Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

EFFECT OF TWO NEWLY SYNTHESIZED RIBOFURANOSE DERIVATIVES ON THE PLASMA XANTHINE OXIDASE ACTIVITY IN PATIENTS WITH CHRONIC RENAL FAILURE

* Lubna Suleiman,
** Israa Ghassan Zainal
University of Kirkuk, College of Science, Chemistry Department, Iraq
* lobnna2021@gmail.com ; ** israaz@uokirkuk.edu.iq

Abstract

Background: Renal failure claims the lives of humans worldwide**.** Several studies suggest that oxidative stress is a major factor in deterioration of renal function. Oxidative stress seems to play a pivotal role in this process, and purine metabolism may be involved in renal failure-related oxidative stress. Xanthine oxidase is an enzyme involved in purine metabolism and is also responsible for producing reactive oxygen species.

Objective: This study aimed to evaluate the activity of xanthine oxidase activity and study the effect of *in vitro* two newly synthesized ribofuranose derivatives including (B-D-Ribofuranose-3-morpholine methyl thymine, and (B-D-Ribofuranose-3-morpholine methyl pyrrolidine) on the activity of the enzyme in patients with chronic renal failure. The study includes 110 plasma samples divided to (60) samples as patients with chronic renal failure and (50) samples appeared healthy subjects as control.

Results: the results indicated that there was significant ($p \leq 0.05$) decrease in total protein levels, and a significant ($p \leq 0.05$) increase in xanthine oxidase activity and specific activity in patients as compared to control. The two used compounds caused inhibitory effect on the enzyme activity; and the high inhibition% equal to (91.72) % obtained with 0.11 M from B-D-Ribofuranose-3-morpholine methyl thymine and (92.19) % with 0.11 M from of B-D-Ribofuranose-3-morpholine pyrrolidine**.** This study also estimated the kinetic parameters (K_m and V_{max}) with different concentrations from each inhibitor under the same conditions by using Line weaver-Burk equation and the results indicated that the level of Km was not affected by adding the inhibitor to the enzyme reaction, while the level of Vmax was decreased when the reaction of enzyme include the inhibitor. Finally, the type of inhibition was found as non-competitive inhibition in the two ribofuranose derivatives.

Conclusions: the results

Keywords: Xanthine oxidase, ribofuranose derivatives**,** chronic renal failure**,** inhibition**.**

Introduction

The term chronic renal failure (CRF) denotes inability of the renal to perform excretory functions leading to retention of nitrogenous waste products from the blood. Chronic renal failure, also known as end-stage renal disease, is a medical condition in which the renal are functioning at less than 15% of normal levels(1). Complications of CRF also include heart disease, high blood pressure, and anemia(2). Renal disease diagnosis is based on the presence of renal damage such as albuminuria or decreased

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

renal functions (i.e. Glomerular filtration rate (GFR). Chronic renal failure is a global general medical issue, it is one of the leading causes of morbidity and mortality worldwide and is increasingly recognized as a global public healthcare challenge, especially in developing countries. The occurrence of CRF in low-income countries increases by about 20% / year. Xanthine oxidase (Xo) is a superoxide-producing enzyme found normally in serum, lungs, and is more abundant in the intestine and liver and its activity increases during infection. Plasma Xo activity was associated with hypertension in general population, speculating that the activation of Xo may promote oxidative stress-related injury to endothelial cells and the renal, leading to the elevation of blood pressure (BP) [3]. A report showed that Xo genetic polymorphisms predict changes in BP and the risk of hypertension supports the crucial role of Xo in the incidence of hypertension [4]. The lack of Xo leads to high concentration of xanthine in blood and can cause health problems such as CRF [5]. Xanthine oxidase activity was also an independent predictor of cardiovascular events in CRF and hemodialysis patients. Several investigations showed that in various pathological conditions, both in experimental animals and in humans, there is an increased plasma level of Xo because of release into circulation from liver or other tissues. In view of their good efficacy and long-term proven safety, xanthine oxidase inhibitors (Xoi) currently represent the first-choice treatment of hyperuricemia associated with various diseases, including CRF [6]. In the latter, (Xoi) administration may also ameliorate renal damage, not only by reducing circulating uric acid levels (indirect benefit), but also through various mechanisms at the renal level (direct benefits),[7] including the reduction of inflammation and oxidative stress and the prevention of glomerular hypertension, afferent arteriolar thickening and ischemic renal histologic changes. Inhibition of Xo has been proposed as a mechanism for improving cardiovascular health. The inhibitor is defined as a substance that reduces the rate of an enzymatic reaction. The inhibitor may be similar to the composition of the substrate, or it may differ from it. The effect of inhibitors differs according to their different types, some of which affect the substrate itself, and others combine with the active site on the surface of the Xo and thus it reduces the tendency of the enzyme towards the substrate. The aim of this study was to evaluate the activity of human plasma Xo in CRF patients compared to healthy subjects, and then determine the *in vitro* effects of two newly synthesized ribofuranose derivatives on human plasma Xo activity.

