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In Vitro Inhibition Effects of 2-Amino Thiazole Derivatives on Lactoperoxidase Enzyme Activity

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ABSTRACT

Lactoperoxidase (LPO E.C. 1.11.1.7) is a member of the peroxidase family and is an important glycoprotein containing heme group in its structure and showing the antimicrobial effect on disease causing microorganisms in the digestive system of newborn babies. Thiazoles are the simplest members of heterocyclic compounds containing nitrogen and sulfur atoms in their structure. Many active pharmaceutical substances such as vitamin B1, penicillin, and those obtained by synthesis, contain a thiazole ring. It is desirable to evaluate the biological activities of thiazole derivatives, such as antiprotozoal antibacterial, antifungal, antituberculosis, and anthelmintic, with emphasis on their potential medical applications. The aim of this study was to determine the in vitro inhibition profiles of 2-amino thiazole derivatives against bovine LPO enzyme. In this study it was determined that all amino thiazole derivatives inhibited the LPO enzyme competitively. When the results were compared with each other, the 2-Amino-4-(4-chlorophenyl) thiazole compound showed the best inhibition effect against LPO with the Ki value of 250±100 nM.

Keywords: Lactoperoxidase, Amino thiazole, Pharmaceutical, Inhibition.

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Introduction

Peroxidases (POD: E.C.1.11.1.7), is a member of the oxidoreductase enzyme family, which has the potential to be used in many fields; one of the most common enzymes in all cell types [1]. Peroxidases is found in many sources, including bacteria, yeast, and higher plants, fungi, milk, and dairy products. Hydrogen peroxide (H_2O_2), which is formed during biotransformation in biological systems and has oxidizing properties, must be removed from the environment. This task in ensuring the removal of H_2O_2 from the environment in metabolism; It performs enzymes such as catalase and peroxidase, which are enzymes that show antioxidant properties in cells [2].

Lactoperoxidase (LPO); found in milk, saliva, tears, and the digestive system of newborns. It is a glycoprotein consisting of a single polypeptide chain containing the heme group (protoheme 9) as a prosthetic group [3]. This enzyme, which is found in large amounts in milk and dairy products, has a very important position in the prevention of pathogens in the gastro tract of infants during the lactation process. In other words, the lactoperoxidase system functions as a natural defense mechanism against invading microorganisms [4]. Thiazoles, one of the most important representatives of the quintuple natural ring system carrying sulfur and nitrogen. They are functional groups that are frequently encountered in the structure of drug molecules. It is an important component of the pharmacophores of many molecules of medical importance. The fact that the thiazole ring system is in the structure of compounds with important physiological effects such as penicillin and vitamin B1 and its similarities with other heterocyclic ring systems and the role of the ring in different pharmacological activities have increased the studies on ring structure and derivatives [5,6].

For the thiazole ring system, antimicrobial [7,8], antiviral [9], antiprotozoal [10], anticancer [11], antioxidant [12], anticonvulsant [13], antidiabetic [14], antihypertensive [15], anticholinesterase [16, 17], analgesic and anti-inflammatory [18] activities have been reported.

In this study, the inhibition effects of 2-amino thiazole derivatives on the LPO enzyme activity were determined. The IC_{50}, K_i values and the inhibition type of 2-amino thiazole derivatives were found.

Materials and Methods

Chemicals and Experimental Procedure

LPO enzyme was isolated by procuring bovine milk from local dairy farms in Erzurum. 2,2´-Azino-bis(3-ethylbenzthioazoline-6-sulfonic acid) (ABTS) used for activity studies, hydrogen peroxide and 2-Amino-4-(2,4-difluorophenyl) thiazole [a], 2-Amino- 4-(4-bromophenyl) thiazole [b], 2-Amino-5-(methyl)-4-phenyl) thiazole [c], 2-Amino-4-(5,6,7,8-tetrahydro-2-naphthyl)thiazole [d], 2-Amino-4-(4-chlorophenyl)thiazole [e], 4-(3,4-difluorophenyl)-1,3-thiazol-2-amino [f] compounds were commercially available from Sigma-Aldrich.

