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Synthesis and biological activity of some new flavonyl-2,4-thiazolidinediones

Oya Bozdağ-Dündar [a,*], Eugen J. Verspohl [b], Net Daş-Evcimen [c], Rebecca M. Kaup [b], Katrin Bauer [b], Mutlu Sarıkaya [c], Begüm Evranos [a], Rahmiye Ertan [a]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandoğan, Ankara, Turkey
[b] Department of Pharmacology, Institute of Medicinal Chemistry, Münster University, Münster, Germany
[c] Department of Biochemistry, Faculty of Pharmacy, Ankara University, Tandoğan, Ankara, Turkey

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ABSTRACT

A new series of flavonyl-2,4-thiazolidinediones (**Va–c**, **VIa–c**) was prepared by Knoevenagel reaction. The synthesized compounds were tested for their ability to inhibit rat kidney aldose reductase (AR) and for their insulinotropic activities in INS-1 cells. Compound **Vb** was able to increase insulin release in the presence of 5.6 mmol/l glucose. Compounds **VIa–c** displayed moderate to high AR inhibitory activity levels. Particularly, compound **VIa** showed the highest AR inhibitory activity (86.57%).

1. Introduction

Aldose reductase (AR), the key enzyme of the polyol pathway, has been demonstrated to play important roles not only in the cataract formation in the lens but also in the pathogenesis of diabetic complications such as neuropathy, nephropathy, and retinopathy.[1] The elevated glucose concentration in blood activates the polyol pathway (Fig. 1), of which the first enzyme is AR reducing glucose into sorbitol.[2,3] 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.[4–6] There is some evidence that the blockade of AR can have beneficial effects in some diabetic complications.[7,8]

Although a large number of synthetic aldose reductase inhibitors (ARI) have been shown to inhibit the enzyme and have been tested in clinical trials, the clinical efficacy of these compounds is not satisfactory, and some of them have also shown deleterious 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 Other promising ARIs such as tolrestat, zopolrestat, zenarestat, and ponalrestat were also withdrawn from clinical trials.[9]

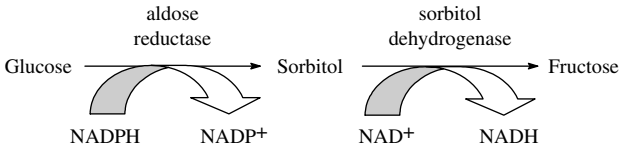

Figure 1. Polyol pathway.

On the other hand, a number of structurally diverse naturally occurring and synthetic ARIs have been studied in vivo to clarify their effectiveness for prevention of cataract formation as well as diabetic complications in experimental animals as well as in clinical trials. It is well established that natural products are an excellent source of chemical structures with a wide variety of biological activities.[1]

Flavonoids are polyphenols ubiquitously found in a wide variety of edible plants, fruits, nuts, seeds, and plant-derived beverages, such as juice and tea.[10] They are also called vitamin P[11] and have been described as health-promoting, disease-preventing dietary supplements.[12] They are extremely safe and associated with low toxicity, making them excellent candidates for chemopreventive agents,[13] and may exert an antihyperglycemic effect by promoting peripheral utilization of glucose or enhancing the sensitivity of insulin in diabetic animals.[14] In addition, it was reported that the health benefits of flavonoids are usually linked to two

* Corresponding author. Tel.: +90 312 2033077; fax: +90 312 2131081.
E-mail address: bozdag@pharmacy.ankara.edu.tr (O. Bozdağ-Dündar).

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properties: (i) inhibition of certain enzymes such as xanthine oxidase, AR; and (ii) antioxidant activity.[15]

Thiazolidinediones (TZDs) are a newer class of antidiabetic drugs, which improve glycemic control in type 2 diabetic patients by increasing insulin action in skeletal muscles, liver, and adipose tissue through activation of the peroxisome proliferator-activated receptor γ.[16,17] Following extensive evaluation of numerous compounds, several other agents were developed, including pioglitazone and rosiglitazone.

The structural characteristic common to all TZDs is a thiazolidinedione ring, to which divergent molecular moieties are attached. The thiazolidinedione ring is assumed to relate to antihyperglycemic TZD action.[18] Beside, there is a great interest in 2,4-TZD derivatives as ARIs,[19,20] since they can be viewed as hydantoin bioisosters potentially free of the hypersensitivity reactions which are linked to the presence of the hydantoin system. On the other hand, it was reported that some 2,4-TZDs have been patented as antihyperglycemic and AR inhibitory agents with dual activity.[19,21]

Previously, we reported that the AR inhibitory effects of some insulinotropic flavonyl-4′,[22] 3′,[23] and 6-TZDs[24] showed significant inhibitory activity on rat kidney AR.[25] Herein, in our screening program to search for antihyperglycemic and AR inhibitory compounds with dual activity, some new derivatives containing flavone and acetic acid/acetic acid ethyl ester groups on N-3 position of the 2,4-TZD ring system were synthesized, and their insulin releasing activities in INS-1 cells and AR inhibitory effects were evaluated.