Material and Methods

Subjects

Sixty CRF patients which subdivided to (31 male and 29 female), those patients visited Kirkuk general hospital/Kirkuk city during the period from (December 2021 to July 2022) with age ranged between (20-40) years were selected in this study. All patients were subjected to a personal interview using specially designed questionnaire format of full history with detailed information. And (50) subjects appeared healthy volunteer were selected, subdivided to (27 male and 23 female) with the same age range as patients' group, selected from Kirkuk city regions as control. Any subject diagnosed with diabetes mellitus, heart diseases, individuals with a history of drug addiction and alcohol consumption and participants with anti-hyper urinemic drug intake were excluded from the study, also excluded participants with self-reported renal and hepatic diseases, hypothyroidism, and any infectious diseases.

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

The study protocol was approved by the internal ethics committee which exists at the department of chemistry, Kirkuk University, college of science and the ethics committee of Kirkuk general hospital. A peripheral venous blood sample (5ml) was collected in EDTA tube using a disposable syringe, and blood was taken by vein puncture without using the tourniquet, then centrifuged the tubes for (10) minutes at (308 X g). The plasma was collected and stored at -20 C° until use.

Methods

Xanthine oxidase activity assay:

The most frequently used method for the determination of Xo activity is described by Marcocci *et al*[8] and Cos *et al*[9]. The measurement is carried out using phosphate - EDTA buffer (0.1M, pH 7.4). Spectrophotometric determination of XO activity is based on measuring uric acid production from xanthine as substrate, read the absorbance at 295nm.

Total protein determination:

Quantitative plasma TP determination was achieved by absorbance measurements at 660 nm according to Lowry *et al,* method 1951[10], using bovine serum albumin as a standard.

Purification methods:

Xanthine oxidase was partially purified from plasma of CRF patients using ammonium sulphate with 75% saturation percent, the solution dialyzed against buffer phosphate (pH 7.5) (changed each 8hours) to remove the trace of AS. Finally, the enzyme activity.

Inhibition of xanthine oxidase:

Inhibitors prepared by dissolving (0.14gm) of (β-D-Ribofuranose-3-morpholine methyl thymine, I-1) and (0.15gm) of (β-D-Ribofuranose-3-morpholine methyl pyrrolidine, I-2) in (1ml) of phosphate buffer (0.0467 M, pH=7.5) as a stock solution, this solution was diluted with buffer solution to obtain concentrations (0.009, 0.033, 0.07, 0.11) M. Table 1 represented the structure of I-1 and I-2.

