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In Vitro Aldose Reductase Inhibitory Activity of Some 2,4-Thiazolidinedione Derivatives

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ABSTRACT

Aldose reductase (ALR2; AR; EC 1.1.1.21) is the first enzyme of the polyol pathway, which reduces glucose to sorbitol, in the presence of NADPH. The pathway is completed by sorbitol dehydrogenase, which catalyzes the NAD-linked oxidation of sorbitol to fructose. Synthesis and accumulation of sorbitol in cells due to increased aldose reductase activity is one of the main causes of diabetic complications. The aim of our study is to determine the potential inhibitory effect of some 2, 4-TZD derivatives on aldose reductase enzyme activity. Aldose reductase enzyme is isolated from bovine lenses and the activity of the enzyme is determined by spectrophotometric method. In our study 13 thiazolidinedione (TZD) derivatives which are containing benzyl and phenacyl groups were studied. Maximum inhibitory activity was found with 2,4- TZD-N-unsubstituted compound (86 %). Compound **13**, unsubstituted compound which was showed the maximum inhibitory activity, will be the key formula of our future studies.

Keywords: Aldose reductase, polyol pathway, 2,4- thiazolidinedione derivatives

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INTRODUCTION

The Aldose reductase (AR) is the first enzyme of the polyol pathway[1], which reduces glucose to sorbitol is demonstrated to play an important role not only in the cataract formation but also in the pathogenesis of diabetic complications such as neuropathy, nephropathy, and retinopathy[2]. Elevated glucose concentration in blood activates the polyol pathway, through AR enzyme[3] (Figure 1). The excess accumulation of intracellular sorbitol is linked to the pathogenesis of diabetic complications[4-6] such as cataract formation. Prevention of sorbitol accumulation by inhibiting an AR activity might be an effective treatment[7-9]. AR inhibitors (ARIs) attenuate diabetic complications in several tissues like lens, retina, kidney, blood vessels, striated muscle and peripheral nerve.

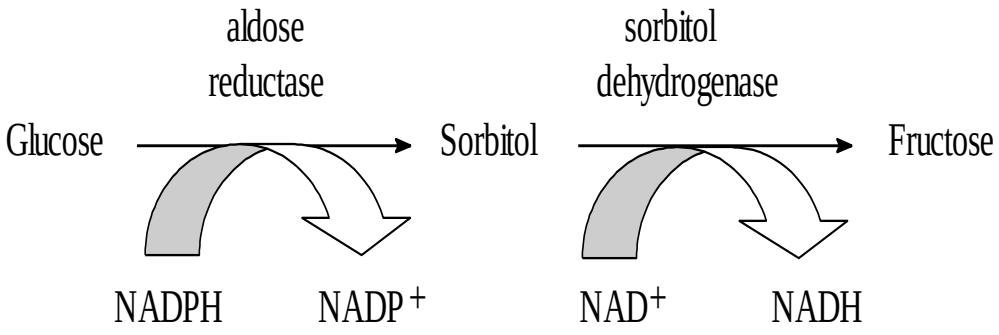


Figure 1: Polyol pathway

Although a large number of synthetic ARIs have been shown to inhibit the enzyme and have been tested in clinical trials, the clinical efficacy of these compounds were not successful and also some of them were showed side effects. Sorbinil, an extensively studied ARI, induced hypersensitivity reactions. Other promising ARIs, such as tolrestat, zopolrestat, zenarestat and ponalrestat were also withdrawn from clinical trials because of their side effects[10]. On the other hand, number of structurally diverse synthetic ARIs has been studied *in vivo* in our laboratory, to clarify their effectiveness for prevention of cataract formation by inhibition AR[11-16].

Thiazolidinediones (TZDs) are a class of antidiabetic drugs, which improves glycemic control in type 2 diabetic patients' skeletal muscles, livers, and adipose tissues by activating the peroxisome proliferator–activated receptor γ[17, 18].

