

Alterations of aldose reductase and superoxide dismutase activities by some chromonyl-2, 4-thiazolidinedione derivatives

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Summary: Persistent hyperglycemia in diabetes mellitus (DM) leads to progression of secondary complications, such as neuropathy, nephropathy and retinopathy, which cause irreversible damage once initiated. During states of hyperglycemia, the polyol pathway has increased activity. As a result of the increased polyol pathway activity and the overutilization of NADPH by the enzyme aldose reductase (AR), a number of other homeostatic mechanisms are compromised. In view of the complex metabolic changes induced by hyperglycemia in which AR is critically involved and the prominent role performed by oxidative stress, derivatives endowed with dual activity as AR inhibitors (ARIs) and antioxidant agents could thus represent a promising way forward in the search for useful drugs to treat long-term complications associated with DM. However, many of the clinically tested aldose reductase inhibitors (ARIs) proved to be inadequate as drug candidates because of adverse pharmacokinetics, toxic side effects or low efficacy. For these reasons, nowadays the design of the ARIs which do not cause side effects is still carried on. In our study, AR enzyme was purified from bovine lens tissues. Then, the probable effects of 17 different chromonyl-2,4- thiazolidinedione derivatives on aldose reductase and superoxide dismutase (SOD) enzymes were investigated. Depending upon the results, compounds named **1** and **8** showed the best AR inhibitory activity at the ratio of 52.56% and 58.73 %, respectively. The most activator effect on SOD was found at the ratio of 24.74 % in compound **7**.

Key words: Aldose reductase, diabetes, inhibition, superoxide dismutase, TZD derivatives.

Bazı kromonil-2,4-tiyazolidindiyon türevleri ile aldoz redüktaz ve süperoksit dismutaz aktivitelerindeki değişiklikler

Özet: Diabetes Mellitus (DM) hastalığındaki uzun süreli hiperglisemi nöropati, nefropati ve retinopati gibi geri-dönüşümsüz sekonder komplikasyonlara neden olmaktadır. Hiperglisemi sırasında polyol yolağının aktivitesi artmaktadır. Artan polyol yolağı aktivitesi ve aldoz redüktaz (AR) tarafından NADPH'ın aşırı tüketimi diğer homeostatik mekanizmaları etkilemektedir. AR'ın dahil olduğu hiperglisemiye bağlı kompleks metabolik değişiklikler ve oksidatif stres sonucunda oluşacak olan diyabetik komplikasyonların tedavisinde hem AR inhibitör etkisi olan hem de antioksidan özelliği bulunan dual etkili ajanların kullanılabilmesi olasılığı, araştırmalar ve tedavi açısından umut vericidir. Bunun yanısıra, klinik olarak denenmiş olan pekçok AR inhibitörünün (ARI) olumsuz farmakokinetikleri, toksik yan etkileri ve yetersiz etkileri nedeni ile ilaç adayları olarak yetersiz oldukları tespit edilmiştir. Bu nedenle, günümüzde hala yan etkisi az olan ARI leri üzerinde çalışmalar devam etmektedir. Çalışmamızda, AR enzimi sığır lenslerinden saflaştırılmıştır. Daha sonra, 17 farklı kromonil-2,4-tiyazolidindiyon türevlerinin AR ve süperoksit dismutaz (SOD) enzim aktiviteleri üzerine olası etkileri incelenmiştir. Sonuçlara göre; **1** ve **8** numaralı bileşikler en iyi ARI aktivitesini sırası ile % 52.56 ve % 58.73 oranları ile göstermiştir. SOD üzerinde ise **7** nolu bileşik % 24.74 oranında aktivatör etki göstermiştir.

Anahtar sözcükler: Aldoos redüktaz, diyabet, inhibisyon, süperoksit dismutaz, TZD türevleri.

Introduction

Diabetes mellitus (DM) is a chronic disease caused by deficiency in production of insulin by pancreas, and by resistance to insulin's effects. Diabetic complications, such as neuropathy, retinopathy, nephropathy or cataract, are serious and disabling pathologies associated with DM (2). Hyperglycemia is a typical condition of DM and plays a crucial role in the development and advancement of these complications which arise from acute and

reversible changes in cellular metabolism as well as from irreversible long-term damage in biological macromolecules. Among the numerous mechanisms triggered by the chronic exposure to high levels of glucose the ones that are very clearly related to hyperglycemia are increased advanced glycation end product (AGE) formation, glucose auto-oxidation, the activation of protein kinase C (PKC) isoforms and increased aldose reductase (AR)-related polyol pathway