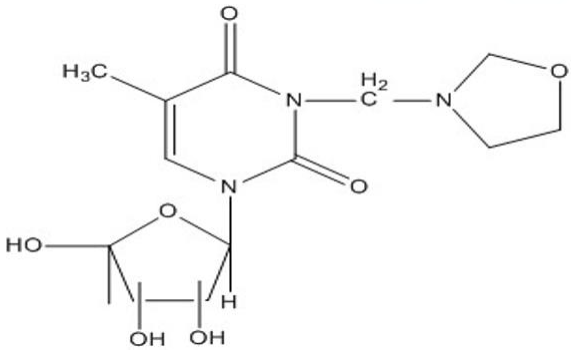
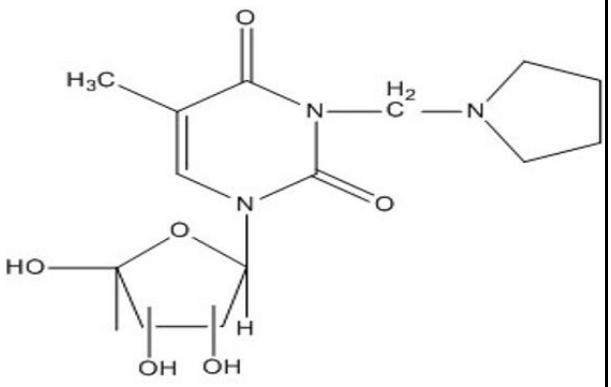
The rote of inhibitory was computed by comparing the enzymatic efficacy with the presence and absence of inhibitor as in the equation below:

$$\text{Inhibition } \% = \frac{Activity\ without\ inhibitor - Activity\ with\ inhibitor}{Activity\ without\ inhibitor} * 100\%$$

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

Table (1): Ribofuranose derivatives used as inhibitors to Xo.

Compound name	Structure of compound
β-D-Ribofuranose-3-morpholine methyl thymine	tha 
β-D-Ribofuranose-3-morpholine methyl pyrrolidine	

Statistical Analysis

Statistical analysis was done using graph pad prism version 6 and values were expressed as (mean ±SD). The comparison of mean ± SD was performed using Student t – test. Statistical significance was defined as P≤ 0.05. and this study aimed to elucidate the kinetic parameters for xanthine as substrate and two ribofuranose derivatives as inhibitors to the XO activity, as well as determine the inhibition type.

Results

The plasma levels of TP and the (activity & specific activity) of the Xo were determined in CRF patients compared to control, the results were mentioned as (mean ± SD) as present in Table 2:

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

Table 2: The TP and the (activity with specific activity) of Xo in the plasma of CRF patients and subdivided to (males and females) compared to control.

Parameters	Control mean ± SD	Patients mean ± SD	P value
Xo activity (U/ml)	3.24±0.53	3.61±0.23	<0.0001
Specific activity of Xo (U/mg)	0.47± 0.085	0.71± 0.13	<0.0001
TP (gm/dl)	6.90±0.62	5.27±1.27	<0.0001

The results indicated that there was significant ($p \leq 0.05$) decrease in TP levels, and significant ($p \leq 0.05$) increase in XO activity and specific activity in patients group compared to control. The sequential steps to partially purify plasma XO from CRF patients' using 75% AS precipitation and dialysis, against phosphate buffer, presented in the table (3):

Table (3) Purification steps of XO of chronic renal failure patients

Steps	Total volume (ml)	protein mg/ml	T.P mg	Activity U/ml	Total activity U	Specific activity U/mg	Fold of purification	Yield %
Crude plasma	8	58	464	3	24	0.0517	1	100%
Ammonium sulphate 75%	5.7	30	171	3.7	21.09	0.1233	2.38	87.88%
Dialysis	5.9	27	159.3	4	23.6	0.1481	2.86	98.33%

After using the AS (75% saturation) precipitation method, most XO activity was recovered in the soluble fraction of plasma contaminants of proteins which were precipitated by addition solid AS. The results indicated that the specific activity of the crude plasma of patients was (0.0517 U/mg). After adding AS, it increased to (0.1233 U/mg), and after dialysis, it was (0.1481 U/mg), while the folds of purification

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

were (2.38, 2.86). The yield % was (87.88, 98.33) % respectively. The purified XO can be stored as a precipitate at 75% saturation of AS without any addition of reducing agents such as DTT.
Also this study examined the in *vitro* inhibitory effect of two newly synthesized ribofuranose derivatives using different concentrations on the plasma XO activity reaction in CRF patients. Xanthine oxidase activity without a ribofuranose compound was accepted as 100% activity, as cleared in table 4.

Table (4) Effect of different concentrations from I-1 and I-2 compounds on the activity of plasma of Xo.

