Study the effect of oxazepine derivatives of alkaline phosphatase in normal persons sera

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Abstract :

The inhibitory effect of new organic compound which is derivative of oxazepine on the activity of alkaline phosphatase in normal persons serum have been studied

This compound was :
2 (p-toyl) 3 – (2- traizole) -2,3 dihydro {1,3} – oxazepine – 4,7 dione

The percentage of inhibition caused by oxazepine derivative was to be arranged between 22.5 – 69 %

The result from line weaver – burk plot indicated that the inhibition was competitive which maximum velocity V_{max} while Micheal menten constant Km is decreased in presence of inhibitor 9 m mol/L while the value of Km without inhibitor
is 10 m mol/L .
synthesis new 1,3 oxazepine 4-7 dion derivatives which are expected to have biological activity which are most active anticonvulsant.

دراسة تأثير مشتق oxazepine على انزيم الفوسفاتيز القاعدي في مصول الاصحاء

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الخلاصة:

تم دراسة تأثير مركب 2 (p-toyl) 3 – (2- traizole) -2,3 dihydro {1,3} – oxazepine – 4,7 dione

وهو من مشتقات مركب oxazepine في فعالية انزيم الفوسفاتيز القاعدي في مصول الاشخاص الاصحاء مختبريا .

اعطى مشتق oxazepine نسبة تثبيط بين (22.5 - 69) % .

3

درس نوع التثبيط من خلال تثبيت تركيز الماده العضوية وتغير تركيز الماده الاساس m (10 × 10^{-3}, 9×10^{-3} ,8 × 10^{-3}, 7× 10^{-3} 6 × 10^{-3}) mol/L

وعند رسم معادلة line weaver – burk plot وجد ان التثبيط من نوع تنافسي حيث بقيت السرعه القصوى vmax ثابتة بمقدار 6.66 U/L بينما حصل تغير في قيمة ثابت السرعه لمايكل Km وقد كان للتفاعل بدون وجود المثبط m mol/L 10 m mol/L بينما في حالة وجوده 9 m mol/L .

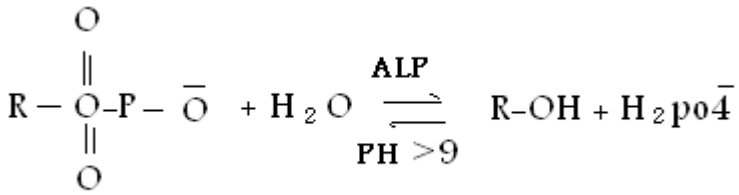
تحضير مشتقات جديدة من 1,3 oxazepine 4-7 dion من المتوقع ان تكون لها فعالية بايولوجية في معالجة التشنجات العصبية .

Introduction

The alkaline phosphatase are agroup of enzymes which hydrolyze organic phosphatase at high PH .

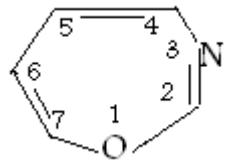
They are present in most tissue but are in particularly high concentration in osteoblasts of bone , the cells of the hepatobilliary tract , the intestinal wall , the renal tubules and placenta [1] , the molecular weight varies with the tissue source of enzyme and ranges from 70.000 to 120.000 adlton [2] .

ALP is a family of dimeric metalloenzymes and requires Mg^{+2} and Zn^{+2} for stability and maximum activity . ALP is group of nonspecfic phosphates that catalyze the reaction as shown below :



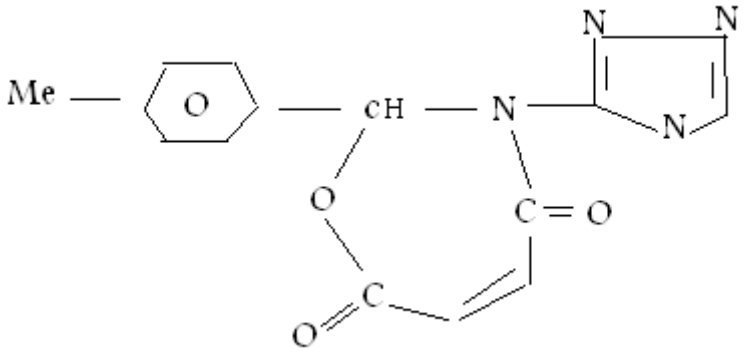
Oxazepine compound :

Oxazepine is non-homologous seven membered ring that contain two hetro atoms oxygen and nitrogen



1,3 oxazepine

Oxazepine – dione prepared from shiff's bases with selected anhydride **5 , 6**



2 (p-toyl) 3 – (2- traizole) -2,3 dihydro {1,3} – oxazepine – 4,7 dione

Synthesis new 1,3 oxazepine 4-7 dion derivatives which are expected to have biological activity like oxapam (serax) and diazepam (valium) which are the most active anticonvulsant **7**.