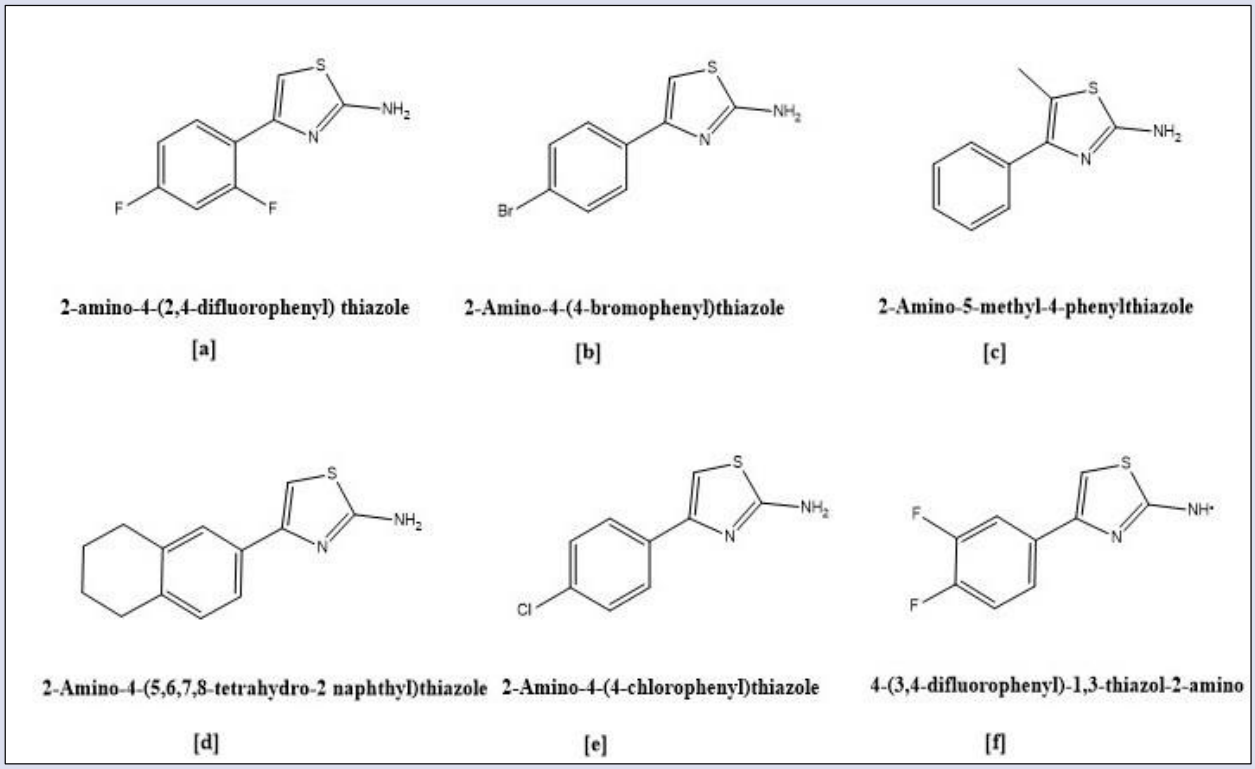

Figure 1. The chemical structures of 2-amino thiazole derivatives

Measurement of LPO Activity

LPO activity was determined by making minor modifications to the procedure performed by Shindler and Bardsley. According to previous study, 2.895 mL of ABTS (1.0 mM) in KH_2PO_4 (100 mM, pH 6.0) was mixed with 0.1 mL of 3.2 mM H_2O_2 with 0.005 mL of enzyme. It is determined according to the increase in absorbance at 412 nm of the compound formed as a result of oxidation of the ABTS molecule, which is the substrate that provides the formation of pigment or colorant [19,20]. An enzyme unit (E.Ü.); It is defined as the amount of enzyme that catalyzes the oxidation of 1 µmol ABTS at 298 K° in 1 minute (Ɛ: 32.600 M-1 cm-1) [21].

Purification of LPO

As reported in our previous study, the LPO enzyme was isolated from Sepharose 4B-L-tyrosine-sulfanilamide column. The prepared affinity column was equilibrated with the 10 mM, pH 6.8 Na_2HPO_4 solution. The homogenate was then packed onto the column and the column was washed with Na_2HPO_4 400 mL, 25 mM, pH 6.8. In the last step, eluents were collected by elution with NaCl/Na_2HPO_4 solution (1 M/0.25 M, pH:6.8) [22].