Compounds	[I] M	[S] M	Activity without inhibitor U/ml	Activity with Inhibitor U/ml	The inhibition %
I-1	0.009	0.05	3.66	0.303	91.72%
	0.033			0.357	90.25%
	0.07			0.5	86.34%
	0.11			0.83	77.32%
I-2	0.009	0.05	3.66	0.286	92.19%
	0.033			0.4	89.07%
	0.07			0.435	88.11%
	0.11			0.769	79.15%

The results revealed that the two used compounds caused inhibitory affect on the Xo activity. The highest %inhibition obtained for I-1 compound equal to 91.72% with concentration of inhibitor equal to 0.009M, also the higher %inhibition of I-2 compound equal to 92.19%, with concentration of inhibitor equal to 0.009 M, as shown in figures 1 and 2.

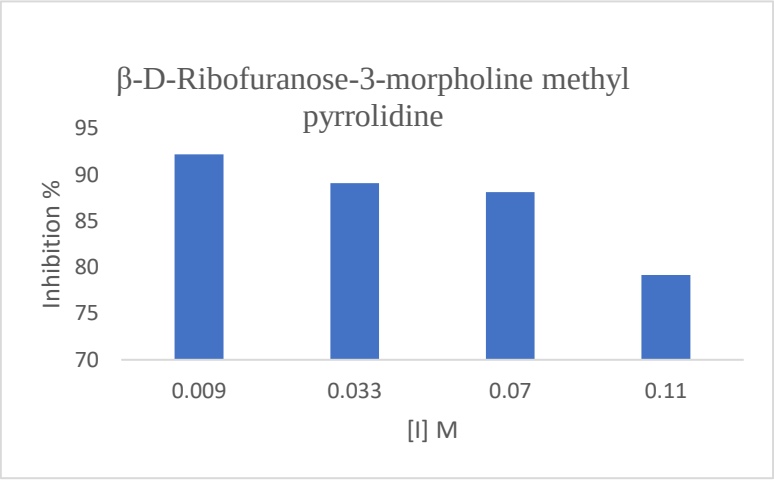


Figure 1: The inhibition effect of I-1 compound with % inhibition.

Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

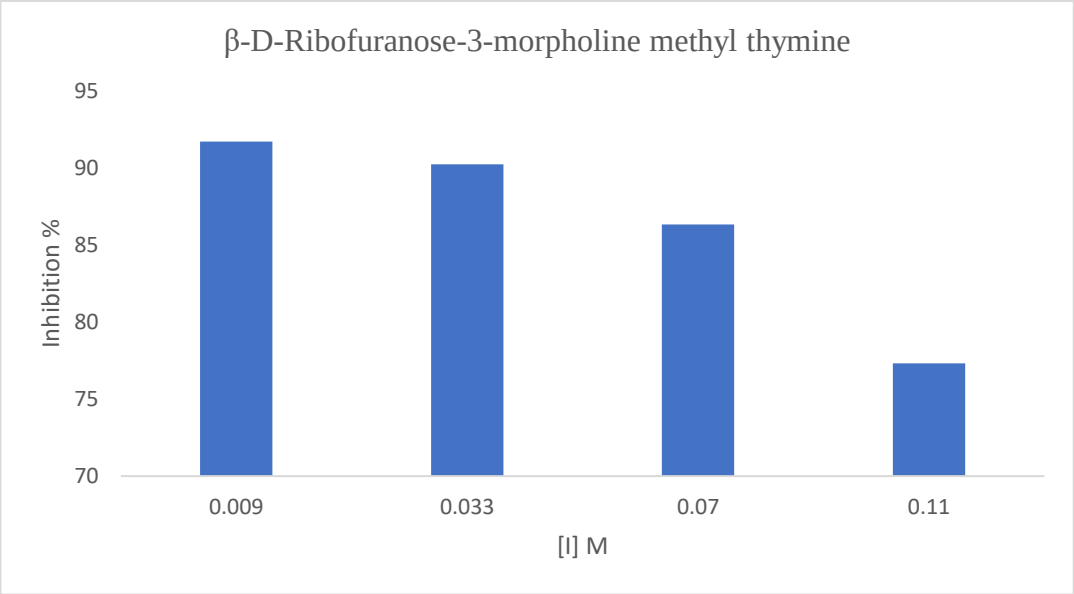
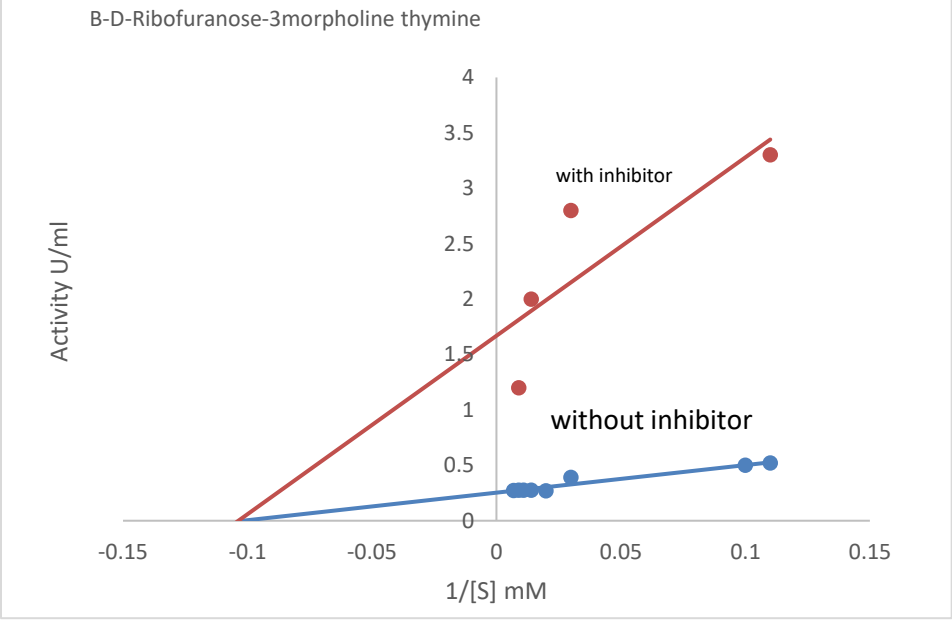


Figure 2: The inhibition effect of I-2 inhibitor with the % inhibition.

The XO kinetic parameters (with and without inhibitor) have been calculated from Line weaver - Burk plot as shown in figure 3 which shows that the inhibition type in this study was non-competitive inhibitor to the XO activity.



Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

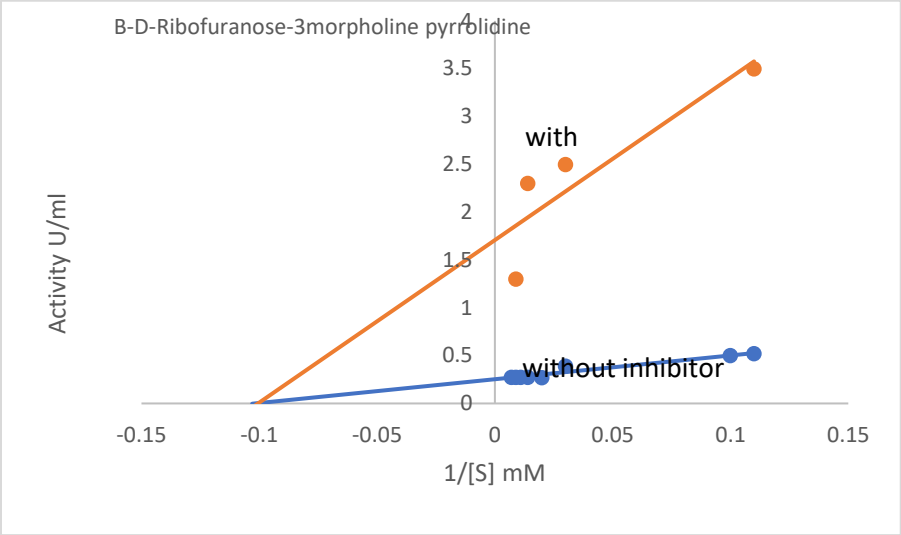


Figure 3: Line weaver - Burk plots of the studied inhibitors on XO in CRF patients.

