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Studying the Effect of Some Quinoline Derivatives on the Activity of Prostate Acid Phosphatase (PAP) in Human Serum

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الخلاصة:

تضمن البحث دراسة تأثير بعض مركبات الكوينولين على فعالية إنزيم الفوسفاتيز الحامضي في مصل دم الإنسان، وأظهرت النتائج بان لكل من المركبات (1) و (2) تأثيرا مثبطا لفعالية الإنزيم وكانت النسبة المئوية للتثبيط تتراوح بين (31−60%). وعند دراسة الخواص الحركية لهذه المركبات ظهر أنها تسلك كمثبطات غير تنافسية وكذلك تم دراسة تأثير المركبات (3−6) كمركبات معيدة لتنشيط الإنزيم المثبط وأظهرت النتائج أن هذه المركبات تسبب نقصان في النسبة المئوية للتثبيط واسترجاع فعالية الإنزيم.

Abstract:

This work involved studying the effect of some quinoline derivatives on the activity of PAP enzyme in human serum.The results showed that the inhibition effect of compounds (1) and (2) on enzyme activity was in the range (31-60%). Studying kinetic properties showed that these compounds behave as non-competitive inhibitors.Also, the effect of compounds (3-6) was studied, these compounds showed reactivation effect on the inhibited enzyme, they caused a decrease in the percentage of inhibition.

Introduction:

Inflammation of the prostate gland is known as prostasis. If the prostate grows too large it may constrict the urethra and impede the flow of urine, making urination difficult and painful and in extreme cases completely impossible. Prostatitis is treated with antibiotics, prostate massage or surgery.In older men, the prostate often enlarges to the point where urination becomes difficult. This is known as benign prostatic hyperplasia and can be treated with medication or with surgery that removes part of the prostate. The surgery most often used in such cases is called transurethral resection of the prostate (TURP or TUR). In TURP, an instrument is inserted through the urethra to remove prostate tissue that is pressing against the upper part of the urethra and restricting the flow of urine[(1)].Acid phosphatase is an enzyme found throughout the body, but primarily in the prostate gland. Like all enzymes, it is needed to trigger specific chemical reactions[(2)].The male prostate gland has 100 times more acid phosphatase than any other body tissue. When prostate cancer spreads to other parts of the body, acid phosphatase levels rise, particularly if the cancer spreads to the bone. One-half to three-fourths of persons who have metastasized prostate cancer have high acid phosphatase levels. Levels fall after the tumor is removed or reduced through treatment[(3)].Tissues other than prostate have small amounts of acid phosphatase, including bone, liver, spleen, kidney, and red blood cells and platelets. Damage to these tissues causes a moderate increase in acid phosphatase levels[(4)].Laboratory testing measures the amount of acid phosphatase in a human blood, and can determine from what tissue the enzyme is coming. For example, it is important to know if the increased acid phosphatase is from the prostate or red blood cells. Acid phosphatase from the prostate, called prostatic acid phosphatase (PAP), is the most medically significant type of acid phosphatase.Subtle differences between prostatic acid phosphatase and acid phosphatases from other tissues cause them to react differently in the laboratory when mixed with certain chemicals. For example, adding the chemical tartrate to the test mixture inhibits the activity of prostatic acid phosphatase but not red blood cell acid phosphatase. Laboratory test methods based on these differences reveal how much of a person's total acid phosphatase is derived from the prostate. Results are usually available the next day.The highest levels of acid phosphatase activity are found in metastasized prostate cancer[(2)]. Diseases of the bone, such as Paget's disease or hyperparathyroidism; diseases of blood cells, such as sickle cell disease or

multiple myeloma; or lysosomal disorders, such as Gaucher's disease, will show moderately increased levels.Certain medications can cause temporary increases or decreases in acid phosphatase levels. Manipulation of the prostate gland through massage, biopsy, or rectal exam before a test can increase the level[5].Any chemical compound that can inhibit acid phosphatase making it unable to break down p-nitro phenyl phosphate, is called acid phosphatase inhibitor.

Experimental:

Acid phosphatase (E.C. 3.1.3.2) activity was assayed by manual procedures [6,7]. The principle of the method is the measurements of the rate of production p-nitro phenol as p-nitro phenyl phosphate is hydrolyzed[8]

p-nitro phenyl phosphate + H_2O $\rightarrow$ p-nitro phenol + phosphate

The rate of color production is measured at 405 nm.

