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Inhibitory Effect Of Oxadiazooles And Thiadiazoles In Vitro On Serum Alkaline Phosphatase Enzyme Of Pregnant Woman

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الخلاصة:

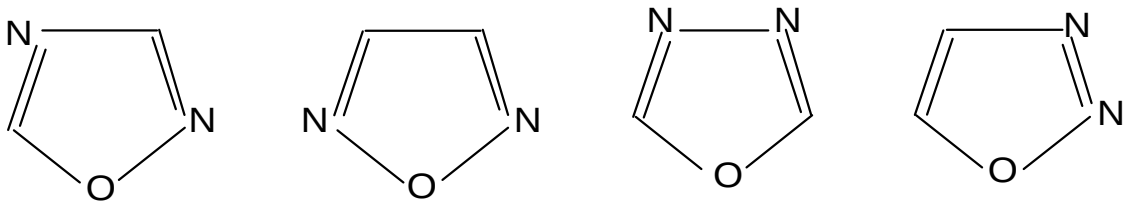
لغرض تقييم التأثير المثبط لمركبات الأوكسيديازول والثياديازول على مستويات أنزيم الفوسفيتيز القاعدي في مصل الدم للنساء الحوامل مختبريا. صممت الدراسه بشكل دراسه مقطعيه على 34 امرأه حامل في مستشفى ألكاظميه التعليمي. تبين من الدراسه ان آليه التثبيط لمركبات الاوكساديازول والثياديازول لأنزيم الفوسفتيز القاعدي مختبريا تحدث نتيجة لتفاعل أزاله الفسفره والتي تعيق فعاليه أنزيم الفوسفتيز القاعدي.

Abstract:

To assess the inhibitory effect of oxadiazole and thiadiazole derivatives on serum alkaline phosphatase level of pregnant *in vitro*. A cross-sectional study on 34 pregnant women was carried out at Al-Kadhumia Teaching Hospital. The mechanism of Inhibitory effect of Oxadiazoles and Thiadiazoles derivatives in vitro on alkaline phosphatase enzyme of pregnant women due to the dephosphorylation reaction which block the ALP activity.
Key Words: Oxadiazoles, Thiadiazoles, serum alkaline phosphatase.

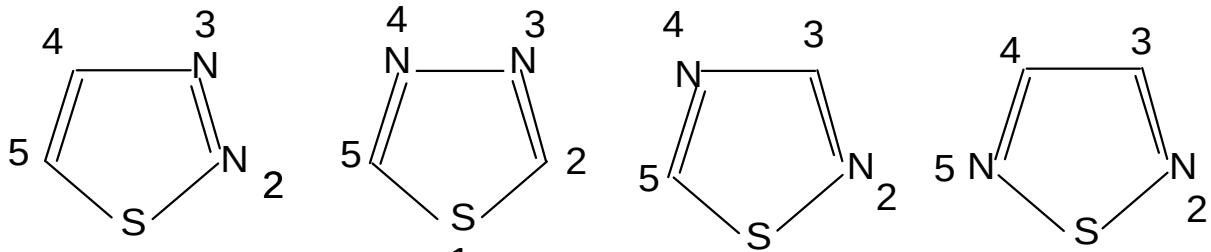
Introduction:

Simple aromatic rings are aromatic organic compounds, which also known as simple arenes or simple aromatics, they have many trivial names. They are usually found as substructures of more complex molecules ("substituted aromatics"). Simple aromatic rings can be heterocyclic if they contain non-carbon ring atoms, e.g. oxygen and nitrogen or sulfur. Simple monocyclic aromatic rings are usually five-membered rings like oxazole and thiazole or six-membered rings like pyridine. Fused aromatic rings consist of monocyclic rings that share their connecting bonds forming other aromatic compounds like oxadiazoles and thiadiazoles with two nitrogens instead of one. Oxdiazoles and Thiadiazoles are the parent compounds for vast class of heterocyclic aromatic organic compounds. These are azoles with oxygen, or sulfur, and nitrogen separated by one carbon atom.[1, 2, 3] (Figure: 1, 2)



1,2,4-oxadiazole 1,2,5-oxadiazole 1,3,4-oxadiazole 1,2,3-oxadiazole [2]

Figure-1: chemical structure and formula of some Oxadiazoles.