The Inhibition Studies of 2-Amino Thiazole Derivatives on LPO

Inhibition of 2-amino thiazole derivatives was determined from the graph of % Activity-inhibitor concentration drawn by working at five different inhibitor concentrations. IC_{50} values were determined for inhibitors with inhibitory effect, and the Lineweaver-Burk plot was drawn from the results with the activity measurements carried out at three different 2-amino thiazole derivatives and five different ABTS concentrations, and the K_i values and inhibition types of 2-Amino thiazole derivatives were determined [23].

Results and Discussion

The benefits of compounds containing milk and dairy products to human health at every stage of human life are known to date. Milk has many components that protect itself and the newborn from harmful microorganisms. The most important of these components is LPO [21]. LPO is an enzyme found in abundance in milk, which is responsible for forming an antimicrobial compound that catalyzes the reaction of thiocyanate ion and hydrogen peroxide in the living body [24-28]. LPO obtained from many animal sources contributes greatly to the destruction of bacteria by suppressing growth by causing bacterial inhibition [29].

In the literature, it was seen that there is no study to determine the inhibition parameters on LPO in studies involving thiazoles. In the light of this information, the parameters of the effects of amino thiazole derivatives on LPO enzyme activity were determined (Table 1).

Table 1. Inhibition data of 2-Amino thiazole derivatives

Compound	R^2	IC_{50} (nM)	K_i (nM)	Inhibition type
[a]	0.9068	340	390±50	Competitive
[b]	0.9968	490	670±60	Competitive
[c]	0.9175	890	480±50	Competitive
[d]	0.9132	470	540±140	Competitive
[e]	0.9035	320	250±100	Competitive
[f]	0.9418	550	600±290	Competitive

2-Amino-4-(2,4-difluorophenyl) thiazole [a], 2-Amino-4-(4-bromophenyl) thiazole [b], 2-Amino-5-(methyl)-4-phenyl) thiazole [c], 2-Amino-4- (5,6,7,8-tetrahydro-2-naphthyl) thiazole [d], 2-Amino-4-(4-chlorophenyl) thiazole [e], 4-(3,4-difluorophenyl)-1,3-thiazole-2-amino [f] IC_{50} values were found from the graphs drawn from the activity measurements of the compounds at different dose concentrations (Table 1). These values are (a), (b), (c), (d), (e) and (f); 340, 490, 890, 470, 320 and 550 nM for amino molecules, respectively.