(13). Aldose reductase (AR; EC 1.1.1.21), a member of the NADPH dependent aldo-keto reductase family, is a cytosolic, monomeric oxidoreductase enzyme that catalyzes the conversion of glucose to sorbitol in the first and rate-limiting step of the polyol pathway of glucose metabolism (9, 10, 22). AR enzyme not only reduces glucose to sorbitol but also decreases the formation of toxic aldehydes (4, 11, 16). Under normal conditions, aldose reductase has a low affinity for glucose, with a very small percentage of total glucose (less than 3%) converted to sorbitol via this pathway. Under hyperglycemic conditions, there is an increase in the enzymatic activity and production of sorbitol, resulting in an overall decrease in NADPH (15). Since the increased polyol pathway flux leads to accumulation of sorbitol in the lens fiber, lead to osmotic imbalances and oxidative stress that result in fiber cell swelling, liquefaction, and eventually development of cataracts (21). Thus, reduction of the hyperglycemia induced polyol pathway flux by AR inhibitors (ARIs) could be a potential therapeutic opportunity in the treatment and prevention of diabetic complications (8).

In addition, superoxide dismutases (SODs) are very important antioxidant enzymes. They catalyze the dismutation reaction of superoxide radical into molecular oxygen and hydrogen peroxide in all oxygen metabolising organisms, and therefore are an essential part of the antioxidant defence system. An excessive build up of free radicals often leads to oxidative stress, resulting in the oxidation of lipids, sugars, proteins and DNA. The oxidative stress caused by free radicals may relate to aging and diseases, including cancer, cardiovascular disease, neurodegenerative disease and diabetes (20). Thus SODs, and especially MnSODs, play a crucial role in maintaining health and in the prevention of diseases caused by free radicals (7).

Previously, we reported the synthesis and antidiabetic activity of some chromonyl-thiazolidinedione derivatives (6). Some of them have been shown to have good antidiabetic activities. As part of this ongoing research, herein, the probable inhibitory effects of these compounds on aldose reductase enzyme and the probable activator effects on superoxide dismutase enzyme were investigated on the purpose of both preventing/delaying formation notably of cataract and the other long term complications of diabetes (Tables 1-2).

Materials and Methods

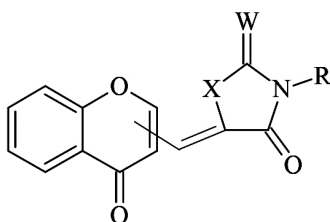
Bovine lenses were used for experiments. Bovines were received standard diet. AR enzyme was isolated from the lens tissues and enzyme activity as determined following the isolation. All the enzyme experiments were performed in triplicate. Procedures involving the animals and their care conformed to institutional guidelines, in

compliance with national and international laws and guidelines for the use of animals in biomedical research.

The AR enzyme was isolated by a method (Cerelli et al. 1986) described below. 60 bovine lenses, were thawed on ice and homogenized with 3 volumes of distilled water, homogenate were centrifugated at 10,000 xg for 20 min. Saturated ammonium sulfate was added to the supernatant for 40 % saturation. The thick suspension was stirred for 15 min, and, was centrifugated at 10,000 xg for 20 min. The inert protein left in the supernatant was removed by increasing the ammonium sulfate concentration to 50 % saturation followed by centrifuging the mixture at 10,000 xg for 20 min. The aldose reductase enzyme was precipitated from the 50 % saturated solution by adding powdered ammonium sulfate to 75 % saturation and was recovered by centrifugation at 10,000 x g for 20 min. Protein concentration was measured by the method of Bradford (1976) (3) using bovine serum albumin as a standard. Aldose reductase enzyme activity of the freshly prepared supernatant was assayed spectrophotometrically. The activity determining the decrease in NADPH concentration at 340 nm by a UV-1700 Visible spectrophotometer (5). The enzyme was dissolved in 5 ml 0,05M NaCl solution. 840 µg protein was added to a quartz cuvette which contains 0,1 ml phosphate buffer (0,067M, pH:6,2), 0,1ml NADPH (2 x 10⁻⁵ M final concentration), 0,1 ml of the test drug (10⁻⁴ M solutions prepared in 50 % DMF and 50 % methanol) and 2,3 ml distilled water to obtain final volume 2,9 ml solution. The reaction is started by the adding of 0,1 ml DL-glyceraldehyde (5 x 10⁻⁵ M final concentration) to the cuvette and the decrease in NADPH concentration was recorded at 340 nm for 5 minutes at 37°C. Readings were taken at intervals in the periods when the changes in absorbance were linear. The results represent three individual experiments.