1,2,3-Thiadiazole 1,3,4-Thiadiazole 1,2,4-Thiadiazole 1,2,5-Thiadiazole [3]

Figure-2: chemical structure and formula of some Thiadiazole.

In biomolecules, oxazoles result from the cyclization and oxidation of serine or threonine nonribosomal peptides. Oxazoles are not as abundant in biomolecules as the related thiazoles with oxygen replaced by a sulfur atom. Oxazoles are aromatic compounds but less so than the thiazoles.[4]

Oxadiazole and Thiadiazole derivatives have been evaluated and proved for wide range of pharmacological, biochemical, clinical uses and applications. They prepared and evaluated as orally active anti-inflammatory and analgesic agents with reduced side-effects [5, 6] ; their activity against hepatitis B virus (HBV) has been tested, [7] as well as its antiviral activity against measles virus.[8]

To date some of these compounds have shown antibacterial, antimycotic and antimitotic activity. [9] Some compounds showed an interesting activity against *Mycobacterium tuberculosis*; five of its clinical isolates were more active than isoniazid, streptomycin and more potent than ethambutol against drug-resistant strain. [10] Also synthesis and biological evaluation of new 1,2,5-oxadiazole N-oxide derivatives with potential cytotoxic effects are proved to be very active, although non-selective. [11] Some derivatives of phenomethyl-1,2,4-oxadiazole with the oxadiazole cycle at the o-position of the aromatic ring have a significant beta-adrenoceptor blocking activity associated with alpha-adrenoceptor blocking properties. [12]

Alkaline Phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. [13, 14] The amount of placental isoenzyme of alkaline phosphatase (PLAP) began to increase dramatically around 13th week and probably continued to increase gradually until term. The continuous increase in the expression of PLAP throughout pregnancy suggests

that PLAP may play a role in feto-maternal metabolism and placental differentiation. [15] The bone isoenzyme of alkaline phosphatase (BALP) contributes significantly to the increased ALP in normal pregnancy, and to determine the gestational age at which the increase occurs.[16] Women with elevated placental alkaline phosphatase levels are at increased risk for preterm delivery.[17] After delivery of a healthy baby the alkaline phosphatase level returned to the reference range.[18]

In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone, and the placenta. [13, 14] The optimal pH for the enzyme activity is pH=10 in standard conditions (310K,1 atm) . [19, 20] During the second and third trimester the ALP level increased and be double or more than its level before gestation. [21, 22]

Objectives:

To asses the effect of oxadiazole and thiadiazole derivatives on serum alkaline phosphatase level of pregnant in vitro.

Design and Setting:

A cross-sectional study on 34 pregnant women, 30.33±3.9 weeks of gestation, aged 28.47±6.1 years, was carried out in Al-Kadhumia Teaching Hospital from 1st July to 31st August 2006, after obtaining their agreements according to the medical research and ethical regulations, thus an oral consent was taken from all enrolled participants.

Materials and Method:

The following Oxadiazle derivatives were used to measure its effects on pregnant serum alkaline phosphatase:

- N-Benzoyl-2-(3-Indolyl)-1-(2-thio-1,3,4-oxadiazole-5-yl) ethylamine.
- N-Benzoyl-2-(3-Indolyl)-1-(2-thiopropynl-1,3,4-oxadiazole-5-yl) ethylamine.

Also the following Thiadiazle derivatives were used:

- N-Benzoyl-1-[(2-thio-1,3,4-thiadiazole-5-yl) methylamine].
- N-Benzoyl-1-[(2-thiobenzyl-1,3,4-thiadiazole-5-yl)-methylamine].
- N-Benzoy-1-[(2-ethylSulphonyl-1,3,4-thiadiazole-5-yl) methylamine].
- Bis-1,4-[(5,5-thio-1,3,4-thiadiazole)-2-yl]butane.