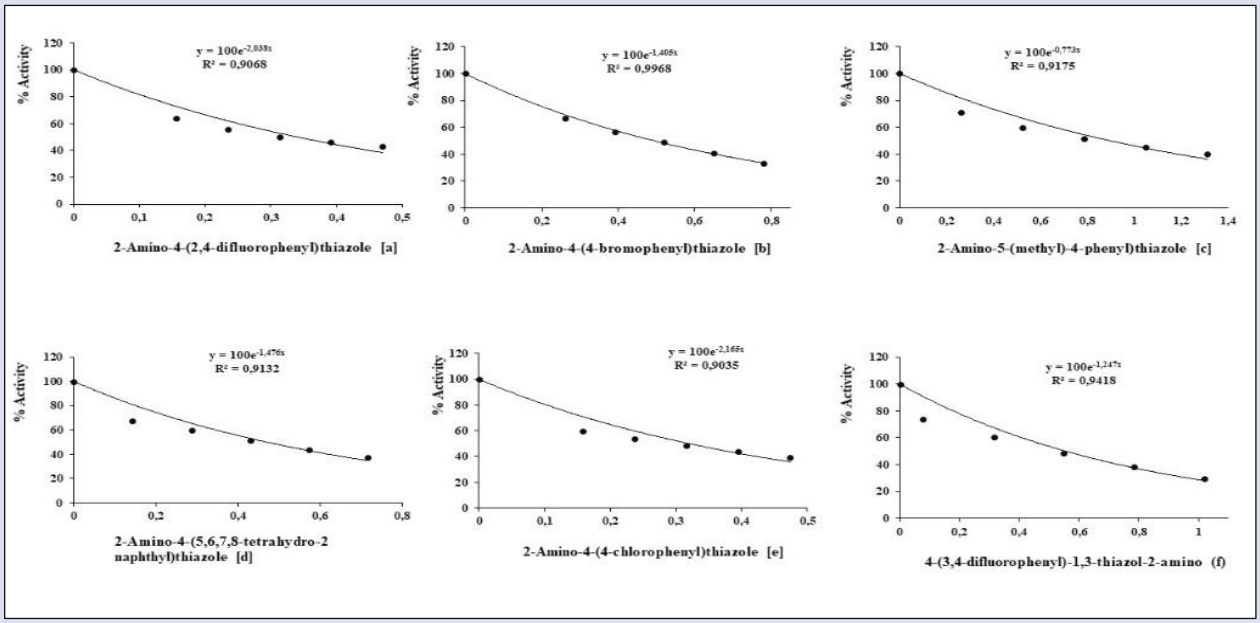

Figure 2. Activity(%)-[Inhibitor] graphs of 2-Amino thiazole derivatives with LPO enzyme

As a result of the activity measurements, the K_i values and inhibition types of 2-amino thiazole derivatives were determined by the Lineweaver-Burk curves (Figure 2). K_i values are (a), (b), (c), (d), (e) and (f); 390±50, 670±60, 480±0.05, 540±140, 250±100 and 600±290 nM for amino molecules, respectively. It was determined from the graphs that the inhibition types were competitive for all studied thiazole compounds.

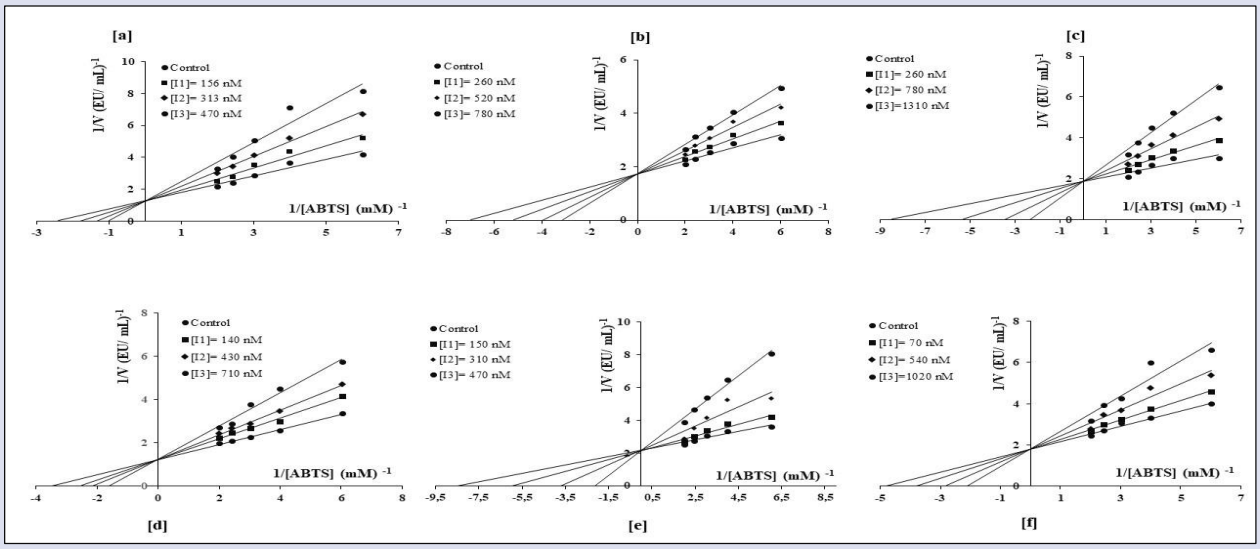

Figure 3. Graphs of 1/V and 1/[S] for 2-amino thiazole derivatives of LPO enzyme ([a]: 2-Amino-4-(2,4-difluorophenyl) thiazole; [b]: 2-Amino-4-(4-) bromophenyl) thiazole [c]: 2-Amino-5-(methyl)-4-phenyl) thiazole; [d]: 2-Amino-4-(5,6,7,8-tetrahydro-2-naphthyl) thiazole; [e]: 2-Amino-4-(4-chlorophenyl) thiazole; [f]: Lineweaver-Burk plots for 4-(3,4-difluorophenyl)-1,3-thiazol-2-amino)

In a study by Wang et al., the α-glucosidase inhibitory activities of several coumarin thiazole derivatives were evaluated. When the results were compared with acarbose used as a standard inhibitor, it was determined that coumarin thiazole compounds exhibited potent inhibitory activities at the micromolar level [30].

