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In vitro aldose reductase inhibitory activity of some flavonyl-2,4-thiazolidinediones

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Abstract
Aldose reductase (AR) is implicated to play a critical role in diabetes and cardiovascular complications because of the reaction it catalyzes. AR enzyme appears to be the key factor in the reduction of glucose to sorbitol. Synthesis and accumulation of sorbitol in cells due to AR activity is the main cause of diabetic complications, such as diabetic cataract, retinopathy, neuropathy and nephropathy. Aldose reductase inhibitors have been found to prevent sorbitol accumulation in tissues. Numerous compounds have been prepared in order to improve the pharmacological prophile of inhibition of aldose reductase enzyme. In this study, seventeen flavonyl-2,4-thiazolidinediones (flavonyl-2,4-TZD) (**Ia–e, IIa–e** and **IIIa–g**) were tested for their ability to inhibit rat kidney AR. Compound **Ib** showed the highest inhibitory activity (88.69 ± 1.46%) whereas **Ia**, **IIa**, **IIIa**, **IIIb** also showed significant inhibitory activity (49.26 ± 2.85, 67.29 ± 1.09, 71.11 ± 1.95, 64.86 ± 1.21%, respectively).

Keywords: *2,4-thiazolidinediones, flavone derivatives, antidiabetic, aldose reductase, inhibition*

Introduction

The hyperglycemia observed in diabetes mellitus seems to be the primary instigator for the pathogenesis of long-term diabetic complications such as retinopathy, neuropathy, nephropathy, and cataracts [1,2]. The elevated glucose concentration in blood activates the polyol pathway (Figure 1), of which the first enzyme is AR reducing glucose into sorbitol [3,4]. As the excessive accumulation of intracellular sorbitol through the polyol pathway is linked to the pathogenesis of diabetic complications, prevention of sorbitol accumulation by inhibiting the AR activity would be an effective treatment [5,6].

Aldose reductase inhibitors (ARIs) have been shown to reduce tissue sorbitol accumulation in diabetic animals [7]. Studies have shown evidence which provides support for a major role for AR in the manifestations of various diabetic complications and it has become apparent that inhibitors of AR enzyme may be able to prevent, retard, or reverse the complications of chronic diabetes [8].

ARIs (Figure 2) can be grouped into two chemical classes: hydantoins (such as sorbinil) and carboxylic acids (such as epalrestat, tolrestat) [9]. Nevertheles, many of them have shown to be clinically inadequate because of adverse pharmacokinetics or toxic side-effects [10].

As a new class of antidiabetic agents 2,4-thiazolidinediones (2,4-TZDs) are differ markedly from other antidiabetic agents in that they are effective in normalizing glucose and lipid metabolism associated with insulin resistance and are therefore expected to be useful in the treatment of both type 2 diabetes mellitus and obesity [11–14]. Some of 2,4-TZDs such as pioglitazone, rosiglitazone (Figure 2) have been marketed for the treatment of type 2 diabetes. On the other hand, it was reported that some 2,4-TZDs have been patented as antihyperglycemic agents and also have an inhibitory effects on AR enzyme [9,13]. In addition, topical ARI,

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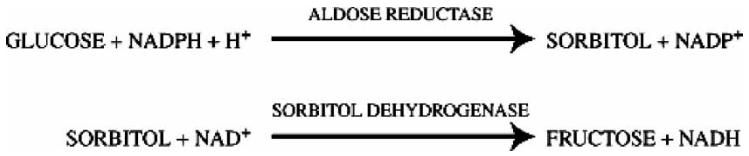


Figure 1. Polyol pathway.

CT-112 (5-[3-ethoxy-4-pentyloxyphenyl]-2,4-thiazolidinedione) improves corneal sensation in clinical cases [15] and corneal epithelial barrier function in galactose fed rats [16] There is a great interest in 2,4-TZD derivatives as ARIs [9,17], since they can be viewed as hydantoin bioisosters potentially free of the hypersensitivity reactions which are linked to the presence of the hydantoin system.

