spectrophotometer, Varian Techtron Moder 635 series).Blood samples were obtained from Al-shroq laboratory-Kirkuk for untreated Leukemic samples. Samples were obtained by Venipuncture and kept to clott for 2 hours at room temperature, and then the serum was separated by centrifugation at 3000 round per minutes for 10-20 min.

3.METHODS

The activity was determined colorimetrically by Retiman and Frankel method [8].The effect of the Uracil derivatives for the activity of GOT enzyme in the presence of buffer with conc.(0.1) M at pH 7.4 was investigated. Concentration of intented inhibitors were (0, 0.1, 0.05, 0.025, 0.005) M, while the concentrations of substrate aspartic acid were (10, 15, 18, 36,

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55.5,) X 10^{-3} M This procedure was also repeated with various concentrations of substrate α-ketoglutrate (0.3, 0.66, 1.02, 1.33, 1.66) X 10^{-3} M. For the measurement of the activity, the method was followed with the exception that the inhibitor was added to the substrat buffer and kept at pH 7.4.The inhibition percentage was calculated by comparing the activity with and without using. The inhibitor and under same condition, the inhibition percentage was measured in normal human and in the patient according the equation:

$$\% \textbf{ Inhibition} = \frac{\boldsymbol{the\ activity\ with\ using\ (I)}}{\boldsymbol{the\ activity\ without\ using\ (I)}} \times \mathbf{100}$$

The type of inhibition was determined according to Lineweaver-Burk method by a plot of 1/V &1/(S) [9] and the Dixon method by a plot of 1/V & (I) [10].

4.RESULTS AND DISCUSSION

There are many studies on uracil derivatives showed that there are acting an important roles and its active biologically, study on this molecules showed it were importance as anticancer and antiviral agents. [11] Recently a study reported that these compounds should play an important role in biological systems and have considerable attention in pharmacology.[12] In addition anther study illustrated that series of new carbocyclic uracil derivatives evaluated as potential anti tuberculosis agents and its completely inhibited the growth of mycobacterium tuberculosis. [13]

In order to study the effect of some uracil derivatives as inhibitor of the activity of GOT in the serum of patients of various kind of Leukemia and according to the method above we found that these synthesized uracil derivatives act as inhibitor as illustrated in the Table (2) which was showed the values of activity of GOT in the serum of patients with Leukemia of various types, values of protein concentration, and specific activity, also we measured the activity of GOT in the normal serum in order to compare with the serum of patients and these was showed that some GOT activity values for normal human are surprisingly higher than that reported [14] and these data was showed in Table (2).Our estimate for the recorded high values of GOT may be that these persons are suffering from anther diseases.It is observed the increase of enzyme activity in 6 cases upon 11 cases of Acute Lymphatical Leukemia (ALL) and it is 71 I.U/L, the other type of cancer it was observed light increase in the activity in Chronic Lymphatic Leukemia (CLL) and this agree with the literature result in the case of

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Acute Mylocytic Leukemia (AML).The concentration of compounds that used as inhibitor showed a direct proportion with the activity of the enzyme, Table (3) showed the inhibitory effect of the compounds at (0.l) M concentration on the GOT activity. Compounds (1), (2) and (4) were found to have higher inhibition effect on serum GOT in normal than patients. Compounds (5) and (6) were found to have higher inhibition effect on serum GOT in patients than normal. Compound; (3) showed same inhibition effect on serum GOT in normal and patient. Compounds (6) was found to have higher inhibition effect on serum GOT in patients, while compound (1) was found to have higher inhibition effect on serum GOT in normal . The k_i value of the compounds in this study with (0.1 M) concentration for (Aspartic and α-ketoglutrate) and the type of inhibition summarized in Table (4). Figures (l), (2), (3) and (4) illustrates the types of the inhibitory effect of the compounds (1), (3), (4) and (6) individually. The inhibitors used (1), (2), (3), (4) and (5) in aspartic causes non-competitive inhibition while compound (6) causes un-competitive inhibition. The inhibitors used (2),(3)and (6) in α- ketoglutrate causes non competitive inhibition while compound (l),(4)and(5) causes un -competitive inhibition .Finally we can say that uracil derivatives can be used as inhibitor for the GOT activity .

5.CONCLUSION

In this research it was established that the data on GOT inhibition confirmed that the Uracil's derivatives showed that inhibition was significantly higher in the sera of patient with leukemia.