Procedure:

A) Acid phosphatase activity was measured in human serum using the modified manual method (ACP-Kit) as following:

Preparation of solutions:

1. Buffer (pH = 4.8):
   Content is ready for use stored at +2 to +8 °C.

2. Substrate (p-nitro phenyl phosphate):
   Reconstitute the contents of one bottle 2 ml to 10 ml of buffer (1). Stable for 5 days at +2 to +8 °C.

3. Tartrate:
   Content is ready for use. Stable up to the expiry date when stored at +2 to +8 °C.

4. Sodium hydroxide:
   For 10 assays dilute 10 ml of solution (4) with 90 ml redistilled water. Stored at +2 to +8 °C.

Pipette into test tubes according to:

Solution	Reagent blank	Sample 1(ACP)	Sample 2(PAP)
Solution 2(ACP) Solution 3(PAP)	1.0 ml -	1.0 ml -	1.0 ml 0.1 ml
Incubate for exactly 5 min at 37 °C			
Sample (serum)	-	0.2 ml	0.2 ml
Incubate for exactly 30 min at 37 °C			
Diluted NaOH Sample (serum)	1.0 ml 0.1 ml	10.0 ml -	10.0 ml -

The above solutions were mixed and the absorbance were recorded at 405 nm.

Calculation:

Acid phosphatase activity was calculated by use the following:

Total acid phosphatase(ACP)= 101×A sample 1
Prostate phosphatase(PAP)= 101×(A sample 1 - sample 2)

Normal Acid phosphtase (ACP)=11(Unit\L)
Normal Prostatic acid (PAP)=4(Unit\L)

B) A stock solution (10^{-2} M) concentration of each compoud table (1) was prepared and then the following concentrations (1×10^{-4}, 1×10^{-6} M) were prepared. The different concentrations of the derivatives were prepared by diluting with dimethyl sulfoxide (DMSO) solvent using stock solution. Acid phosphatase activity was measured in human serum in the presence of inhibitor (0.8 ml buffer mixed with 0.2 ml of inhibitors).

The method described in section (1) was repeated for the measurement of the activity of enzyme.

The inhibition percentage was calculated by comparing the activity with and without inhibitor under the same conditions, according to the equation[9]:

$$\% \text{ Inhibition} = 100 - \left[\frac{\text{The activity in the presence of inhibitor}}{\text{The activity in the absence of inhibitor}} \right] \times 100$$

C) Tow concentration of each inhibitor (1×10^{-4}, 1×10^{-6} M) were used with different substrate concentrations (2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5 mmole) to study the type of inhibition. These different concentrations were prepared from stock solution of (11.0 mmole) p-nitro phenyl phosphate.

The enzyme activity was determined with and without the inhibitor using the Lineweaver-Burk method by plotting 1/V vs. 1/[S], the following values were calculated:

a) K_i
b) V_{maxi}
c) Type of inhibition

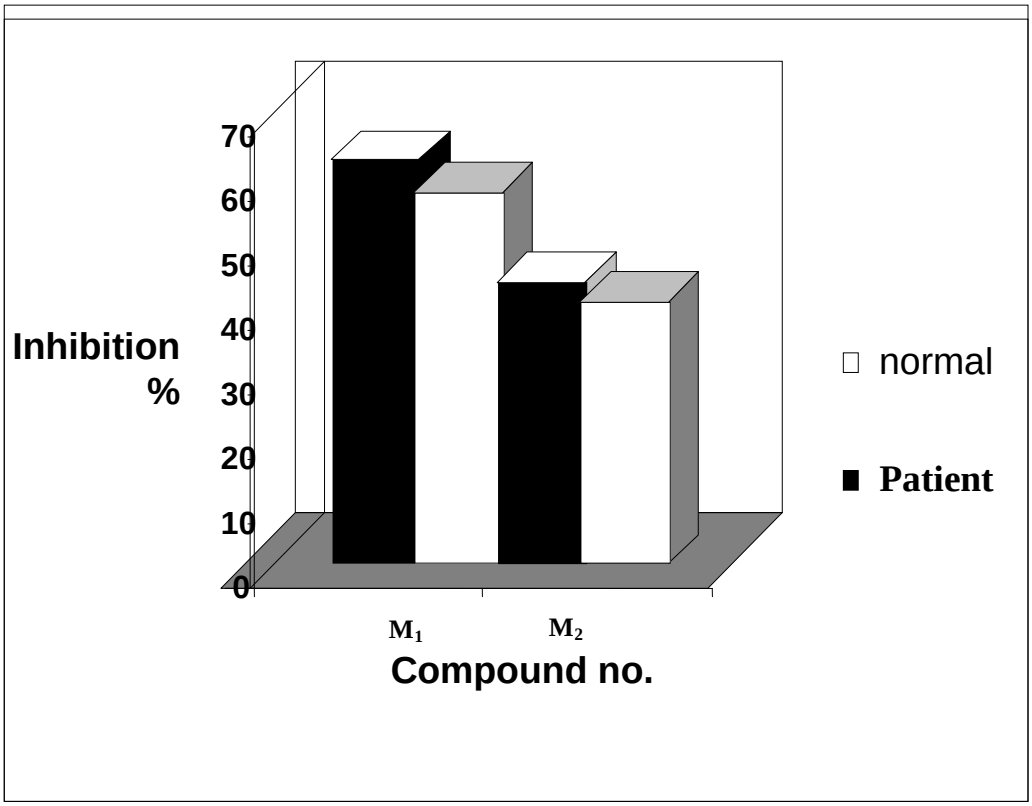
D) A stock solution (10^{-2} M) concentration of compounds (M_3, M_4, M_5, M_6) were prepared and the following concentrations (1×10^{-4}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7} M) were prepared. A concentration of (1×10^{-4} M) from all inhibitors was selected to determine the enzyme activity with and without using the inhibitor and under the same conditions according to the method described in section (2).