Materials and chemicals

Phosphatase alkalin – kit biomerieux company / ethanol 99% BDH company / England

Sample :

the samples were collected from the bank of blood of the normal persons

Principle :

The activity of ALP was measured by [7,8] employ a colorimetric method to the following reaction :

$$\text{Phenylphosphate} \xrightarrow[\text{PH 10}]{\text{ALP}} \text{phenol + phosphatae}$$

The liberated phenol is measured in the presence of 4-amino and pyrine and potassium ferriccyanid

Determination of ALP activity before and after addition of inhibitor

The activity of ALP enzyme in sera of normal persons without inhibitor was determined according to (kind and Belfield etal) [7,8]
The activity of ALP enzyme was calculated from following equation :

$$\text{Sample ALP} = \frac{\text{OD sample} - \text{OD sample blank}}{\text{OD standard}}$$

OD = absorbance = A
n= 142 U/L
determination of ALP enzyme activity with inhibitor (I) while the concentration of substrate (S) and enzyme were fixed

1- Aliquate 50 µl of sample was pipetted in each four test tube
2- Aliquate from inhibitor concentration (10^{-3} , 10^{-5} , 10^{-6}, 10^{-7} and 10^{-8}) were placed in separate tube .
3- the Steps were repeated according to (kind and Belfield etal) [7,8].
4- The effect of inhibitor was calculated according to the following equation

$$\text{Inhibitor} = 100 - \frac{\text{activity with inhibitor}}{\text{Activity without inhibitor}}$$

5- The highest percentage of inhibition was used to obtain the type of inhibition . the type of inhibitor was determined using different concentration of substrate (6×10^{-3} ,7×10^{-3} ,8×10^{-3} ,9×10^{-3}, 10×10^{-3}) m mol / L concentration of (I) and (E) were fixed . lineweaver – Burk plot was used to find the type of inhibition .

Table -2- explain the inhibition percentage of oxazepine derivative on ALP activity of normal persons sera

Inhibitor concentration (M)	Enzyme activity U/L	% inhibition
0.0	76.69	-
10^{-3} M	59.3	22.5 %
10^{-4} M	30.90	69 %
10^{-5} M	117.579	150 %
10^{-6} M	32.12	59 %
10^{-7} M	36.74	29.2 %
10^{-8} M	50.22	34.52 %

Table -3- explain the concentration values of substrate and activity of ALP without inhibitor .

Substrate concentration m Mol m mol/L	Enzyme activity U/L
6×10^{-3}	15.0468
7×10^{-3}	24.214
8×10^{-3}	24.983
9×10^{-3}	25.001
10×10^{-3}	46.408

Table (4) –A- explain the ALP activity with oxazepine derivative

V^{-1}	0.0215	0.0259	0.040	0.041	0.005
S^{-1}	0.10	0.11	0.125	0.142	0.166

Table (4) –B- explain the ALP activity without oxazepine derivative .

V^{-1}	0.0478	0.0486	0.0645	0.036	0.049
S^{-1}	0.1	0.11	0.125	0.142	0.166

Fig -1- explain Michle and meanten plot between substrate concentration and ALP activity without inhibitor