The effect of pyrazolyl-thiazole compounds on the inhibition of aldose reductase and α-glycosidase, which is accepted as a standard approach in the treatment of diabetic complications, was investigated. It was reported from the results that all compounds exhibited an inhibition profile at the micromolar level [31].

LPO, which has the potential to be used in many areas, is abundant in milk and dairy products because it reduces microflora [32,33]. It has an important position in the prevention of bacterial growth and is considered a very important compound in the natural defense mechanism against bacterial infections [29,34].

In another study, secondary sulfonamides containing acetoxybenzamide, triacetoxybenzamide, hydroxybenzamide and trihydroxybenzamide and containing thiazole, pyrimidine, pyridine, isoxazole and thiadiazole groups were synthesized. In vitro inhibitory effects of these synthesized derivatives against LPO were investigated. The results obtained determined that secondary sulfonamide derivatives are effective LPO inhibitors [35].

Xanthine oxidase (XO) inhibitors are widely used in the treatment of gout. In another study, a series of new tri-substituted 2-(indol-5-yl) thiazole derivatives with an indole skeleton were synthesized and their in vitro inhibitory activities against xanthine oxidase were determined. It has been reported that the 2-(3-cyano-2-isopropylindol-5-yl)-4-methylthiazole-5-carboxylic acid derivative exhibits the strongest XO inhibitory activity at the nm level [36].

The synthesis of sulfonamide-containing thiazole compounds and the inhibitory effects of these derivatives on the activity of human carbonic anhydrase I and II were evaluated by Kılılçaslan et al. The inhibitory effects of 12 synthesized sulfonamides on the hydratase and esterase activities of these isoenzymes (hCA-I and hCA-II) were investigated in vitro. They observed that all synthesized compounds inhibited CA isoenzyme activity at µM level [37].

In a different study, in vitro inhibition parameters of sesamol on bovine lactoperoxidase system were determined.

LPO enzyme was purified 467.51 fold with 75.99% yield in one step by an affinity chromatography technique using sulfanilamide as ligand. It was determined that sesamol strongly inhibited the LPO enzyme at the nM level and showed a competitive inhibition effect [38].

Conclusion

Newborn health needs to determine how and in what way this enzyme will act if chemicals are used in living things. As a result, the inhibitory effects of 2-amino thiazole derivatives on the purified LPO enzyme *in vitro* were investigated in detail. As can be seen here in the study, some 2-amino thiazole derivatives seem to inhibit lactoperoxidase at the micromolar level. The LPO enzyme found in milk and dairy products, which is important for the innate immune system, plays an important role in breaking down bacteria and removing them. A problem in the LPO system triggers the immune system significantly. Although it is not a desirable situation especially for newborn babies, parents in the lactation process must be careful against the problems caused by inhibitions to keep the immune system of the babies strong.

Conflicts of interest

The authors state that did not have conflict of interests.

References

[1] Erdem H.U., Kalın R., Özdemir N., Özdemir H., Purification and Biochemical Characterization of Peroxidase İsolated From White Cabbage (Brassica oleracea Var. Capitata F. Alba), *International Journal of Food Properties.*, 18 (2015) 2099- 2109.

[2] Halliwell B., Oxygen radicals: A Commonsense Look at Their Nature and Medical Importance, *Medical Biology*, 62 (1984) 71-77.

[3] Wolfson LM., Sumner SS., Antibacterial Activity of the Lactoperoxidase System: A Review, *Journal of Food Protection*, 56 (1993) 887-892.

[4] Reiter B., Review of the Progress of Dairy Science: Antimicrobial Systems in Milk, *Journal of Dairy Research*, 45 (1978) 131-147.

[5] Mayhoub A.S., Marler L., Kondratyuk T.P., Park E.J., Pezzuto J.M., Cushman M., Optimizing thiadiazole analogues of resveratrol versus three chemopreventive targets, *Bioorg. Med. Chem.*, 20(1) (2012) 510-20.