Di Methyl Sulphoxide (DMSO) or Ethanol was used as solvents to prepare, 10^{-3}, solutions of above derivatives to study their effects on alkaline phosphatase; before and after dissolving the oxadiazole and thiadiazole derivatives.

Alkaline Phosphatase kit (Bio Merieux) was used to measure the enzyme activity according to Kind [23] and Belfield [24] method.

The resulting color of samples, blanks and standards were measured, at 510 nm, by UV-Visual spectrophotometer. The alkaline phosphatase activity calculated according to the following equation:

$$ALP = [(OD_{sample} - OD_{blank}) / OD_{standard}] \times n \qquad (n=142\ U/L)$$

Statistical analysis and reporting of obtained data were carried out by using Microsoft Excel - Windows XP professional program. Statistical tests were performed using a null hypothesis of no diffirence with a two-tails paired student t-test; the level of signifecance of P value was ≤ 0.05 and of high significance was ≤ 0.01.

Results:

Primarily, the mean serum alkaline phosphatase (ALP level) activity of studied pregnant women who enrolled in the study, at 30.33±3.9 weeks of gestation, was found to be 162.13±69.34 U/L.

Solvents, Di Methyl Sulphoxide (DMSO) and Ethanol, used to dissolve the crystal forms of oxadiazoles and thiadiazoles were found to be of marked inhibitory effects on the ALP activity in vitro as 129.91±31.02 U/L (20%) for DMSO and 127.47±14.35 U/L (21%) for the Ethanol (Table-1).

Oxadiazole and Thiadiazoles solutions of 10^{-3} concentration were found to be of obvious inhibitory effects on ALP activity in vitro ranging from 122.20±86.14 U/L, 24% for the N-Benzoyl-2-(3-Indolyl)-1-(2-thiopropynl-1,3,4-oxadiazole-5-yl) ethylamine, up to 162.13±69.34 U/L, 62% for the N-Benzoyl-1-[(2-thiobenzyl-1,3,4-thiadiazole-5-yl)-methylamine] (Table-2).

Discussion:

The mean Alkaline Phosphatase activity of studied pregnant women, 162.13±69.34 U/L, was elevated more than the normally accepted concentrations of serum levels of ALP, which are typically 20-70 U/L at the normal lower limits and 38-126 U/L at the normal upper limits, depending on the assay and local normal guidelines; [13, 14] this elevation was due to the progressive increase of ALP activity during pregnancy. [21, 22]

Di Methyl Sulphoxide (DMSO) or Ethanol, solvent solutions for the Oxadiazole and Thiadiazole derivatives, effects on alkaline phosphatase was measured to avoid its contributory or biased effect on the ALP activity. Alkaline phosphatase activity measured before and after dissolving the oxadiazole and thiadiazole derivatives in the solvents to find its merit effects on alkaline phosphatase activity which had been 20-21% inhibitory effect on the alkaline phosphatase enzyme (Table-1).

Adding of the Oxadiazole and Thiadiazole compounds to the pregnant serum showed inhibitory effect on the alkaline phosphatase activity ranged between 24-64% of their initial effects (Table-2). Alkaline phosphatase was the first zinc enzyme to be discovered in which three closely spaced metal ions (two Zn^{+2} ions and one Mg^{+2} ion) are present at the active center, forming its triangular base

pyramidal shape of its molecule, the Zn^{+2} ions at all three sites produce a maximally active enzyme; [25, 26] at the same time Oxadiazole and Thiadiazole derivatives had three active inorganic radicals (two N^{-2} and one O^{-2} in Oxadiazole or one S^{-2} in Thiadiazole).[2, 3, 26] The mechanism of activity of alkaline phosphatase was explained by Chappelet-Tordo who was give for the first time acceptable mechanism for the ALP activity;[27] since the determination of the initial crystal structure of alkaline phosphatase, Oxadiazoles and Thiadiazoles involved in phosphate ester hydrolysis have been known,[26] and the Alkaline Phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules including nucleotides, proteins, and alkaloids by the process called dephosphorylation, so that process of dephosphorylation give us an idea about a triad of closely spaced zinc ions bonds present at the active centers of ALP to form a new resulting blocked ALP molecules which is not detected by the procedure of measuring ALP activity. [13, 14, 26] (Figure- 3, 4, 5)