After that, the enzyme activity was determined in the presence of inhibitor and possible reactivator (0.8 ml buffer mixed with 0.2 ml of possible reactivator). The method described in section (2) was repeated for the measurement of the activity of enzyme.

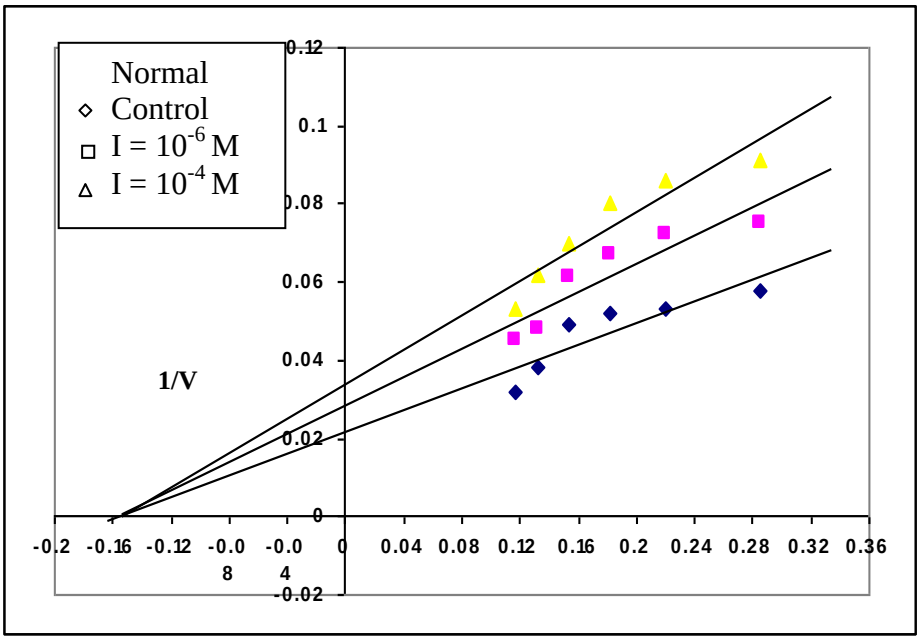
Results & Discussion:

In the light of the great importance of quinoline in biological application, 2 compounds derived from quinoline (table 1) were examined regarding their effect on the activity of PAP enzyme.The activity of PAP in human serum was measured *in vitro* according to the modified manual method.Compounds (1 &2) showed inhibitory effects on the enzyme activity and the relationships between compounds concentration versus percentage of inhibition are shown in Fig.(1).The enzyme activity decreased with