Flavonoids are polyphenols ubiquitously found in a wide variety of edible plants, fruits, nuts, seeds and plant-derived beverages, such as juice and tea [18]. They, also referred to as vitamin P [19], have been described as health-promoting, disease-preventing dietary supplements, and have many biological activities as antibacterial and antiviral agents [20]. They are extremely safe and associated with low toxicity, making them excellent candidates for chemopreventive agents [21]. In addition, it was reported that the health benefits of flavonoids are usually linked to two properties: (i) inhibition of certain enzymes such as xanthine oxidase, AR; and (ii) antioxidant activity [22].

In our previous studies, the synthesis and insulinotropic activity in INS-1 cells evaluation of flavonyl-2,4-TZD derivatives have been described. A significant insulinotropic effect was seen with some of the compounds [23–25].

In this study, we have determined the probable inhibitory effects of some flavonyl-2,4-TZD derivatives (Figure 2), already known to have antidiabetic activities, on rat kidney homogenates.

Materials and methods

Chemistry

The Compounds **Ia** [23], **IIa–b** [24], **IIIa** [25], a methylene group linker between the flavone and the 2,4-TZD rings were synthesized by dilithio-2,4-TZD with appropriate bromomethylflavone. The compounds **Ib–e** [23], **IIc–e** [24] and **IIIb–g** [25] were synthesized by Knoevenagel reaction of 2,4-thiazolidinediones / 2,4-imidazolidinediones / thiohydantoine with flavone-3′ / 4′ / 6-carboxaldehydes using acetic

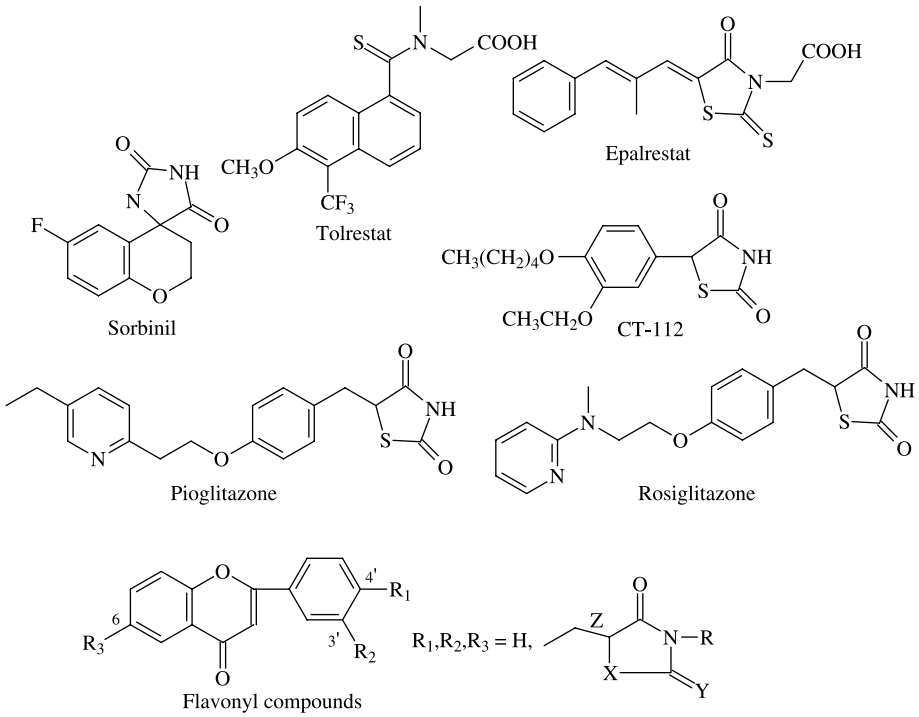


Figure 2. Formula of the compounds.

acid / sodium acetate mixture. Condensation products were N-alkylated with alkyl iodide in alkaline medium. All reagents were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA).

