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Synthesis and Inhibition effect of 1,3-Oxazepine derivative on AST and ALT activities *in Vitro* study

تحضير مركب الاوكسازبين ودراسة تأثيره على فعالية انزيمات ALT , AST خارج جسم الانسان .

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Abstract:

1,3-Oxazepine derivative is an antidepressant with a mild sedative component to its action. The mechanism of its clinical action in man is not well understood. In animals,amoxapine reduced the uptake of noradrenaline and serotonine and bloked the response of dopamine receptors to dopamine.

In order to study the Kinetic of human serum alanine amino transferase (ALT) and aspartate amino transferase (AST). Anovel oxazepine derivative prepared in our Lab. Was used to study its effect on ALT and AST since both enzymes are involed in the evaluation of hepatic disorder, on the other hand oxazepine among other drugs that are eliminated by the liver.

The kinetic study confirmed that this oxazepine derivative act as non competitive inhibitor for both ALT (GPT) and AST(GOT). The Vmax was found to be 113.5 U/mL and 85.18 U/mL for the non – inhibited and inhibited ALT respectively with Km value of 2.5×10^{-3} mol/L. As for AST, the Vmax was found to be 207 U/mL for the non-inhibited enzyme and 164.1 U/mL for the inhibited AST. The Km Value Which is the same for the uninhibited and the inhibited enzyme was found to be 1.2×10^{-3} mol/L.

الخلاصة :-

لاجل دراسة حركية انزيمات الانين امينو ترانسفريز (ALT) واسبارتيت امينو ترانسفريز (AST) استخدم مشتق الاوكسازبين المحضر مختبرياً لدراسة تأثيره على الانزيمات (AST,ALT) .

حيث ان هذه الانزيمات تستخدم في تقييم الخلل الوظيفي للكبد وان الاوكسازبين يتم التخلص من بقاياه بعمليات الايض التي تحدث في الكبد .

اكدت الدراسة الحركية بأن المشتق العضوي المحضر يعمل كمثبط لا تنافسي لانزيمي ALT (GPT) و AST(GOT) وان السرعة القصوى Vmax كانت 113.5 U/mL و 85.18 U/mL لانزيم ALT الغير مثبط والمثبط على التوالي وان قيمة ما يكلس Km كانت 2.5×10^{-3} M/L .بينما كانت Vmax لانزيم AST الغير مثبط 207 U/mL والانزيم المثبط 164.1 U/mL وقيمة Km والتي لم تتغير وجدت بأنها تساوي 1.2×10^{-3} M/L .

Introduction :-

Oxazepine is non-homologous seven membered ring that contains two heteroatoms(Oxaygen and Nitrogen).Discovery of the activity of 1,4-benzodiazepine on the Central Nervouse System(CNS)[1],encouraged further searching for new ways to build up this 7-membered heterocyclic ring system[2,3].For example:-

Diazepam(Valium) is a substituted benzodiazepine introduced 1964 which was used for the control of anxiety and tension stetes.

Oxazepam(Serax) is anew benzodiazepine derivative introduced in 1965 for use in the relief of psychoneuroses characterized by anxiety and tension[4].

The new Oxazepine in this work has been synthesized by reaction of schiff's base and maleic anhydride.

The removal of α- Amino groups of most of L- amino acid is promoted by enzymes called transaminases or amino transferases, in these reactions, the amino group is enzymatically transferred from the amino acid to the α- carbon of α- Ketoglutarate, leaving behind the corresponding α- keto acid analog of the incoming amino acid and causing the amination of the α – ketoglutarate to form glutamate, they by which the amino groups from many different amino acids collected. The glutamate then functions as amino group donor for biosynthetic pathway or excretion pathways that lead to the elimination of nitrogenous waste products. All transaminases have prosthetic group, pyridoxal phosphate (PLP), a derivative of pyrodoxine or vitamin B_6 . PLP functions as an intermediate carrier of amino group[5].

Measurment of alanine amino transferase (ALT) and aspartate amino- transferase (AST) levels in blood serum is important in some medical diagnosis [5].