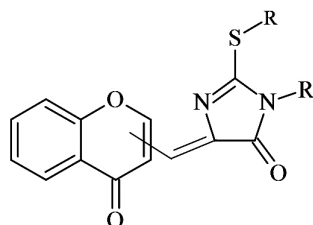
In the experiments, as well as the aldose reductase enzyme, the bovine lens was used. SOD enzyme has been isolated from lens tissues, and then activity of the enzyme was determined. For isolation, lenses taken from the bovine eyes which stored at -80°C weighted, and then were homogenized by adding 3 volumes phosphate buffer (pH 7.4). Homogenized tissue centrifuged at 10,000 x g and +4°C for 20 minutes. As described in our previous paper (20) supernatant which obtained by centrifugation was used to determination of superoxide dismutase enzyme activity. Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was measured by the method of Kostyuk and Potapovich (1989) (12) in which quercetin is used as the substrate after suitable dilution. The SOD enzyme activity calculations were made by using a rate of standard solutions absorbance in 20 minute to quercetin absorbance. The effect of compounds 1-17 on SOD enzyme is calculated with the aid of a standard graph of SOD.

Table 1. Structures of TZD derivatives and effects of chromonyl compounds (1-13) on AR SOD enzyme activities
 Tablo 1. TZD türevlerinin yapıları, kromonil bileşiklerinin (1-13) AR enzim inhibisyonu ve SOD aktivasyon aktiviteleri



Code	Position	X	W	R	Inhibition of AR (%)	Activation of SOD (%)
1	3	S	O	H	52.56 ± 3.09	No activation
2	3	NH	O	H	No inhibition	6.50 ± 3.10
3	3	NH	S	H	No inhibition	No activation
4	3	S	O	CH ₃	7.57 ± 1.87	No activation
5	3	S	O	C ₂ H ₅	No inhibition	14.8 ± 1.28
6	3	N-CH ₃	O	CH ₃	3.07 ± 2.12	11.20 ± 6.00
7	3	NH	O	C ₂ H ₅	1.66 ± 3.98	24.74 ± 2.95
8	2	S	O	H	58.73 ± 2.40	No activation
9	2	NH	O	H	23.81 ± 0.00	No activation
10	2	NH	S	H	11.74 ± 5.42	No activation
11	2	S	O	CH ₃	6.67 ± 3.81	3.84 ± 1.76
12	2	S	O	C ₂ H ₅	No inhibition	8.85 ± 2.00
13	2	N-CH ₃	O	CH ₃	No inhibition	No activation

Table 2. Structures of TZD derivatives and effects of chromonyl compounds (14-17) on AR SOD enzyme activities
 Tablo 2. TZD türevlerinin yapıları, kromonil bileşiklerinin (14-17) AR enzim inhibisyonu ve SOD aktivasyon aktiviteleri



Code	Position	X	W	R	Inhibition of AR (%)	Activation of SOD (%)
14	3	-	-	CH ₃	No inhibition	No activation
15	3	-	-	C ₂ H ₅	No inhibition	No activation
16	2	-	-	CH ₃	0.94 ± 5.25	No activation
17	2	-	-	C ₂ H ₅	3.14 ± 3.31	No activation

Compounds 1-3 and 8-10 were prepared via the Knoevenagel reaction in the presence of sodium acetate/glacial acetic acid between the 2,4-thiazolidinedione/2,4-imidazolidinedione/2-thioxo-imidazolidine-4-one and 2/3-formyl chromones. Compounds 4-7 and 11-17 were prepared by alkylation of compounds 1-3 and 8-10 with methyl / ethyl iodide in the presence of anhydrous sodium carbonate/ dimethyl formamide (DMF). The synthesis and antidiabetic activities of these compounds were reported in our previous paper (6, 14). All other chemicals used were analytical grade.