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Table (1): The compounds used as inhibitor on the GOT enzyme activity in serum with stock solution (0.1) M concentration.

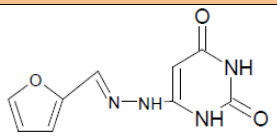
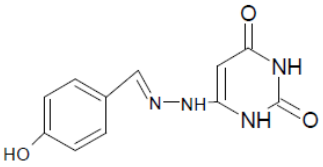
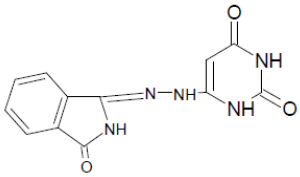
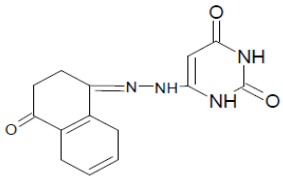
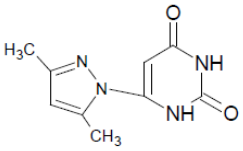
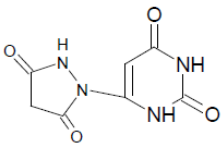
Comp. No.	Structures of the compounds	Name of the compounds	M.Wt.
1.		6-(2-(furan-2-ylmethylene) hydrazinyl)pyrimidine-2,4(1H,3H)-dione	220
2.		6-(2-(4-hydroxybenzylidene) hydrazinyl)pyrimidine-2,4(1H,3H)-dione	246
3.		6-(2-(3-oxoisoindolin-1-ylidene)hydrazinyl)pyrimidine-2,4(1H,3H)-dione	271
4.		6-(2-(4-oxo-3,4-dihydronaphthalen-1(2H)- ylidene)hydrazinyl) pyrimidine-2,4(1H,3H)-dione	282
5.		6-((3,5-dimethyl-1H-pyrazol-1-yl)amino)pyrimidine-2,4(1H,3H)-dione	206
6.		6-((3,5-dioxopyrazolidin-1-yl)amino)pyrimidine-2,4(1H,3H)-dione	210

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Table (2): GOT activity in sera of different types of Leukemia

Type of Leukemia	No. of Cases	Age	GOT activity rang	Average activity	Protein Conc.	Specific activity
		Year	I.U/L	I.U/L	mg/ml	I.U/mg
Acute lymphatic ALL	11	10-69	69-71	70	80	0.875
Acute mylosyticl AML	4	11-40	35-48	41.4	50	0.828
Acute mylomonocyticl AMOL	2	12-39	30-38	34	49	0.694
Acute pro-mylocyticl APL	3	14-35	25-35	30	46	0.652
Chronic mylobid CM	4	15-48	10-35	22.5	60	0.375
Chronic lymphatic CLL	4	13-46	35-40	37.5	60	0.625

Table (3): The degree of inhibition of total GOT enzyme from sera of Leukemia

The results are the average of three samples

Inhibitor	Degree of inhibition in normal	Degree of inhibition in patient
1	73%	65%
2	60%	40%
3	51%	55%
4	33%	25%
5	31%	52%
6	55%	80%

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Table (4): Ki (Aspartic and α- ketoglutrate)for total GOT enzyme in sera of acute Leukemia patient.

Inhibitor	Ki(mM)			Ki(mM)		
	Aspartic			α- ketoglutrate		
	1/v vs.I/(s)	1/v vs.I	Type of Inhibition	1/v vs.I/(s)	1/v vs.I	Type of Inhibition
1	0.011	0.013	Non. Comp.	0.27	0.27	Un.Comp.
2	0.057	0.054	Non. Comp.	0.08	0.07	Non. Comp.
3	0.004	0.003	Un.Comp.	0.046	0.05	Non. Comp.
4	0.280	0.320	Non. Comp.	0.19	0.22	Un.Comp.
5	0.410	0.450	Non. Comp.	0.23	0.21	Un.Comp.
6	0.0027	0.002	Non. Comp.	0.055	0.057	Non. Comp.

Ki values were determined separately in presence of 0.1 M of compounds. The type of inhibition and the inhibitor constants were determined by plot of Dixon, Linweaver Burk.