The levels of the kinetic parameters (K_m and V_{max}) with different concentrations from substrate and inhibitors under the same conditions were shown in table 5.

Table 5: The values of K_m and V_{max} for XO reaction.

Inhibitor	K_m mM	V_{max} U/ml Without inhibitor	V_{max} U/ml With inhibitor
I-1	9.09	4	0.57
I-2	9.09	4.1	0.59

Discussion

The enzyme is highly versatile flavoprotein enzyme ubiquitous among species from bacteria to human and within the various tissues of mammals [11]. It is a key in degradation of DNA and RNA [12] and it is believed to be one source of reactive oxygen species in the failing heart [13]. Xanthine oxidase also plays an important role in cellular oxidative status, detoxification of aldehydes, various forms of ischemic and other types of tissue and vascular injuries. Many studies reported that plasma XO activity was associated with the speculation that the activation of XO may promote oxidative stress-related injury to endothelial cells and the renal[14]. The activity of XO was found to be elevated in heart disease and peritoneal dialysis patients, independently of dialysis modality [15]. It was reported that XO activity in the vasculature is elevated considerably in patients with coronary artery diseases[16]. It has also been suggested that local XO activities in a variety of organs and tissues are likely to elevate under a line of stimuli including hypoxia, inflammatory cytokines and glucocorticoids[17]. Increases in endogenous XO

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

may be germane to vascular conditions that render tissues chronically hypoxic. In addition, tissues or species that have low basal levels of XO may be prone to XO-related vascular injury and inflammation under hypoxic or other conditions that increase XO activity[18].

Proteins are important targets for oxidation reactions described as a mismatch in how free radicals are formed and the antioxidant reaction within cells, because they are abundant in tissues, extracellular cells, and physiological fluids, as well as their quick reaction rates with oxidants. The natural filtration of the renal changing results in the accumulation of toxic wastes, and higher amounts of TP lost through urine. The reduction in TP levels can also be explained as the lack of natural feedback inhibition of gluconeogenesis in the liver that causes an increase in the breakdown of lipids and proteins, as well as the conversion of glucogenic amino acids to glucose which leads to an increase in glucose levels; in addition, hemodilution could also be a cause of this reduction.

The results of purification cleared that every step in the enzyme purification resulted in the removal of undesirable fractions of protein therefore, decline in enzyme yield was found after the process of purification. The differences may be due to essentially lowest XO activity that CRF patients and highest found in control, these results can be attributed to the fact that the presence of inflammatory factors in patients sample may effect XO purified and activity. Thus compared with other mentioned studies, present purification procedure takes less time and good specific activity, yield and fold of purification. The procedure of purification was considered imperative as it resulted in a significant increase in the specific activity of XO.

The inhibitor is defined as a substance that reduces the rate of an enzymatic reaction. The inhibitor may be similar to the composition of the substrate or it may differ from it. The effect of inhibitors differs according to their different types, some of which affect the substrate itself, and others combine with the active site on the surface of the enzyme and thus it reduces the tendency of the enzyme towards the substrate. Others affect reactions by combining with other sites on the enzyme, and this type of union may not affect the tendency of the enzyme, but it affects the rate at which the substrate is converted into a product[19]. This means that this type of inhibitor is associated with a site other than the active site of the XO, and this type of inhibitor cannot be reduced by increasing the concentration of the substrate and these types of inhibitors are called non-competitive inhibitors. These types of inhibitors are characterized by their effect on the maximum velocity V_{max}[20]. This type of inhibition occurs when the substrate and inhibitors bind to different sites of the enzyme and the inhibitor binds either with the free enzyme or with the enzyme-substrate [ES] complex to form an enzyme-substrate inhibitor [ESI] complex. This complex can be degraded to give the product, but at a slower rate than it is for the decomposition of the [ES] substrate enzyme complex. Thus, the reaction will be slower than it is in the absence of this type of inhibitor[21].