Results and Discussion

A variety of structurally different compounds have already been identified as potent in vitro ARIs (4, 13, 17). However, many of the clinically tested ARIs proved to be therapeutically inadequate because of adverse pharmacokinetics, toxic side effects or low efficacy. They can be classified into three general groups based on their structures: acetic acid derivatives (e.g. tolrestat and epalrestat), cyclic imides (especially spirohydantoin, e.g. sorbinil) and phenolic derivatives (e.g. quercetin). In our study, the synthesized compounds were obtained as a

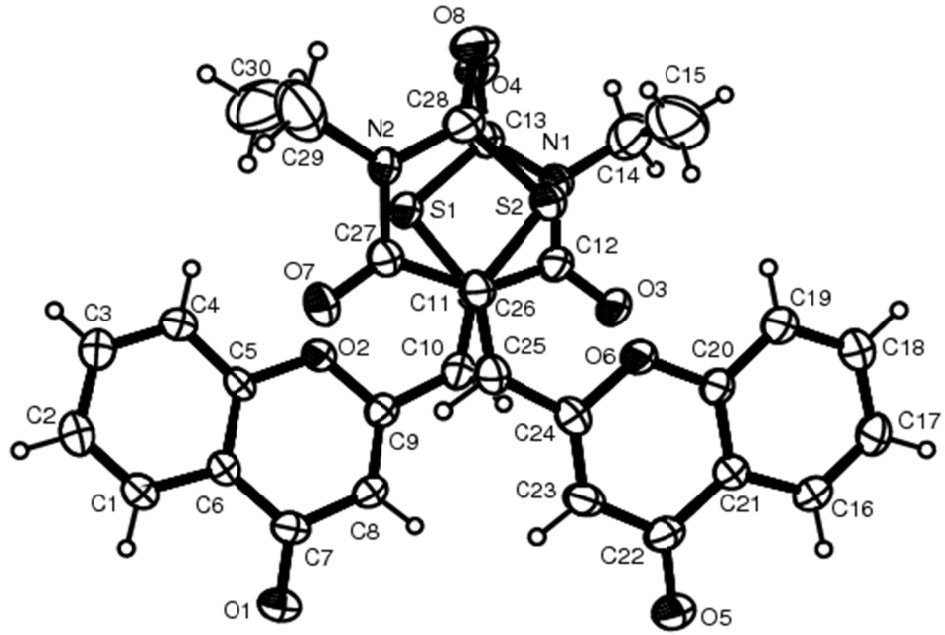


Figure 1. Molecular structure of the title compound with atom-numbering scheme. The thermal ellipsoids are drawn at the 40% probability level.

Şekil 1. Ana bileşiğin atom numaralandırması ile işaretli moleküler yapısı. Termal elipsoidler % 40 olasılıklı olarak çizilmiştir.