Biological activity studies

Animals. Male Albino rats weighing 200–250 g were used for experiments. They received standart diet. 30 rats were sacrified and kidney tissues were obtained. Aldose reductase activity was determined after isolation from kidney tissue. 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 aldose reductase enzyme. The aldose reductase enzyme was isolated by the method described below. 30 pooled kidney which were obtained from 200–250 g albino rats, were thawed on ice and homogenized with 3 volume of distilled water, followed by centrifugation at 10000 × g for 20 min. Saturated ammonium sulfate was added to the supernatant to 40% saturation. The thick suspension had been stirred for 15 min, followed by centrifugation at 10000 × g 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 10000 × g for 20 min. The aldose reductase enzyme was precipitated from the 50% saturated solution by adding powdered ammonuim sulfate to 75% saturation and was recovered by centrifugation at 10000 × g for 20 min. Protein concentration was measured by the method of Bradford [26] using bovine serum as the standard. Protein concentration is 7.18 ± 0.08 mg/mL.

Determination of aldose reductase activity. Aldose reductase 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. DL-glyceraldehyde was used as the substrate. The enzyme was dissolved in 10 mL 0.05 M NaCI and 0.1 mL was added to a quartz cuvette containing 0.2 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 (a 10^{-4} M stock solutions prepared in 50% DMF and 50% Methanol) and 2.4 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 min at 37°C. Reading were taken at intervals in the periods when the changes in absorbance were linear [27].

Results and discussion

In this study, the AR inhibitory effects of the 2,4-TZD series with acidic bioisoster heterocycles such as hydantoin and thiohydantoin substituted at 4′ / 3′ / 6 position of flavone nucleus which has a lipophilic appendage, were determined by using rat kidney homogenates. In our previous study, we have shown that compounds **Ia–e** [23], **IIa–e** [24]**, IIIa–g** [25] had a significant insulinotropic effect. On the basis of these biological results, we tried to identify whether these compounds has dual effect such as ARI activity besides antihyperglycemic effect.

The inhibition obtained by flavone derivatives were studied in vitro and the results are represented in Table I. The enzyme activity was assayed by spectrophotometrically monitoring NADPH oxidation which accompanies the reduction of D-L-glyceraldehyde used as a substrate. The inhibition study was performed merely by using a 10^{-4} M concentration of each drug, and IC_{50} values of compounds **Ia, Ib, IIa, IIIa, IIIb** were studied.

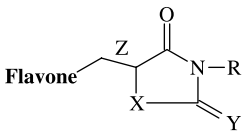
Compound **Ib,** containing 2,4-thiazolidinedione at 4′ position of the flavone ring was showed the highest inhibitory activity. The inhibition rate of **Ib** is 88.69 ± 1.46% inhibition and IC_{50} value is 0.432 ± 0.008 μM. **Ia**, **IIa**, **IIIa**, **IIIb** were also showed significant inhibitory activity, 49.26 ± 2.85, 67.29 ± 1.09, 71.11 ± 1.95, 64.86 ± 1.21% inhibition, respectively.

The inhibitory activity of isomeric 6-flavonyl-2,4-TZD compound **IIIb** is lower than **Ib** (Table I). The other isomeric 3′-flavonyl-2,4-TZD compound **IIc** (22.06 ± 0.19% inhibition) has slight inhibitory activity compared to **Ib** and **IIIb**.

Compounds **Ia**, **Ic**, **IIb**, **IIc** were showed moderate decrease in inhibitory activity, they are not potent as the **Ib**. Compound **Ic** which contains methyl substituted at N-3 position of TZD ring was showed slight inhibitory activity compairing with unsubstituted TZD compound **Ib**. Besides, it was shown that the inhibition rate were reduced in the compounds **IIb** and **IIIc** which were derived by the methylation of the N-3 position of TZD ring in **IIa** and **IIIb**, respectively. On the other hand, the inhibitory activity of N-methyl substituted flavonyl-TZD compound **Ic** is higher than N-unsubstituted TZD compound **IIc** that possesses similar inhibition rate with TZD-N-ethyl substituted compound **IIId**.