This study includes the evaluation of the effect of two ribofuranose compounds on the activity of partially purified XO in CRF patients. The values of K_m and V_{max} were calculated from the Line weaver-Burk plot in the absence and the presence of the inhibitor. The results of this study could be explained according to the classical models described that the inhibitor bind to another conformational change that lock the enzyme & prevent the substrate binding or decreasing substrate affinity to enzyme [22].

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

Line weaver - Burk graph showed the type of inhibition for each inhibitor and inhibition constant K_m was estimated. V_{max} was evaluated from the y-intercept of Line weaver - Burk graph, which reflected that Vmax value for control sample (without inhibitor) was higher than in inhibited samples, so it is clear that the amount of active enzyme (V_{max}) present in non-inhibited which in agreement with the study of Zayzafon, *et al* [23].

References

(1) van Wincoop, M., K. de Bijl-Marcus, M. Lilien, A. van den Hoogen et F. Groenendaal (2021). « Effect of therapeutic hypothermia on renal and myocardial function in asphyxiated (near) term neonates: A systematic review and meta-analysis », PLoS One, vol. 16, n° 2, p. e0247403.
(2) Liao, M.-T., C.-C. Sung, K.-C. Hung, C.-C. Wu, L. Lo et K.-C. Lu (2012). « Insulin resistance in patients with chronic kidney disease », Journal of Biomedicine and Biotechnology, vol. 2012.
(3) Furuhashi, M., Y. Higashiura, M. Koyama, M. Tanaka, T. Murase, T. Nakamura, S. Akari, A. Sakai, K. Mori et H. Ohnishi (2021). « Independent association of plasma xanthine oxidoreductase activity with hypertension in nondiabetic subjects not using medication », Hypertension Research, vol. 44, n° 9, p. 1213-1220.
(4) Tsuchihashi, T. (2021). « Which is more important, xanthine oxidase activity or uric acid itself, in the risk for cardiovascular disease? », Hypertension Research, vol. 44, n° 11, p. 1543-1545.
(5) Stiburkova, B., K. Pavelcova, L. Petru et J. Krijt (2018). « Thiopurine-induced toxicity is associated with dysfunction variant of the human molybdenum cofactor sulfurase gene (xanthinuria type II) », Toxicology and Applied Pharmacology, vol. 353, p. 102-108.
(6) Thurston, M. M., B. B. Phillips et C. A. Bourg (2013). « Safety and efficacy of allopurinol in chronic kidney disease », Annals of Pharmacotherapy, vol. 47, n° 11, p. 1507-1516.
(7) Kabul, S., et B. Shepler (2012). « A review investigating the effect of allopurinol on the progression of kidney disease in hyperuricemic patients with chronic kidney disease », Clinical therapeutics, vol. 34, n° 12, p. 2293-2296.
(8) Marcocci, L., L. Packer, M.-T. Droy-Lefaix, A. Sekaki et M. Gardès-Albert (1994). « [46] Antioxidant action of Ginkgo biloba extract EGb 761 », dans Methods in enzymology, Elsevier, p. 462-475.
(9) Cos, P., L. Ying, M. Calomme, J. P. Hu, K. Cimanga, B. Van Poel, L. Pieters, A. J. Vlietinck et D. Vanden Berghe (1998). « Structure– activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers », Journal of natural products, vol. 61, n° 1, p. 71-76.
(10) Lowry, O. H., N. J. Rosebrough, A. L. Farr et R. J. Randall (1951). « Protein measurement with the Folin phenol reagent. », Journal of biological chemistry, vol. 193, p. 265-275.
(11) F Bdaiwi, L. (2012). « Isolation and Characterization of XantIsolation and Characterization of Xanthine Oxidase from Tissues of Benign and Malignant Colon Tumors Patients », Rafidain Journal of Science, vol. 23, n° 7, p. 70-82.
(12) Al-Leheebe, N. I., O. Y. Al-Abbasy et I. S. AL-Flaeyh (2018). « Inhibition of Partially Purified Xanthine Oxidase Activity from renal failure patients by Theophylline and Metronidazole », Tikrit Journal of Pure Science, vol. 22, n° 9, p. 47-52.
(13) B Alsaadon, M., O. Y Al-Abbasy et N. I Al-Leheebi (2008). « Inhibition of Partially Purified Xanthine Oxidase Activity from Sera of Patients with Gout », Rafidain Journal of Science, vol. 19, n° 3, p. 48-55.