TZD ring is the common structure in all TZD derivatives which the divergent molecular moieties are attached. The TZD ring is related with antihyperglycemic TZD action[19]. There is a great interest in 2,4-TZD derivatives as ARIs[20,21]. They can be viewed as hydantoin bioisosters. They are potentially free of the hypersensitivity reactions which are linked to the presence of hydantoin system. In our study, we decided to test 13 TZD derivatives' potential AR inhibition capacity.

MATERIALS AND METHOD

Biological activity studies

Animals

Bovine lenses were obtained from 600-800 kg bovines. They were received standard diet. 30 bovines were sacrificed and lens tissues were discarded. AR enzyme was isolated from lens tissues. After the isolation of the enzyme, AR activity was determined. All the enzyme experiments were performed 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.

Isolation of the aldose reductase enzyme

The AR enzyme was isolated from bovine lenses: 60 pooled lenses, were thawed on ice and homogenized with 3 volumes of distilled water, homogenate was centrifuged at 10.000xg for 20 min. Saturated ammonium sulfate was added to the supernatant for 40% saturation. The thick suspension was stirred for 15 min, and, was centrifuged at 10.000xg 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 10.000xg for 20 min. The AR enzyme was precipitated from the 50 % saturated solution by adding powdered ammonium sulfate to 75 % saturation and was recovered by centrifugation at 10.000xg for 20 min. Protein concentration was measured by the method of Bradford[22] using bovine serum albumin as a standard.

Determination of Aldose Reductase Activity

AR activity of the freshly prepared supernatant was assayed spectrophotometrically by determining the decrease in NADPH concentration at 340 nm by a UV-1700 visible spectrophotometer[23]. DL-glyceraldehyde was used as a substrate. The enzyme was dissolved in 5 ml 0.05 M NaCl solution and 0.2 ml was added to a quartz cuvette containing 0.1 ml phosphate buffer (0.067 M, pH:6.2), 0.1 ml NADPH (2×10^{-5} M final concentration), 0.1 ml of the test drug (10^{-4} M solutions prepared in 50 % DMF and 50 % Metanol) and 2.3 ml distilled water to obtain 2.9 ml solution. The reaction is started by the adding of 0.1 ml DL-glyceraldehyde (5×10^{-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. For calculating IC_{50} values experiments using drug concentrations of 10^{-4}, 10^{-5} and 10^{-6} M were used. The results represent three individual experiments.

Chemistry

Melting points were measured by an Electrothermal 9100 type apparatus (Electrothermal Engineering, Essex, UK. All instrumental analyses were performed in Central Laboratory of Pharmacy Faculty of Ankara University. ^{1}H NMR spectra was measured with a VARIAN Mercury 400 FT-NMR spectrometer (Varian Inc, Palo Alto, CA, USA) in DMSO-d_6. All chemical shifts were reported as δ (ppm) values. Elementary analyses were performed on a Leco CHNS 932 analyzer (Leco, St. Joseph, USA) and satisfactory results ± 0.4 % of calculated values (C, H, N) were obtained. The compounds **1-12** were synthesized according to the literature (24). The chemical reagents used in synthesis were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA).