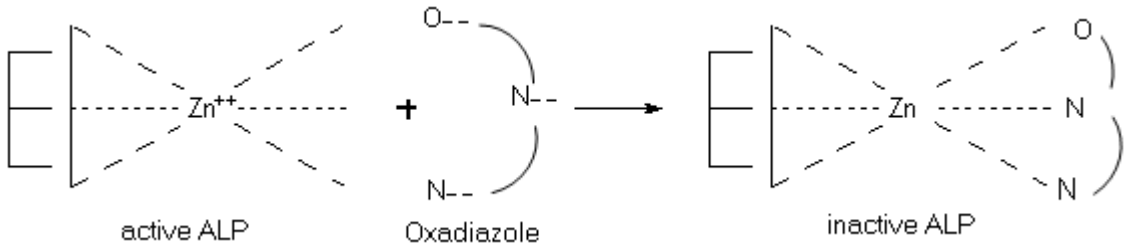


Figure-3: chemical reaction of Oxadiazoles with Alkaline phosphatase. [25]

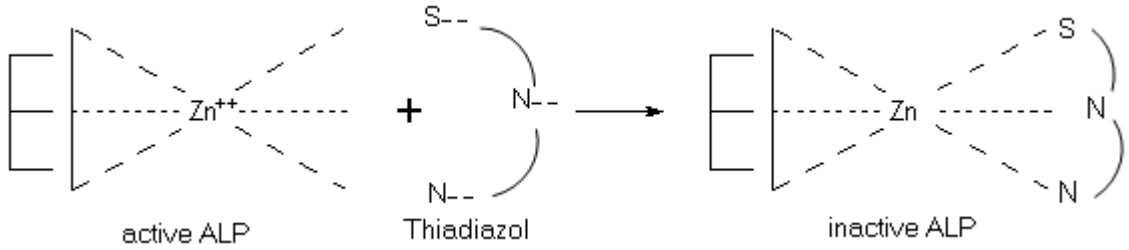


Figure-4: chemical reaction of Thiadiazoles with Alkaline phosphatase. [25]