increased concentrations of inhibitor and Fig. 2 shows the inhibitory effect of the concentration ($1 * 10^{-4}$ M) of each compound on the activity of enzyme[10].From the results in Fig 1 it have been observed that compound (1) exhibited higher percentage of inhibition (63% in patients serum and 58% in normal control) than compound (2) (44% patients serum and 41% in normal control).Type of inhibition, K_i, V_{maxi}, K_{maxi} were estimated by measuring the enzyme activity in absence and presence of inhibitor at different constants of substrate under the same conditions using Lineweaver-Burk equation and plot as shown in Figure 2 and table (2).These results suggest that compounds (1 & 3) act as non-competitive inhibitors.Non-competitive inhibition, K_{mapp}, V_{mapp} and K_i were estimated by measuring the enzyme activity in the absence and presence of inhibitor at different concentrations of substrate under the same conditions using Linweaver-Burk equation and plot, as shown in Fig. 3 and table 2.In non-competitive inhibition Km remains constant and Vmax changes [11,9].Several substances have the ability to reactivate PAP by providing a nucleophilic attack on the enzyme[12].The present work examined the effect of quinoline derivatives (3, 4, 5, 6) that contains a nucleophilic center as reactivator to the inhibited enzyme.Various concentrations of quinoline derivatives (3, 4, 5, 6) with fixed concentration of quinoline derivatives (1 and 2) (1×10^{-6} M) were used. The results showed a decrease in percentage of inhibition with increasing quinoline derivatives (3, 4, 5, 6) concentration and the activity recovered between (65.7%-76.6%) at concentration (1×10^{-6} M)