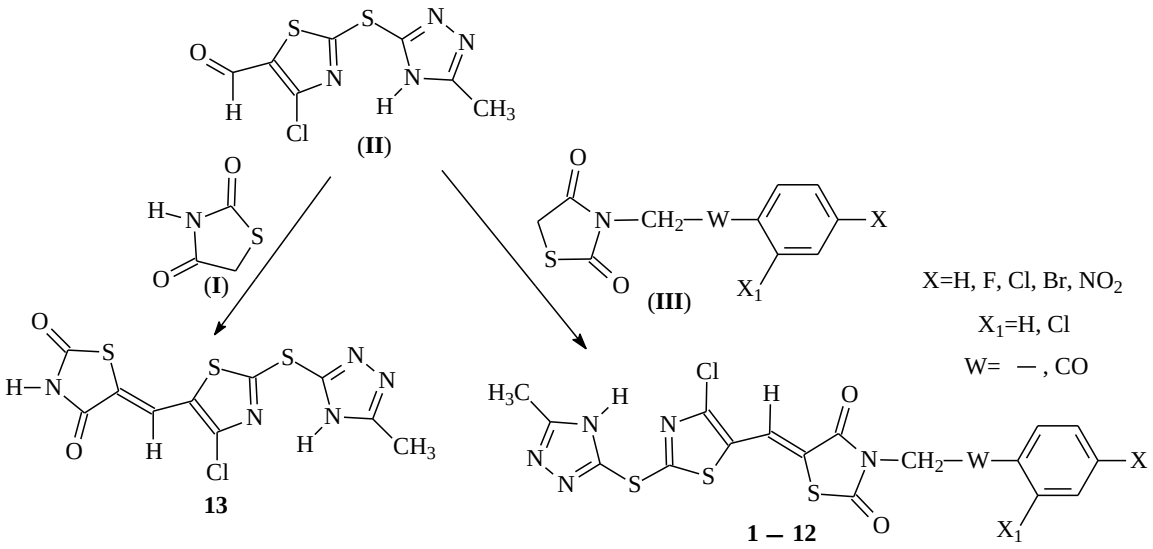
Synthesis of 5-[4-chloro-2-(5-methyl [1, 2, 4] triazol-3-ylsulfanyl)-thiazol-5-ylmethylene]-thiazolidine-2, 4-dione (**13**)

A mixture of 4-chloro-2-(5-methyl[1,2,4]triazol-3-ylsulfanyl)-thiazol-5-carbaldehyde (**IV**) (0.15 g, 0.58 mmol) and 2,4-TZD (0.07 g, 0.58 mmol) was heated at 100° C in the presence of 0.5 ml glacial acetic acid and sodium acetate (0.08 g, 0.58 mmol) for 5 h. The crude product was crystallized from dimethylformamide-ethanol. Yield: 0.103 g, 49.83 %, m.p.: 288° C, ^{1}H-NMR, δ ppm (DMSO-d_6): 2.40 (s, 3H, CH_3), 7. 53 (s, 1H, =CH), 14.44 (s, 1H, NH); Anal. for $C_{10}H_6ClN_5O_2S_3.0{,}5H_2O$: Calc. C: 32.60, H: 1.90, N:19.02, S:26.08; Found C: 32.47, H: 1.87, N:18.89, S:25.92

RESULTS AND DISCUSSION

Thiazolyl-2, 4-TZD compounds **1-13** were synthesized according to the synthetic pathway described in Scheme 1. 4-Chloro-2-(5-methyl[1,2,4]triazol-3-ylsulfanyl)-thiazole-5-carbaldehyde (**II**) were obtained by 2,4-dichlorothiazole-5-carbaldehyde with 5-methyl[1,2,4]triazole-3-thiol in sodium carbonate / acetonitrile[24].

The condensation of **II** with 2,4-TZD (**I**) / substituted benzyl-2,4-thiazolidinediones and phenacyl-2,4-TZD (**III**) in the presence of sodium acetate / glacial acetic acid by Knoevenagel reaction, led to 5-methyl[1,2,4]triazolo-2,4-TZD (**13**), 5-methyl[1,2,4]triazolo-substituted benzyl (**1-6**) and phenacyl-2,4-TZDs (**7-12**), respectively.

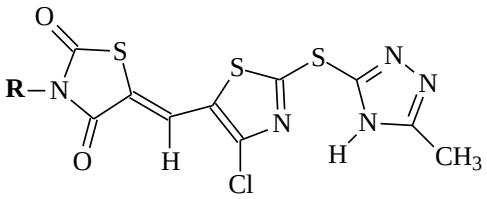


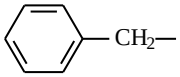
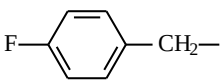
Scheme 1: General synthesis of compounds 1-13.