Alanine Transaminases (ALT) (E.C.2.6.1.2)

Also called serum glutamate- pyruvate transaminase (SGPT). Having molecular weight approximately 101000 KD Catalyze the reaction according to this equation :-

L-Alanine + α-Ketoglutarate $\xrightleftharpoons{\text{ALT PLP}}$ pyruvate + L-glutamate .

In vivo this reaction goes to the right to provide a source of nitrogen for the urea cycle, the pyruvate is available for entry into the citric acid cycle, where as glutamate is deaminated (catalyzed by glutamate dehydrogenase) , yielding ammonia and α- keto glutarate [6].

ALT is present in high concentration in liver and to lesser extent in skeletal muscle, kidney , and heart[7].Measurment of ALT activity in serum used as an indicator of hepato cellular damage[8].It is used as apart of enzymes to establish whether liver damage has occurred [6].

Aspartate Transaminase (AST) : (EC.2.2.1.1).

Also called glutamate oxaloacetate transaminase (SGOT), present in high concentration in heart, Liver, skeletal muscle, kidney and erythrocytes. Damage to any of these tissue may increase plasma AST levels [7]. AST catalyses the following reaction.

L-Aspartate + α-Ketoglutarate $\xrightleftharpoons{\text{AST PLP}}$ oxaloacetate + L-Glutamate .

Also in vivo the reaction goes to the right[6], there are two forms of AST . The mitochondrial and soluble form. The major diagnostic application used AST activities are the investigation of Myocardial in fraction, liver disease, and muscle disease[4].Thus elevation in amino tansferase signify pathology [9].

Markers of liver damage, trunsaminases showed predominate rise in patients with different hematological disorders, cancers [10].

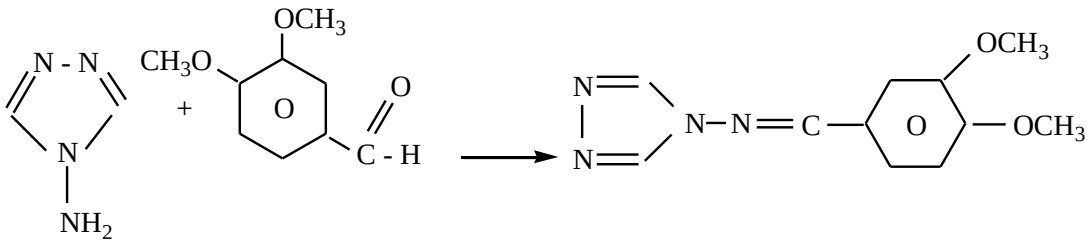
The effect of organic compound which is oxazepine derivative named[2-(3,4- dimethoxy phenyl)-3-(1,3,4- Triazole -1-yl)-2,3- dihydro -5,6-ene-1,3- oxazepine – 4,7- dione] on some hepatic enzymes was studied. The compound is a tricycle antidepressant drug belonging to the dibenzoxazepine class and is used to treat depression , as well as anxiety or agitation associated with depression[11], and also is considered an a typical antipsychotic agent for the treatment of schizophrenia. In mamals, it is metabolized by hepatic biotransformation[12].

Experimental : -

Synthesis of Schiff's base(II).

A mixture of (0.03 mole) of N- Amino – 1,3,4 – Triazole (I) and (0.03mole) of 3,4-dimethoxybenzaldehyde was dissolved in (35 mL) of absolute ethanol and then refluxed in water bath for 2hrs.

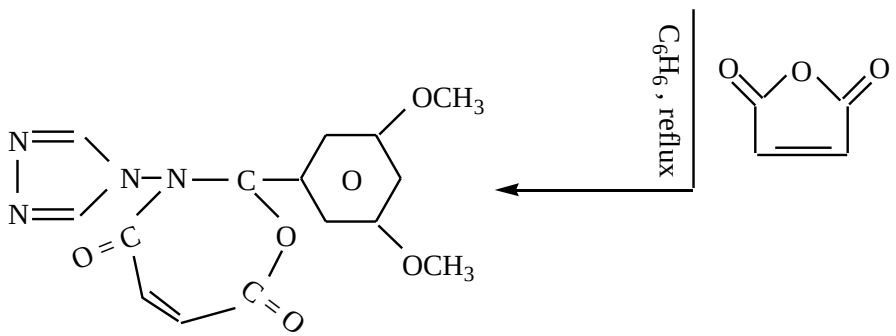
The reaction mixture was then allowed to cool to room temperature , and the solid product was filtered and recrystallized from ethanol (95%) to give the colored crystals of Schiff's base(II),m.p.=(120-122)˚C,yield = 77%.