[6] Mayhoub A.S., Marler L., Kondratyuk T.P., Park E.J., Pezzuto J.M., Cushman M., Optimization of the aromatase inhibitory activities of pyridylthiazole analogues of resveratrol, *Bioorg. Med. Chem.*, 20(7) (2012) 2427-34.

[7] Ozadali K., Tan O.U., Yogeeswari P., Dharmarajan S., Balkan A., Synthesis and antimycobacterial activities of some new thiazolylhydrazone derivatives, *Bioorganic & Medicinal Chemistry Letters*, 24(7) (2014) 1695-1697.

[8] Qin Y.J., Wang P.F., Makawana J.A., Wang Z.C., Wang Z.N., Jiang A.Q., Zhu H., Design, synthesis and biological evaluation of metronidazole–thiazole derivatives as antibacterial inhibitors, *Bioorganic & Medicinal Chemistry Letters*, 24(22) (2014) 5279-5283.

[9] Xu Z., Guo J., Yang Y., Zhang M., Ba M., Li Z., Guo C., 2, 4, 5-Trisubstituted thiazole derivatives as HIV-1 NNRTIs effective on both wild-type and mutant HIV-1 reverse transcriptase: optimization of the substitution of positions 4 and 5, *European Journal of Medicinal Chemistry*, 123 (2014) 309-316.

[10] Makam P., Thakur P.K., Kannan T., In vitro and in silico antimalarial activity of 2-(2-hydrazinyl) thiazole derivatives, *European Journal of Pharmaceutical Sciences*, 52 (2014) 138-145.

[11] Popsavin M., Kojić V., Torović L., Svirčev M., Spaić S., Jakimov D., Popsavin V., Synthesis and in vitro antitumour activity of tiazofurin analogues with nitrogen functionalities at the C-2′ position, *European Journal of Medicinal Chemistry*, 111 (2016) 114-125.

[12] Geronikaki A.A., Pitta E.P., Liaras K.S., Thiazoles and thiazolidinones as antioxidants, *Curr. Med. Chem.*, 20, 4460-4480 (2013).

[13] Mishra C.B., Kumari S., Tiwari M., Thiazole: A promising heterocycle for the development of potent CNS active agents, *European Journal of Medicinal Chemistry*, 92 (2015) 1-34.

[14] Xu Q., Huang L., Liu J., Ma L., Chen T., Chen J., Chen L., Design, synthesis and biological evaluation of thiazole-and indole-based derivatives for the treatment of type II diabetes, *European Journal of Medicinal Chemistry*, 52 (2012) 70-81.

[15] Turan-Zitouni G., Chevallet P., Kilic FS., Erol K., Synthesis of some thiazolyl-pyrazoline derivatives and preliminary investigation of their hypotensive activity, *European Journal of Medicinal Chemistry*, 35(6) (2000) 635-641.

[16] Turan-Zitouni G., Ozdemir A., Kaplancikli Z.A., Altintop M.D., Temel H.E., Akalın Çiftçi G., Synthesis and biological evaluation of some thiazole derivatives as new cholinesterase inhibitors *J. Enzyme Inhib. Med. Chem*., 28(3) (2013) 509-514.

[17] Rahim F., Javed M.T., Ullah H., Wadood A., Taha M., Ashraf M., Khan K.M., Synthesis, molecular docking, acetylcholinesterase and butyrylcholinesterase inhibitory potential of thiazole analogs as new inhibitors for Alzheimer disease, *Bioorganic Chemistry*, 62 (2015) 106-116.

[18] El -Achkar G.A., Jouni M., Mrad M.F., Hirz T., El Hachem N., Khalaf A., Habib A., Thiazole derivatives as inhibitors of cyclooxygenases in vitro and in vivo, *European Journal of Pharmacology*, 750 (2015) 66-73.

[19] Shindler J.S., Bardsley W., Steady-State Kinetics of Lactoperoxidase With ABTS as Chromogens, *Biochemical and Biophysical Research Communications*, 67 (1975) 1307-1312.

[20] Kalin R., Köksal Z., Bayrak S., Gerni S., Ozyürek I.N., Usanmaz H., Gülçin İ., Molecular docking and inhibition profiles of some antibiotics on lactoperoxidase enzyme purified from bovine milk, *Journal of Biomolecular Structure and Dynamics*, (2020) 1-10.