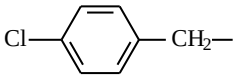
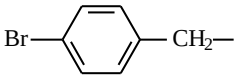
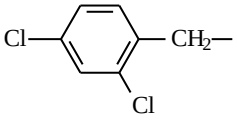
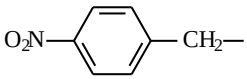
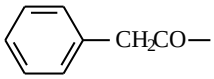
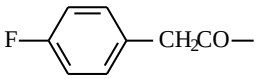
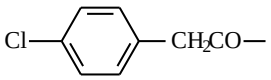
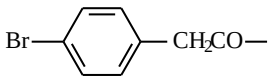
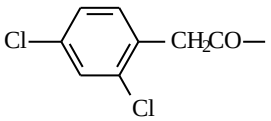
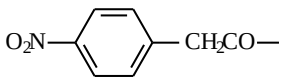
It was reported that by using unsubstituted imidazolidinediones and benzaldehydes in acidic medium, the main product was the Z isomer[25]. In this study, only one isomer of the compounds was obtained, except **3** and **4** which have the chloro and bromo substituents at the para-position of the phenyl ring at N-3 position of the TZD ring, respectively. Methyne protons of the compounds **1-8, 11, 12** were seen at 7.65-7.84 ppm as a singlet. Methyne protons of **9** were observed as a singlet at 7.81 ppm (0.75H) and 8.31 ppm (0.25H). Methyne protons of **10** were seen as a singlet at 7.80 ppm (0.75H) and 8.30 ppm (0.25H)[24]. New compound **13**'s methyne proton was seen as a singlet at 7.53 ppm.

In this study we determined an AR inhibitory activity of these TZD derivatives, which are containing hydrogen, benzyl and phenacyl groups on N-3 position of the 2, 4-TZD ring system (Table1).

Table 1: AR inhibition of 2,4-TZD compounds *



Compounds	R	Inhibition (%)**
1		No inhibition
2		12.70 ± 7.732

3		No inhibition
4		No inhibition
5		0.56 ± 4.81
6		14.55 ± 1.29
7		0.55 ± 3.97
8		0.55 ± 8.77
9		No inhibition
10		4.96 ± 1.12
11		1.65 ± 1.10
12		7.55 ± 0.65
13	-H	86.74 ± 2.21(IC_{50}: 0.0972 µM)

*Values represent the mean ± S. D. of three individual experiments.

**IC_{50} (µM) or % inhibition at the given concentration

DISCUSSION

The inhibition study was performed by using 10^{-4}M concentration stock solution of each drug. Compound **13,** which has unsubstituted 2, 4- TZD ring, was showed significant inhibitory activity (86.74 ± 2.21 %). On the other hand, 2, 4-TZD-N-benzyl substituted compounds (compounds **1-6**) were showed less-poorly or no inhibitory activity, and 2, 4-TZD-N-phenacyl substituted compounds (compounds **7-12**) were also showed slight or no inhibitory activity (Table 1). Compound **13** is a non-substituted TZD compound with imidic structure which gives acidic character to compounds. This imidic structure has given an inhibitory property to compound **13**. Other compounds (compounds **1-12**) that have been substituted with benzyl or phenacyl group instead of hydrogen atom on 2, 4-TZD ring, possess a slight or no inhibitory effect. Thus, we can

say that the potent inhibitory effect of our synthesized compounds might depend on having an acidic proton on the TZD ring.

In this study, we aimed to find more effective compounds as ARIs by adding benzyl and phenacyl groups at N-3 position of the 2, 4-TZD ring. Surprisingly, it was observed that compounds with benzyl and phenacyl groups at N-3 position of the 2, 4-TZD ring were showed less inhibitory effect than their unsubstituted analog (see Table 1). In this series, only unsubstituted analog (compound **13**) showed the highest ARI inhibitory effect. Finally, it can be considered that the acidic hydrogen (imidic or acidic group) instead of lipophilic groups at N-3 position of the 2, 4-TZD ring was played a noticeable role for increasing the ARI effect.

CONCLUSION

In our study we determined the *in vitro* AR inhibitory activity of 2,4-thiazolidinedione-N-benzyl substituted (compounds **1-6**), 2,4-thiazolidinedione-N-phenacylsubstituted (compounds **7-12**) and 2,4-thiazolidinedione-N-unsubstituted compound (compound **13**). Novel synthesized compound **13** was showed the maximum inhibitory activity with the IC_{50} value 0.0972 µM among others. This compound will be the key formula of our future studies.

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