N- amino -1,3,4 - Triazole I

3,4-dimethoxy benzaldehyde

Schiffs base II



2- (3,4- dimethoxyphenyl) -3- (1,3,4-triazol-1-yl)
-2,3- dihydro-5,6-ene-1,3-oxazepine- 4,7-dione

III

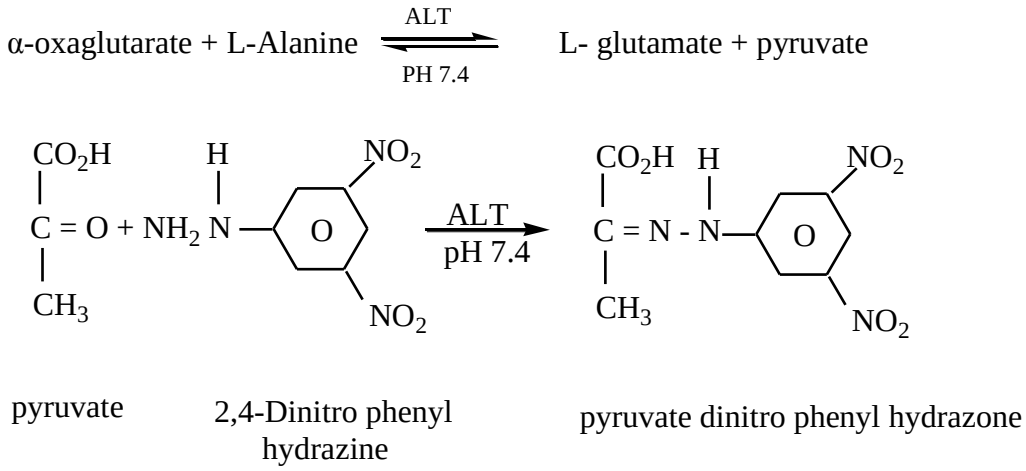
Synthesis of 1,3-Oxazepine derivative(III) :

Amixture of Schiff's base(II) (0.02 mole) and maleic anhydride (0.02 mole) were dissolved in 25mL of dry benzene and then refluxed in water bath for (4-5) hrs. The solvent was removed and the resulting colored crystalline solid was recrystallized from dry 1,4 – dioxan [13],m.p.=(87-88)˚C,yield=81%.

Sampling : Adult human serum was provided by blood bank at Baghdad hospital.

GPT and GOT activity measurements:

A ready kit from Bicmeghreb (France) was used for monitoring the concentration of pyruvate hydrazone formed with dinitrophenyl hydrazine.



The absorbance was recorded at 505 nm [(14)].

Preparation of different dilutions from the organic compound (oxazepine derivation) were prepared by the following steps:-

1- To prepare 10^{-2}M stock solution , 37 mg of the organic compound was dissolved in ethanol and completed to 10 mL with ethanol.
2- One mL of the stock solution was transffered to 10 mL volumetion flask and diluted up to volume with ethanol to get 10^{-3} M.
   Also 10^{-4} , 10^{-5}, 19^{-6} M solutions were prepared respectively.

Determination of the effect of organic compound on GPT and GOT activites:-

Using fixed concentration of the substrate with (0.5)ml of the above prepared different concentration of the organic compound , the activities of GPT and GOT were measured.

The effect of ethanol was determined by adding a quantity equivalent to the sample and all steps completed as in the method of determination of GPT and GOT illustrated by the kit [(13)]. The effect of ethanol as diluent was found to have a negligible effect on GPT and GOT activities.