Our results were showed that, a methylene lipophilic appendage between the flavone core and the 2,4-TZD ring is a different critical requirement for aldose reductase inhibition. 3′-flavonyl compound **IIc** which contains methyne appendage between the flavone core and the 2,4-TZD ring was surprisingly

Table I. Aldose reductase inhibition by Flavonyl compounds*



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Code	Position	X	Z	Y	R	Inhibition (%)**
Ia	4′	S	Single bond	O	H	49.26 ± 2.85 (IC_{50}: 0.432 ± 0.58)
Ib	4′	S	Double bond	O	H	88.69 ± 1.46 (IC_{50}: 0.432 ± 0.008)
Ic	4′	S	Double bond	O	CH_3	34.13 ± 4.39
Id	4′	NH	Double bond	O	H	0.0 ± 0.0
Ie	4′	NH	Double bond	S	H	9.52 ± 2.19
IIa	3′	S	Single bond	O	H	67.29 ± 1.09 (IC_{50}: 0.687 ± 0.015)
IIb	3′	S	Single bond	O	CH_3	18.38 ± 1.58
IIc	3′	S	Double bond	O	H	22.06 ± 0.19
IId	3′	CH_3N	Double bond	O	CH_3	15.18 ± 4.11
IIe	3′	NH	Double bond	S	H	14.26 ± 5.39
IIIa	6	S	Single bond	O	H	71.11 ± 1.95 (IC_{50}: 0.643 ± 0.021)
IIIb	6	S	Double bond	O	H	64.86 ± 1.21 (IC_{50}: 0.683 ± 0.006)
IIIc	6	S	Double bond	O	CH_3	19.30 ± 1.03
IIId	6	S	Double bond	O	CH_2CH_3	21.90 ± 3.68
IIIe	6	NH	Double bond	O	H	13.19 ± 3.21
IIIf	6	CH_3N	Double bond	O	CH_3	14.72 ± 5.61
IIIg	6	NH	Double bond	S	H	19.00 ± 3.84

*Values represent the mean ± S. D. of three individual experiments. **IC_{50} (μM) or % inhibition at the given concentration

showed weak inhibitory activity compairing with the 4′and 6-flavonyl compound **Ib** and **IIIb,** respectively (Table I). On the other hand, compound **IIIa** has methyne and **IIIb** has methylene lipophylic appendage but their inhibition rates do not differ significantly. Therefore, we can say that the difference of the inhibitory capacity of the groups is not affected from the double or single bond between the flavone and TZD ring.

Flavonyl TZD compounds were also showed higher inhibitory activity than TZD isoster heterocyclic compounds **Id**, **Ie**, **IId**, **IIe**, **IIIe–g** (Table I). Surprisingly, 4′-flavonyl compound **Id** which has a hydantoine ring as sorbinil, did not inhibit AR. The results indicate that these flavonyl-2,4-TZD derivatives could display therapeutic potential in the preventation and treatment of diabetic complications.

It was reported that compounds possessed the essential structural requisites (an acidic proton, hydrogen-bond acceptor groups and an aromatic moiety) for aldose reductase inhibitory effect, in accordance with known pharmacophoric requirements [9,28,29]. An aromatic ring with the capability of hydrogen bonding ability also required to afford AR inhibitory activity. It is well known that the presence of an acidic functionality is an important requirement for all ARIs, since they interact, in their ionized form, with the active site of the enzyme [11,28,29] although many compounds may have inhibitory activity with the lack of carboxyl group such as topical ARI CT-112 (5-[3-ethoxy-4-pentyloxyphenyl]-2,4-thiazolidinedione). In our study, it was also found that all our tested compounds except **Id** had an inhibitory effect on AR enzyme at the given concentration even though they had not carboxylic acid group.

As a result, we may conclude that the inhibitory effect of compounds **Ia, Ib, IIa, IIIa, IIIb** might be depend on the acidic hydrogen of the 2,4-TZD imidic moiety and the capability of hydrogen bonding ability of flavone core as aromatic moiety required to afford AR inhibitory activity. In the light of these results, we are still continuing to evaluate TZD derivatives.

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