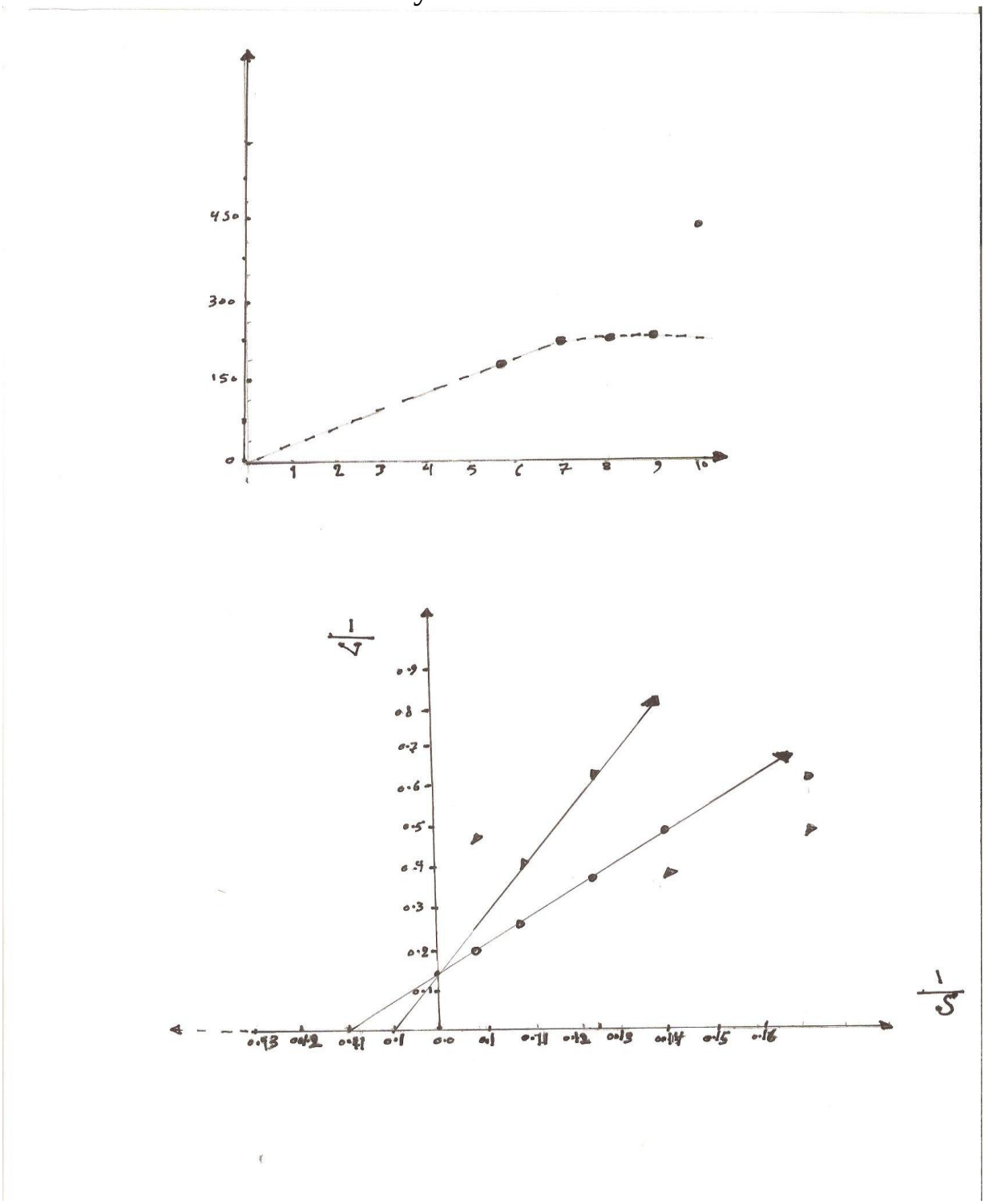


Fig -2- explain line weaver Burk plot of ALP activity in normal person sera

Result and discussion

Table 2 Oxazepine derivative showed an activation effect at concentration of 10^{-5} M/L

With percentage 150% while compound acted as inhibitor at concentration 10^{-3} , 10^{-4} , 10^{-6}, 10^{-7} , 10^{-8} with percentage of inhibition

22.5% , 69 % , 59% , 29.2% , 34.52%

There was inhibition effect of the solvent seen on the enzyme activity with percentage 22.6%

The cause of inhibition compound 2 (p-toyl) 3 – (2- traizole) -2,3 dihydro {1,3} – oxazepine – 4,7 dione

Related to oxazepine ring and triazole ring in position 4 as well pair electrons on nitrogen and oxygen atoms which cause interaction with active sites of amino acid NH_2 and COOH [9]

The oxazepine compound moiety showed liquid crystal lyotropic type result from change in concentration of solution. When the concentration increases transition from non-ordered isotropic solution to an ordered anisotropic solution can take place [11].

These factors were inhibition the activity of enzyme

Table 3 and fig 1 Showed michel menten plot for ALP in normal persons the value of **Vmax** 10 U/L while the Km value was

58×10^{-4} m mol/L

Table 4 A,B and fig 2 :

Explain line weaver Burk plot of ALP in normal persons sera at constant concentration of organic compound (10^{-4}) mMol / L with changed of substrate concentration was competitive with Vmax 6.66 µl and Km value 10 m Mol/L for uninhibited reaction and 9 m Mol/L for inhibited reaction .

This organic compound is new oxazepine derivative so that no available reference.

References

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