The percentage of Inhibition was calculated according to the following equation :-

$$\%\,(\text{Inhibition}=100-(\frac{\text{Activity with inhibitor}}{\text{Activity without inhibitor}}\times 100)$$

Determination of the type of inhibition :-

It was carried out by using different concentration of substrate while a fixed concentration of the organic compound (the nearest value to Km). The same method of GPT and GOT activities were used by utilizing the same concentration of the substrates without the inhibitor (organic compound).

Results of Discussion :-

The synthesized compound (III) was identified by their m.p(87-88)°C,FT.IR spectra and ^{1}H-NMR spectra .

The FT.IR spectra show the disappearance of two absorption band at (3110-3280) cm^{-1} symmetric and asymmetric due to (NH_2) group, also disappearance band at 1618 cm^{-1} due to (C=N) exocyclic group with remain band at 1565 cm^{-1} due to (C=N) endo cyclic group but appearance two band at (1710-1740) cm^{-1} due to (C=O) Lactone 1683 cm^{-1} due to (C=O) Lactame.(15)

The ^{1}H-NMR spectrum of compound (III) showed (DMSO as a solvent): the singlet signal at δ 10.2 that could be attribution to the proton of (-CH-N) and singlet signal at δ 2.3 to the three protons of ($O-CH_3$).

The Conc. of substrate was obtained by multiply the volume pipetted times the total Conc. (202 mmole/L)divided by total volume (24.4).The factor of conversion from ml pipeted to Conc.is 8.28(202/24.4)

The calibration curves for GPT, GOT activities is shown in Fig 1 (A and B) respectively.

To obtain activity U/mL from calibration curve we apply the following equation:

Slope =Abs/(U/mL)

Slope=434.78 for GOT ; Slope=170.36 for GPT

Fig.2 and 3 showed the Michaclis – Menten for GPT and GOT in serum respectively . The values for Vmax were (85.18 u/mL) and (195.65 u/mL) for GPT and GOT respectively While the values for Km were (2.48×10^{-3} molar) and (1.2×10^{-3} molar) respectively .

Percentage inhibition of oxazepine derivative compound on GPT and GOT activities

Conc.	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
GPT(%inh.)	11.5	7.6	3.4	34.1	136.2
GOT(%inh)	25.1	21.6	11.4	18.0	132.9

The compound 2-(3,4-dimethoxy phenyl)-3- (1,3,4- Triazole -1-yl) -2,3- dihydro-5,6-ene -1,3- oxazepine -4,7- dione . Showed an inhibitory effect on GPT activity (11.5, 7.6, 3.4, 34.1 and 136.2 percentage for concentration of (10^{-6}, 10^{-5}, 10^{-4} ,10^{-3} and 10^{-2} molar) while the inhibitory effect on GOT activity is (25.1 , 21.6, 11.4, 18.0 and 132.9 percentage) at the same concentration of organic compound Respectively , consideraing the percentage of the original activity without inhibitor to be 100%.

Figures 4 and 5 showed the type of inhibition of the organic compound (10^{-3} M) on GPT and GOT activities respectively.

The double- reciprocal plots was used to determine the mode of inhibitor binding. From the intercepts on the $X(1/S)$ and $Y(\frac{1}{V})$ axis , V max and Km was calculated .

Both plots showed that the inhibitor (organic compound) is non- competitive inhibitor for both GPT and GOT which effectively reduce the concentration of enzyme and thus Vmax is decreased. The Vmax from Fig 4 for GPT activity in the presence of the inhibitor was found to be 85.18 u/mL compairing with the activity of GPT without the inhibitor to be 113.5 u/mL . the Km values which represent the affinity of the enzyme toward substrates and the inhibitor remains the same which was calculated to be 2.5×10^{-3} mole/L.

Figure 5 showed that the Vmax for the non-inhibited GOT to be 207 U/mL while Vmax in the presence of inhibitor compound to be 164.1 u/mL . The Km value remains constant which is 1.2 $\times 10^{-3}$ mole /L .

Compounds that are these types of inhibitors are more useful as drugs since they can inhibit the enzyme independent of the concentration of substrate[16,17].