ISSN: 2776-1010 Volume 4, Issue 10, Oct. 2023

(14) Kurajoh, M., S. Fukumoto, M. Emoto, T. Murase, T. Nakamura, T. Ishihara, H. Go, K. Yamamoto, S. Nakatani et A. Tsuda (2020). « Independent association of plasma xanthine oxidoreductase activity with serum uric acid level based on stable isotope-labeled xanthine and liquid chromatography/triple quadrupole mass spectrometry: MedCity21 health examination registry », Clinical Chemistry and Laboratory Medicine (CCLM), vol. 58, n° 5, p. 780-786.
(15) Choi, J.-Y., Y. J. Yoon, H.-J. Choi, S.-H. Park, C.-D. Kim, I.-S. Kim, T.-H. Kwon, J.-Y. Do, S.-H. Kim et D. H. Ryu (2011). « Dialysis modality-dependent changes in serum metabolites: accumulation of inosine and hypoxanthine in patients on haemodialysis », Nephrology Dialysis Transplantation, vol. 26, n° 4, p. 1304-1313.
(16) Spiekermann, S., U. Landmesser, S. Dikalov, M. Bredt, G. Gamez, H. Tatge, N. Reepschläger, B. Hornig, H. Drexler et D. G. Harrison (2003). « Electron spin resonance characterization of vascular xanthine and NAD (P) H oxidase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation », Circulation, vol. 107, n° 10, p. 1383-1389.
(17) Tsushima, Y., H. Nishizawa, Y. Tochino, H. Nakatsuji, R. Sekimoto, H. Nagao, T. Shirakura, K. Kato, K. Imaizumi et H. Takahashi (2013). « Uric acid secretion from adipose tissue and its increase in obesity », Journal of Biological Chemistry, vol. 288, n° 38, p. 27138-27149.
(18) Terada, L. S., D. M. Guidot, J. A. Leff, I. R. Willingham, M. E. Hanley, D. Piermattei et J. E. Repine (1992). « Hypoxia injures endothelial cells by increasing endogenous xanthine oxidase activity. », Proceedings of the National Academy of Sciences, vol. 89, n° 8, p. 3362-3366.
(19) Gülçin, İ., B. Trofimov, R. Kaya, P. Taslimi, L. Sobenina, E. Schmidt, O. Petrova, S. Malysheva, N. Gusarova et V. Farzaliyev (2020). « Synthesis of nitrogen, phosphorus, selenium and sulfur-containing heterocyclic compounds–determination of their carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase and α-glycosidase inhibition properties », Bioorganic Chemistry, vol. 103, p. 104171.
(20) Afroz, S., M. A. Abbasi, W. Tanveer, K. M. Khan, M. Ashraf, I. Ahmad, I. Afzal et N. Ambreen (2012). « Synthesis, characterization and biological screening of sulfonamides derived form 2-phenylethylamine. », Pakistan journal of pharmaceutical sciences, vol. 25, n° 4.
(21) Taha, M., F. J. Alshamrani, F. Rahim, N. Uddin, S. Chigurupati, N. B. Almandil, R. K. Farooq, N. Iqbal, M. Aldubayan et V. Venugopal (2021). « Synthesis, characterization, biological evaluation, and kinetic study of indole base sulfonamide derivatives as acetylcholinesterase inhibitors in search of potent anti-Alzheimer agent », Journal of King Saud University-Science, vol. 33, n° 3, p. 101401.
(22) Champe C.P, HarveyM. R, Ferrier D.R.Lippincotts (2008). «illustrated reviews biochemistry, fourth edition . philadelphia, ch;5: 61
(23) Zayzafon N.Nasif (2013) . «Extent of metabolic changes in normal and abnormal pregnancies in iraq.