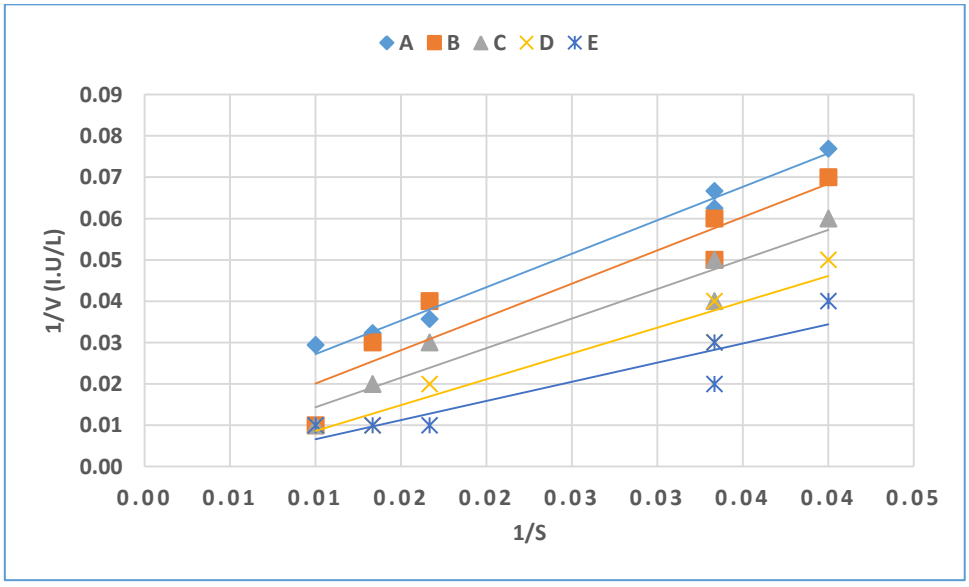

Figure (1): Inhibition of GOT enzyme by compound (1) (0.1— 0.005) M in presence of (1.02) mM α- ketoglutrate and (18-92)mM Aspartic acid in a patient with acute leukemia.

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A- in the absence of compound (1)

B- In the presence of 0.005M compound (1)

C- In the presence of 0.025M compound (1).

D- In the presence of 0.05M compound (1).

E- In the presence of 0.1M compound (1).

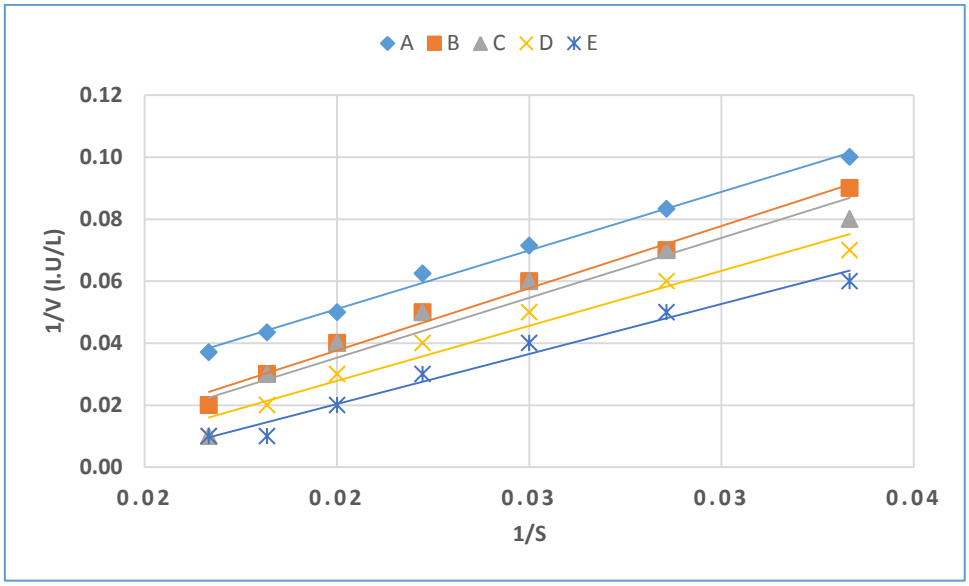

Figure (2): Inhibition of GOT enzyme by compound (3) (0.1— 0.005) M in presence of (1.02) mM α- ketoglutrate and (18-92)mM Aspartic acid in a patient with acute leukemia.

A- in the absence of compound (3)

B- In the presence of 0.005M compound (3)

C- In the presence of 0.025M compound (3).

D- In the presence of 0.05M compound (3).

E- In the presence of 0.1M compound (3).