The non- competitive inhibition is alternatively known as mixed.

In non- competitive inhibition, the substrate can still bind to the enzyme-inhibitor complex.hawever,the enzyme-inhibitor-substrate complex does not proceed to form product. The value of Vmax is decreased to a new value called $Vmax_{app}$ while the value of Km is unchanged. The Vmax lowered while Km remains unchanged. In essence, the inhibitor simple lowers the concentration of functional enzyme[18].

Non- competitive inhibitors bind enzymes at sites distinct from the substrate-binding site and generally bear little or no structural resemblance to the substrate[19].

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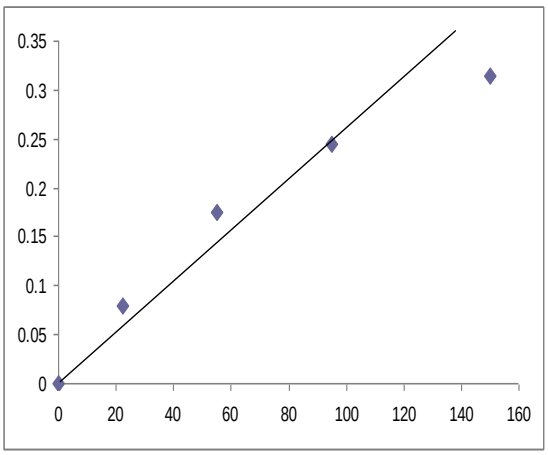
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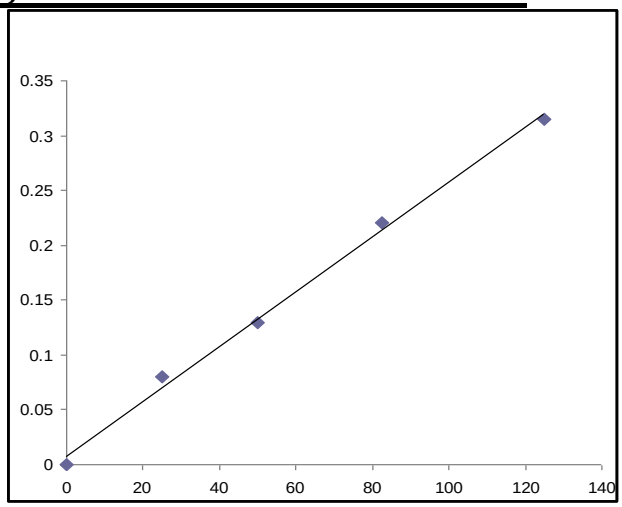
0
0 20 40 60 80 100 120 140 160
U/mL