Fig(1) ; inhibition percentage of compound m_1 m_2 in patient and normal serum



1/S

Fig(2):Inhibition of acid phosphatase enzyme in serum of prostate patients m_2

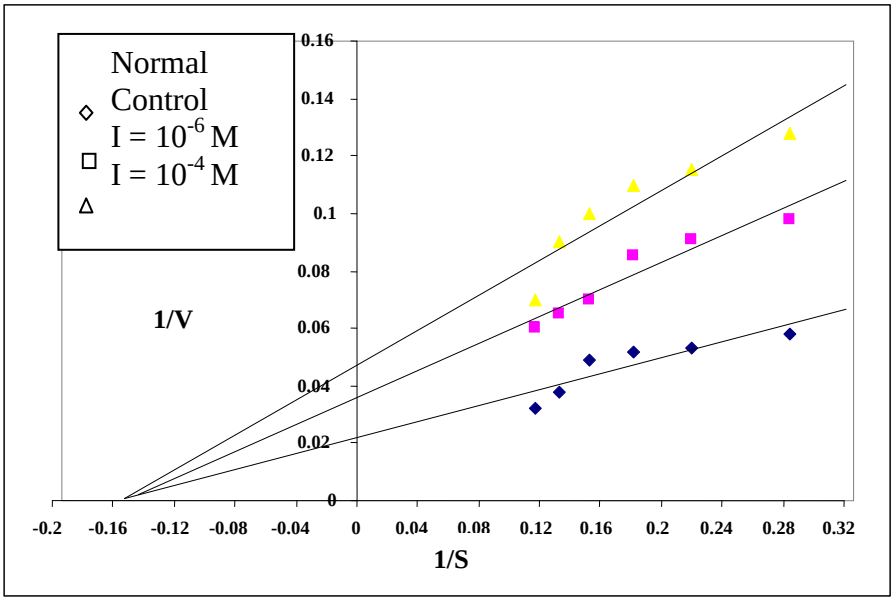


Fig (3): Inhibition of acid phosphatase enzyme in serum of prostate patients m_1

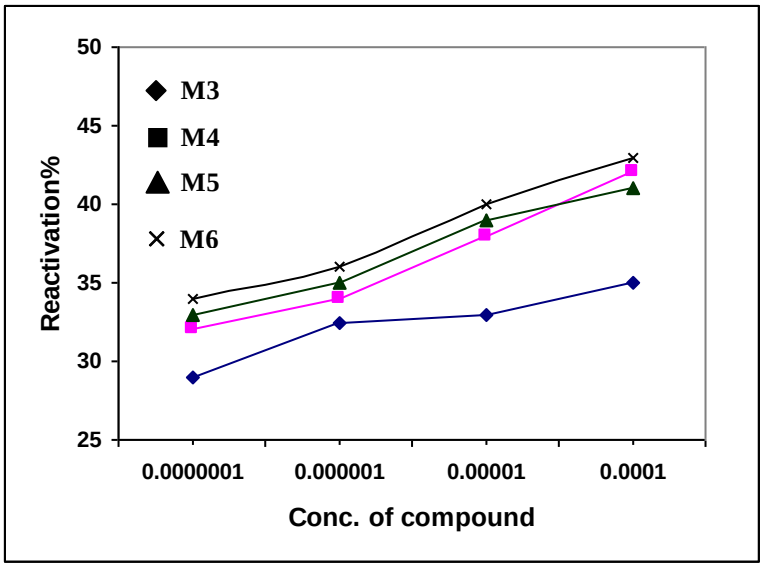
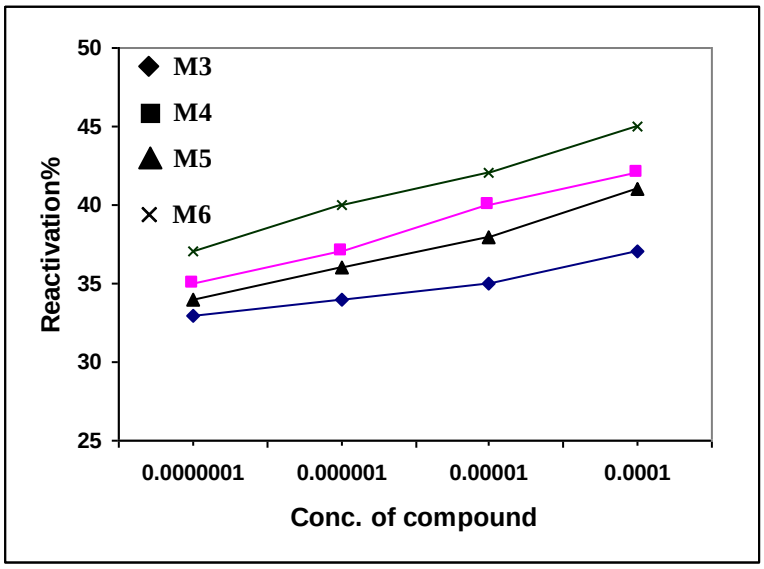
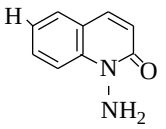
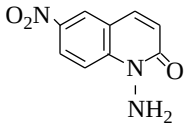
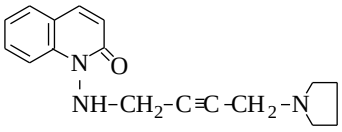
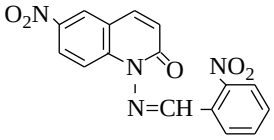
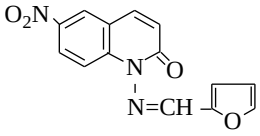
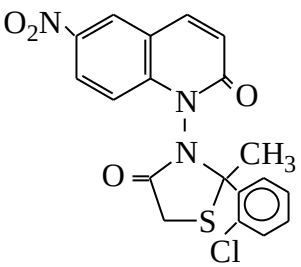


Fig9(4) : Reactivition of acid phosphatase enzyme in serum of prostate patients m_3 ,m_4,m_5,m_6



Fig(5): Reactivition of acid phosphatase enzyme in serum of normal m_3 ,m_4 ,m_5 ,m_6

Table (1): New derivatives of quinoline used for interaction with PAP

Comp. No.	Compound structure	Comp. name
1		1-amino quinolin-2(1H)-one
2		1-amino-6-nitro quinolin-2(1H)-one
3		1-[(4-pyrrolidine-1-ylbut-2-ynyl) amino] quinolin-2(1H)-one
4		6-nitro-1-{[(1E)-(2-nitro phenyl) methylene] amino} quinolin-2(1H)-one
5		1-{[(1E)-2-furyl methylene] amino}-6-nitro quinolin-2(1H)-one
6		1-[2-(2-chloro phenyl)-2-methyl-4-oxo-1,3-thiazolidin-3-yl]-6-nitro quinolin-2(1H)-one

Compound No.	V_{max}	K_m $\times 10^{-3}$ M	[I] = 1 $\times 10^{-4}$ M				[I] = 1 $\times 10^{-6}$ M				Type of inhibition
			V_{maxi}	K_i $\times 10^{-3}$ M	Inh.%	Recovery%	V_{maxi}	K_i $\times 10^{-3}$ M	Inh.%	Recovery%	
M2	**50**	**6.25**	**28.57**	**6.25**	**42.86**	**57.14**	**34.48**	**6.25**	**31**	**68.9**	**Non-competitive inhibition**
M1	**50**	**6.17**	**20**	**6.17**	**60**	**40**	**27.0**	**6.17**	**46**	**54**	**Non-competitive inhibition**

Table (2): Kinetic parameters of M1 and M2 inhibition of PAP with compounds derived from quinoline

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