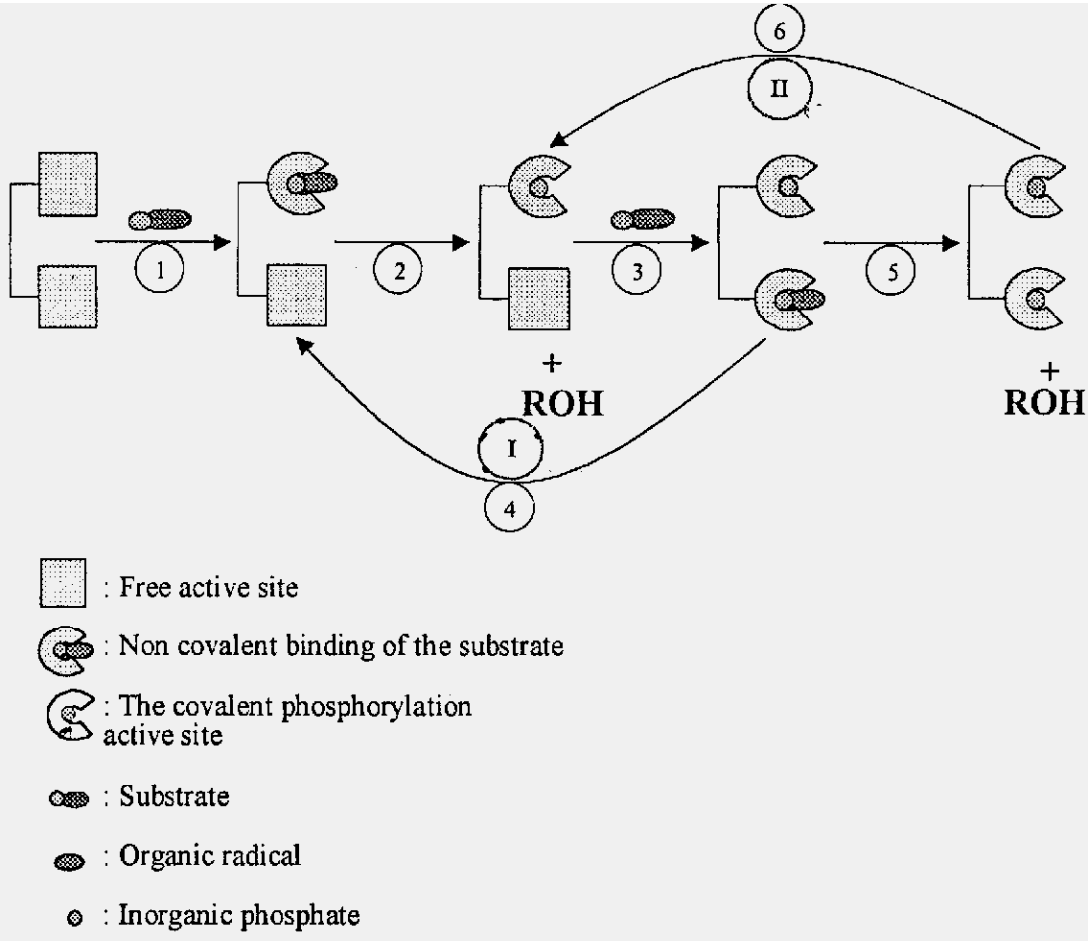


Figure-5: Mechanism of action of alkaline phosphatase enzyme. [27]

Concluosions:

Present study showed obviously the inhibitory effect of Oxadiazoles and Thiadiazoles derivatives in vitro on alkaline phosphatase enzyme of pregnant women due to the dephosphorylation reaction which block the ALP activity.

Recommendations:

Further studies and clinical trials would be conducted to explore the Oxadiazoles and Thiadiazoles derivatives effect in vivo on alkaline phosphatase to utilize these derivatives in the chemical, pharmaceutical and clinical practices.

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Solvent	**ALP before addition of solvent** U/L	**ALP after addition of solvent** U/L	**Inhibitory effect of solvent**
DMSO	162.13±69.34	129.91±31.02	20%
Ethanol	162.13±69.34	127.47±14.35	21%

Table-1: The inhibitory effect of the solvents, used to prepare the solutions on the ALP level.

substances	ALP before addition U/L	solvent	ALP after addition of solved substance U/L	Inhibitory effect of solved substance
N-Benzoyl-2-(3-Indolyl)-1-(2-thio-1,3,4-oxadiazole-5-yl) ethylamine	162.13±69.34	DMSO	105.33±24.13	35%
N-Benzoyl-2-(3-Indolyl)-1-(2-thiopropynl-1,3,4-oxadiazole-5-yl) ethylamine	162.13±69.34	DMSO	122.20±86.14	24%
N-Benzoyl-1-[(2-thio-1,3,4-thiadiazole-5-yl) methylamine]	162.13±69.34	Ethanol	105.34±47.30	35%
N-Benzoyl-1-[(2-thiobenzyl-1,3,4-thiadiazole-5-yl)-methylamine]	162.13±69.34	Ethanol	94.75±76.23	62%
N-Benzoy-1-[(2-ethylSulphonyl-1,3,4-thiadiazole-5-yl) methylamine]	162.13±69.34	DMSO	100.22±69.26	38%
Bis-1,4-[(5,5-thio-1,3,4-thiadiazole)-2-yl]butane	162.13±69.34	DMSO	102.66±51.57	37%

Table-2: The inhibitory effect of Oxadiazoles and Thiadiazoles solutions on the ALP level.