abs
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U/mL

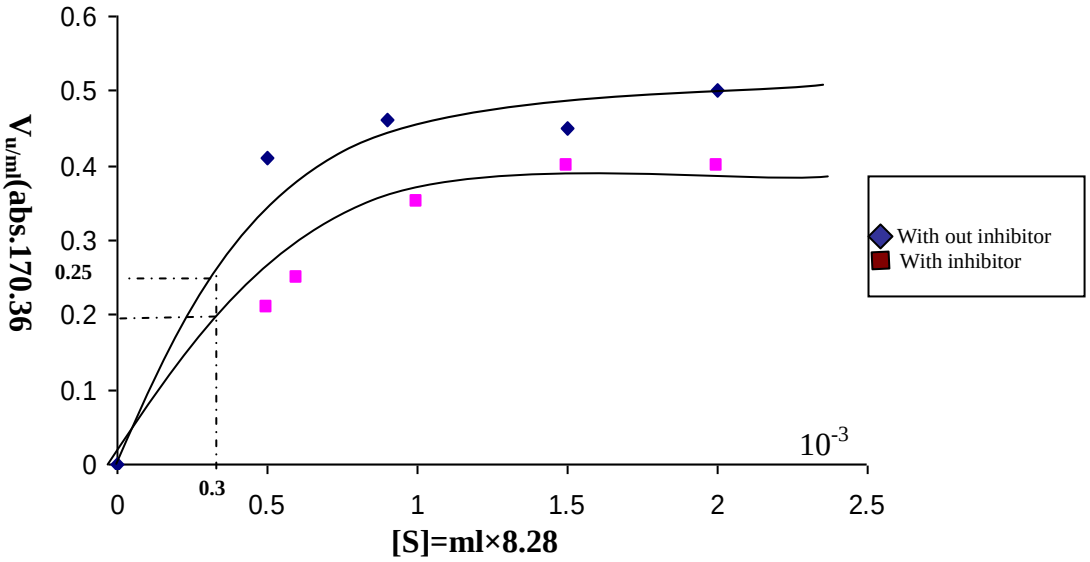

V u/ml(abs.170.36
0.6
0.5
0.4
0.3
0.25
0.2
0.1
0
0.3
0 0.5 1 1.5 2 2.5
10-3
With out inhibitor
With inhibitor
[S]=ml×8.28

Fig.2

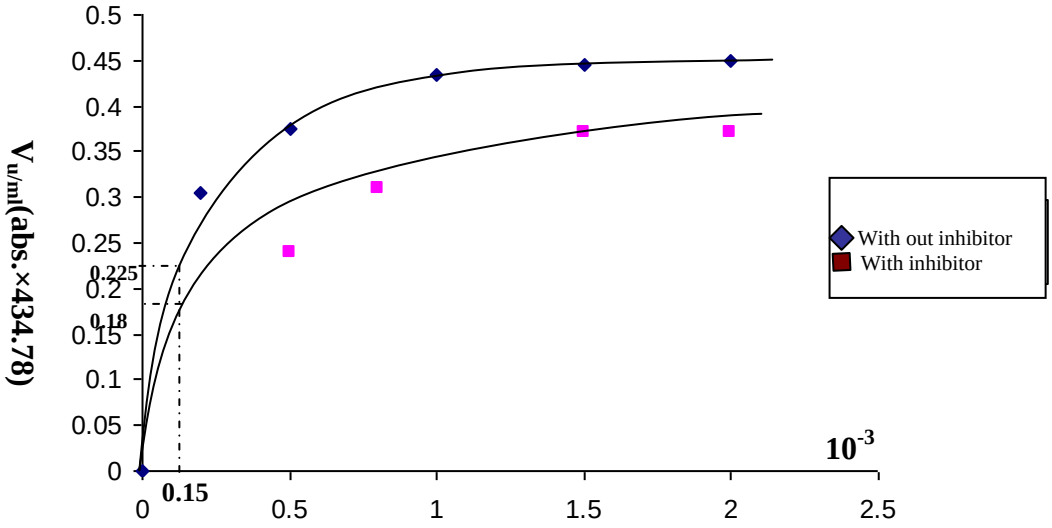

V u/ml(abs.×434.78)
0.5
0.45
0.4
0.35
0.3
0.25
0.225
0.2
0.18
0.15
0.1
0.05
0
0.15
0 0.5 1 1.5 2 2.5
10-3
With out inhibitor
With inhibitor
[S]=ml×8.28