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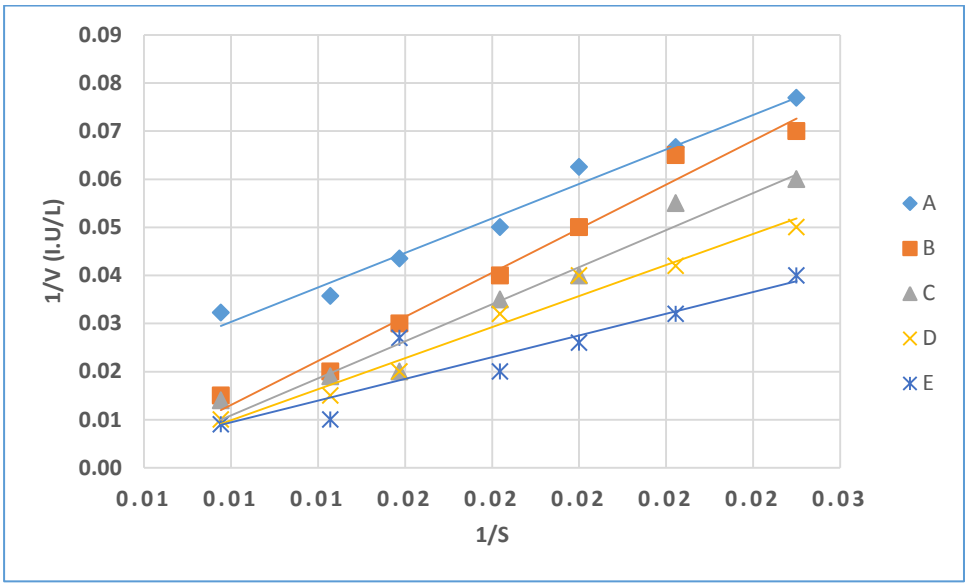

Figure (3): Inhibition of GOT enzyme by compound (4) (0.1— 0.005) M in presence of (0.275-1.0) mM α- ketoglutrate and (55.5) mM Aspartic acid in a patient with acute leukemia.

A- in the absence of compound (4)

B- In the presence of 0.005M compound (4)

C- In the presence of 0.025M compound (4).

D- In the presence of 0.05M compound (4).

E- In the presence of 0.1M compound (4).

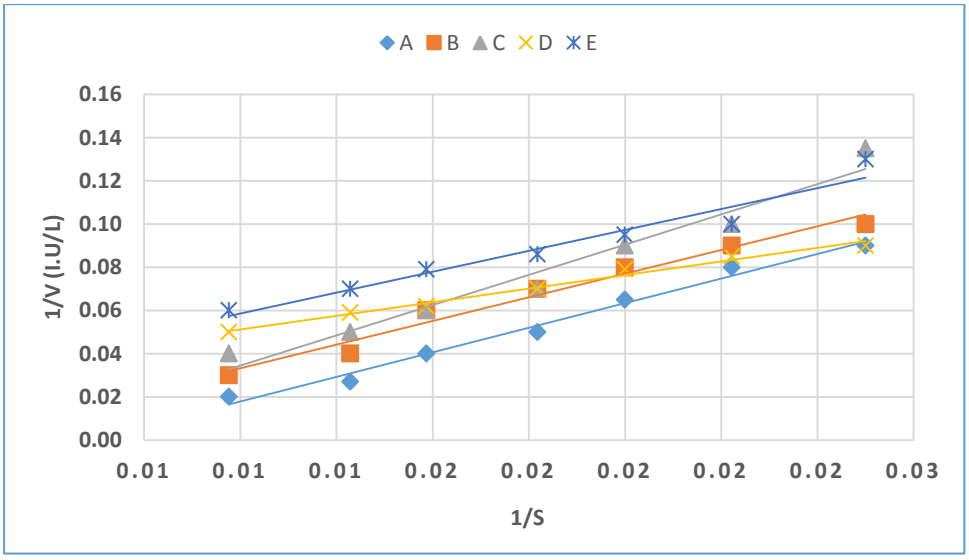

Figure (4): Inhibition of GOT enzyme by compound (6) (0.1— 0.005) M in presence of (0.275-1.0) mM α- ketoglutrate and (55.5)mM Aspartic acid in a patient with acute leukemia.

A- in the absence of compound (6).

B- In the presence of 0.005M compound (6).

C- In the presence of 0.025M compound (6).

D- In the presence of 0.05M compound (6).

E- In the presence of 0.1M compound (6).

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