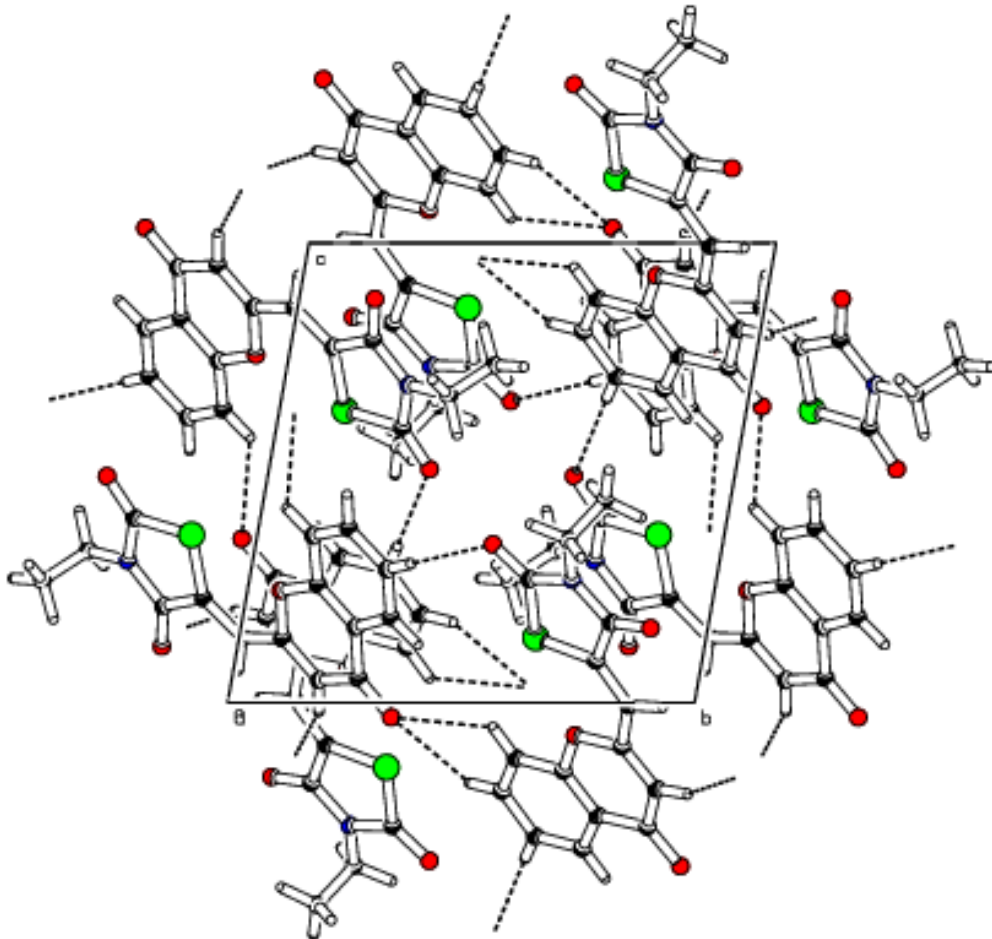


Figure 2. The crystal packing of the title compound along a-axis direction.
Şekil 2. Ana bileşiğin a-ekseni yönündeki kristal paketlenmesi.

result of condensation of chromen ring, which is also found in quercetin with 2,4-thiazolidinedione/2,4-imidazolidinedione/2-thioxo-imidazolidine-4-one rings, which are isostere of rhodanine in epalrestat structure. In these compounds, 2,4-thiazolidinedione/2,4-imidazolidinedione/2-thioxo-imidazolidine-4-one rings are linked to 2nd or 3rd positions of chromen ring. In the literature it was reported that in reactions using unsubstituted imidazolidinediones and benzaldehydes or 3-substituted thiazolidinedione and 3-formyl chromone in an acidic medium, the main product was the Z isomer (15, 18). The coupled ¹³C NMR study of arylidene thiazolidinediones and imidazolidinediones also showed that only the Z isomer was formed (1, 19). After the appropriate crystal of the compound 12 was obtained, X-ray analysis was performed and the compound 12 (CCDC deposition number: CCDC-873382), was found to be Z isomer, as well (Figure 1). It was also observed that crystal packing of the compound 12, whose asymmetric unit contains two independent molecules, is stabilized by C-H...O intra- and intermolecular hydrogen bond interactions (Figure 2).

The probable inhibitory effects of 17 different chromonyl thiazolidinedione derivatives were investigated on the AR enzyme, a member of aldo-keto reductase family, which plays a particular role in the formation of cataract lens in diabetic complications. The probable activatory effects of these compounds on SOD enzyme were investigated, as well. Depending upon the results the best aldose reductase inhibitory effect was found at the ratio of 58.73 % in compound 8. Among these inhibitors, in compound 1 which the TZD ring is linked to 3rd position of chromen ring, was observed 52.56% inhibition while, compound 8 with TZD ring in 2nd position of chromen ring showed inhibition of 58.73% ratio. Compound 4 which was derived from compound 1 as a result of methylation of the nitrogen atom on the TZD ring, showed 7.57% inhibition while compound 11 obtained from compound 8 by methylation of the nitrogen atom on the TZD ring, has shown 6.67% inhibition. Compounds 6 and 7 were obtained by methylation and ethylation of compound 2 showed 3.07% and 1.66% inhibition values, respectively. Compounds 16 and 17 which were derivated by methylation and ethylation of 2-thioxo-imidazolidine-4-one ring in compound 10 showed 0.94% and 3.14% inhibition ratios, respectively. On the other hand, the best activator effect on SOD enzyme was found at the ratio of 24.74 % in compound 7. Compounds 5 and 6 increased SOD enzyme activity by the ratio of 14.8% and 11.20%, respectively and the lack of inhibitory effect on SOD enzyme activity is a desired condition.

As a result, in the synthesized compounds, we observed that presence of TZD ring which is isostere of rhodanine ring in epalrestat shows inhibitory effect on AR while 2,4-imidazolidinedione and 2-thioxo-imidazolidine-4-one rings dose not play a significant role in inhibition of the enzyme. Also, it has been detected that, protection of acidic hydrogen of nitrogen atom on TZD ring is important for inhibition of the AR enzyme. On the other hand, in our compounds we did not observe any correlations between AR enzyme inhibition and SOD activation. Based on these results, we might design and synthesize more selective and effective AR inhibitors which will not affect its detoxification role but increase its antioxidant capacity.

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