Fig.3

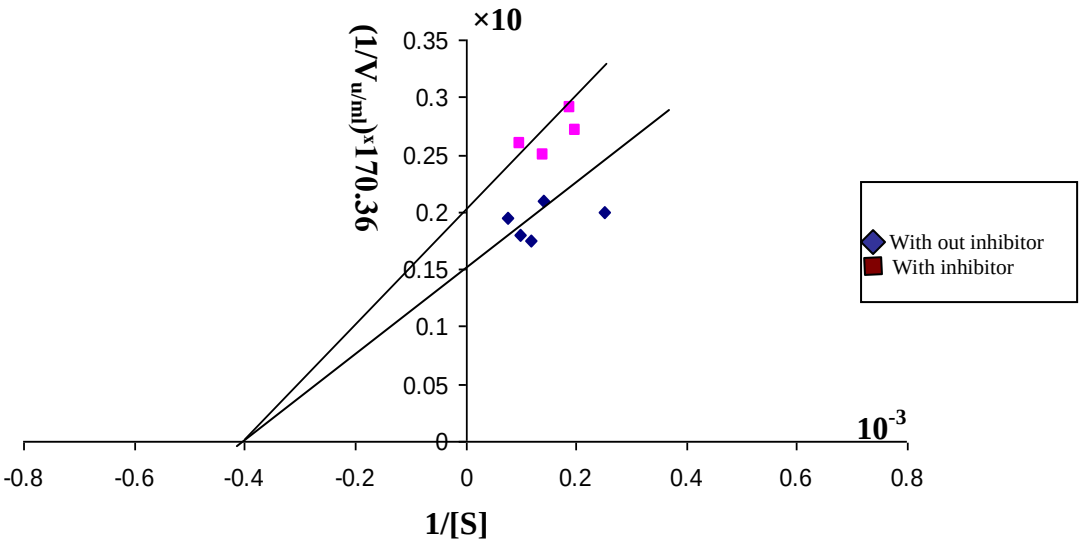

×10
(1/V u/ml)x170.36
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
10-3
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1/[S]
With out inhibitor
With inhibitor