[21] Atasever A., Ozdemir H., Gulcin I., Kufrevioglu O.I., One step purification of lactoperoxidase from bovine milk by affinity chromatography, *Food Chemistry*, 136(2) (2013) 864–870.

[22] Özyürek I.N., Kalın R., Özdemir H., D-Penisilamin, D-Penisilamin disülfit ve N-Asetil-D-penisilamin'in Laktoperoksidaz Enzim Aktivitesi Üzerine İnhibisyon Etkileri, *Journal of the Institute of Science and Technology*, 10(2) (2020) 1146-1153.

[23] Lineweaver H., Burk D.J., The Determination of Enzyme Dissociation Constants, *Journal of the American Chemical Society*, 56 (1934) 658-66.

[24] Berg J., Tymoczko J.L., Stryer L., W. H. Freeman and Company ISBN, Biochemistry, (2002) 0- 7167-4955-6.

[25] Berg J.M., Tymoczko J.L., Stryer L., Biochemistry 6th ed. New York: W. H. Freeman and Company (2007).

[26] Hussain S., Slikker J.R., W., Ali S.F., Age-related changes in antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione in different regions of mouse brain, *International Journal of Developmental Neuroscience*, 13(8) (1995) 811-817

[27] Sievers G., Structure of milk lactoperoxidase. A study using circular dischroism and difference absorption sperctroscopy, *Biochimica et Biophysica Acta*, (1980) 624: 249

[28] Singh AK., Singh N., Sharma S., Kaur P., Srinivasan A., Singh T.P., Crystal structure of lactoperoxidase at 2.4A resolution, *J. Mol. Biol*., 376(1) (2007) 1060– 1075.

[29] Jacob B.M., Antony K.E., Sreekumar B., Haridas M., Thiocyanate mediated antifungal and antibacterial property of goat milk lactoperoxidase, *Life Sciences*, 66 (25) (2000) 2433-2439.

[30] Wang G., He D., Li X., Li J., Peng Z., Design, synthesis and biological evaluation of novel coumarin thiazole derivatives as α-glucosidase inhibitors, *Bioorganic Chemistry*, 65 (2016) 167-174.

[31] Demir Y., Taslimi P., Koçyiğit Ü.M., Akkuş M., Özaslan M.S., Duran H.E., Beydemir Ş., Determination of the inhibition profiles of pyrazolyl–thiazole derivatives against aldose reductase and α-glycosidase and molecular docking studies, *Archiv der Pharmazie.*, 353(12) (2020) 2000118.

[32] Barrett N.E., Grandison A.S., Lewis M.J., Contribution of the lactoperoxidase system to the keeping quality of pasteurized milk, *Journal of Dairy Research.*, 66(1) (1999) 73-80.

[33] Reiter B., Härnulv G., Lactoperoxidase antibacterial system: natural occurrence, biological functions and practical applications, *Journal of Food Protection*, 47(9) (1984) 724-732.

[34] Kussendrager K.D., Van Hooijdonk A.C.M., Lactoperoxidase: physico-chemical properties, occurrence, mechanism of action and applications, *British Journal of Nutrition*, *84*(S1) (2000) 19-25.

[35] Köksal Z., Kalin R., Camadan Y., Usanmaz H., Almaz Z., Gülçin İ., Ozdemir H., Secondary sulfonamides as effective lactoperoxidase inhibitors, *Molecules*, 22(6) (2017) 793.

[36] Song J.U., Choi S.P., Kim T.H., Jung C.K., Lee J.Y., Jung S.H., Kim G.T., Design and synthesis of novel 2-(indol-5-yl) thiazole derivatives as xanthine oxidase inhibitors, *Bioorganic & Medicinal Chemistry Letters*, 25(6) (2015) 1254-1258.

[37] Kılıcaslan S., Arslan M., Ruya Z., Bilen Ç., Ergün A., Gençer N., Arslan O., Synthesis and evaluation of sulfonamide-bearing thiazole as carbonic anhydrase isoforms hCA I and hCA II, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 31(6 (2016) 1300-1305.

[38] Kalın R., Laktoperoksidaz Sistemine Karşı Sesamolün İnhibisyon Kinetiği, *Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi*, 9(4), 389-395.