Fig.4

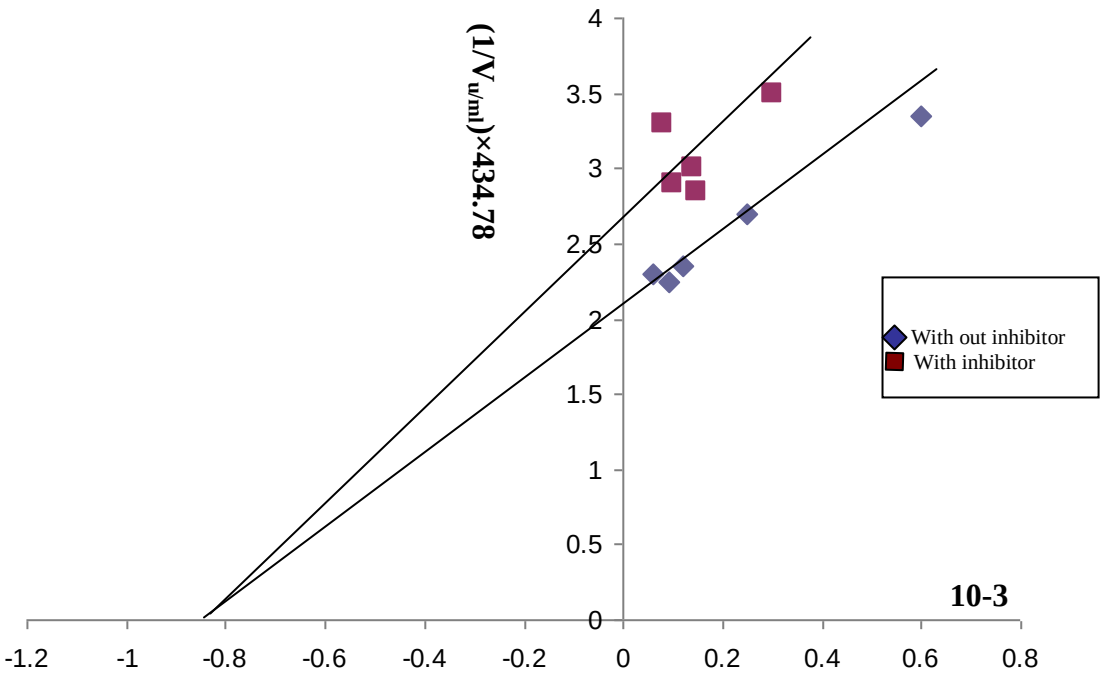
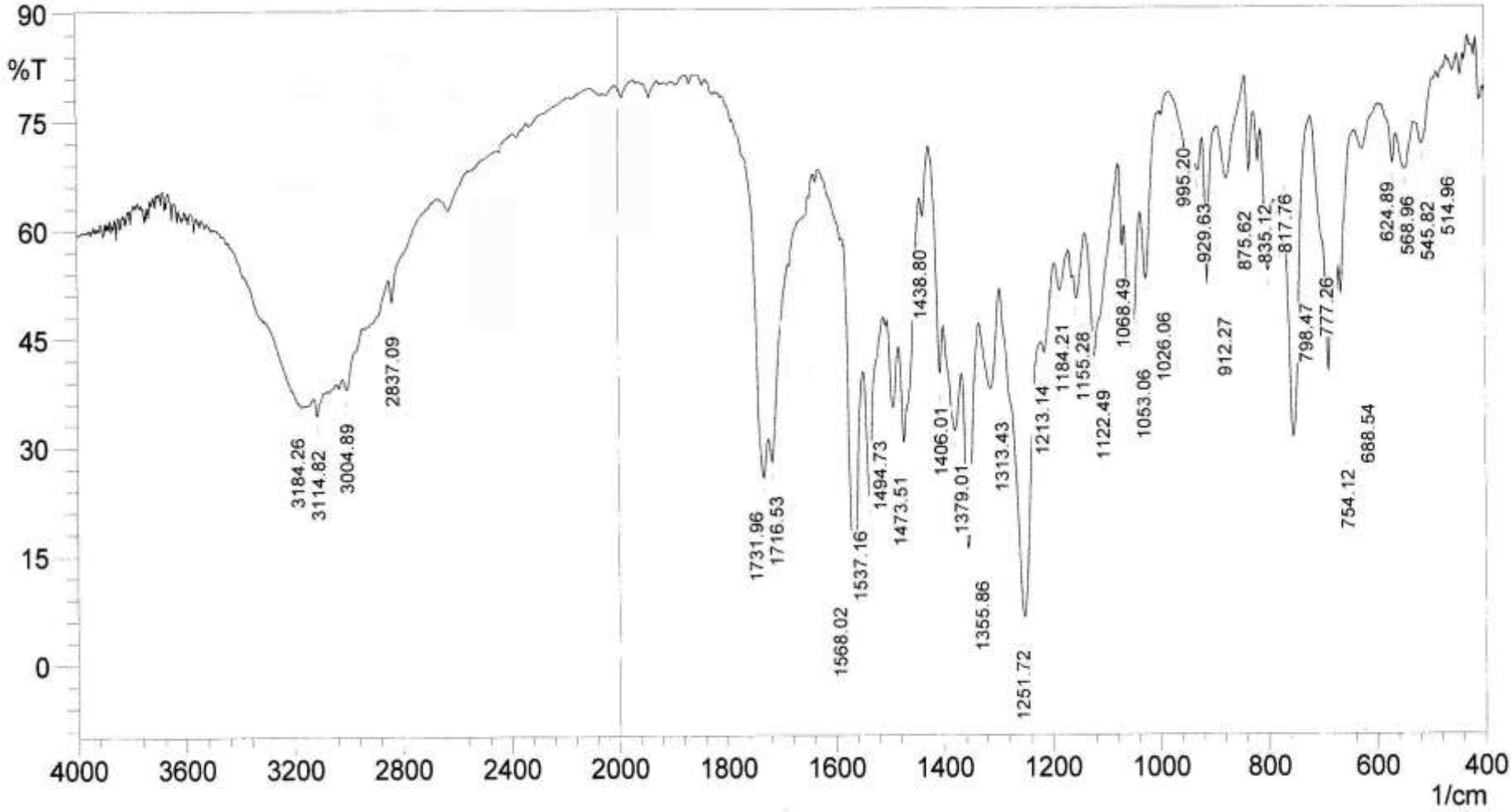

(1/V u/ml)×434.78
4
3.5
3
2.5
2
1.5
1
0.5
0
10-3
-1.2
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1/[S]
With out inhibitor
With inhibitor

Fig.5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%T
75
60
45
30
15
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
3184.26
3114.82
3004.89
2837.09
1731.96
1716.53
1568.02
1537.16
1494.73
1473.51
1438.80
1406.01
1379.01
1355.86
1313.43
1251.72
1213.14
1184.21
1155.28
1122.49
1068.49
1053.06
1026.06
995.20
929.63
912.27
875.62
835.12
817.76
798.47
777.26
754.12
688.54
624.89
568.96
545.82
514.96