2. Results and discussion

2.1. Chemistry

The protocol for synthesis of the presented TZD derivatives is shown in Scheme 1. The general method which is known as Baker–Venkataraman method[26] was used to prepare 3′/4′/6-methyl flavone (**Ia–c**). The methyl group of the flavone was converted to bromomethyl (**IIa–c**) with *N*-bromosuccinimide and a catalytic amount of benzoyl peroxide, and then this group was subjected to the Sommelet reaction that gave rise to aldehyde (**IIIa–c**).

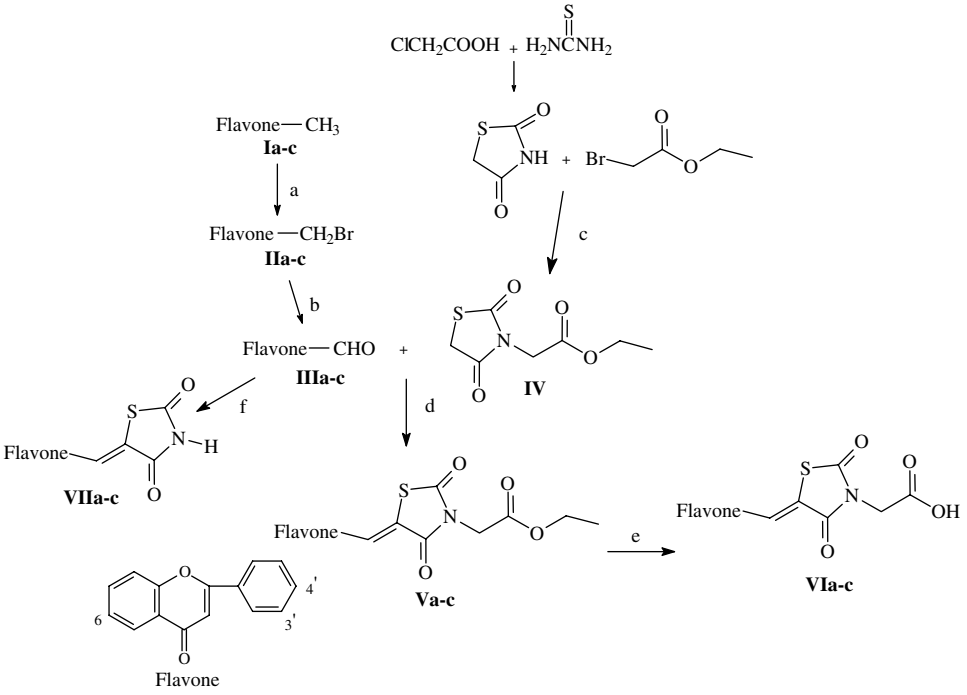
2,4-TZD (**1**) was synthesized with $ClCH_2COOH$ and thiourea in hot water.[27] N-Alkylation of 2,4-TZD with ethyl bromoacetate in THF/NaH furnished ethyl 2,4-dioxothiazolidine-3-ylacetate **IV**,[28] which on Knoevenagel condensation with flavone carbaldehydes **IIIa–c** yielded flavonyl-2,4-TZD acetic acid ethyl ester derivatives **Va–c**. The acidic hydrolysis of **Va–c** provided corresponding carboxylic acids **VIa–c**.

The structure of the synthesized compounds was elucidated by elementary analysis, ^{1}H NMR, mass spectral data, and IR findings. All spectral data were in accordance with assumed structures. IR spectra of the compounds showed γ-pyrone C=O stretching bonds at 1611–1658 cm^{-1}. In ^{1}H NMR spectra, flavone protons were observed between 6.88 and 8.44 ppm; methylidene protons (=CH) for flavonyl-2,4-TZDs were seen 7.97–8.17 ppm as a singlet; N–CH_2 protons in acetic side chain were observed 4.39–4.52 ppm as a singlet. In mass analysis, all the compounds have M+H ion peaks.

2.2. In vitro insulin releasing and aldose reductase inhibitory activities

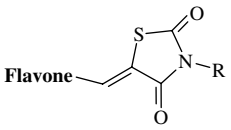
Derivatives of acetic acid ethyl esters **Va–c** and acetic acids **VIa–c** were tested comparing with glibenclamide for their insulinotropic activities in INS-1 cells at two different concentrations (Table 1). Compounds **Va, Vb**, **VIb**, and **VIc** (at lower concentration; 0.001 mg/ml) were able to increase insulin release in the presence of 5.6 mmol/l glucose. Compound **Vb** had a more potent insulinotropic effect at higher concentration (0.01 mg/ml) than at lower concentration (0.001 mg/ml).

In our previous papers, we synthesized novel 4′,[22] 3′,[23] and 6-flavonyl- TZDs[24]and tested their insulin releasing activities. As seen in this study, acetic acid ethyl esters **Va, Vb** and acetic acids **VIb, VIc** had an insulinotropic effect at lower concentration (1 μg/ml), while 3′ (**VIIa**), 4′ (**VIIb**), and 6-flavonyl TZDs (**VIIc**) had no effect on insulin releasing activity in INS-1 cells (Table 1). According to these results, it should be pointed out that when car-



Scheme 1. Reagents: (a) NBS, benzoyl peroxide; (b) HMTA, 50% acetic acid, HCl; (c) NaH, THF; (d) CH_3COONa, glacial acetic acid; (e) glacial acetic acid, HCl; (f) 2,4-TZD, CH_3COONa, glacial acetic acid.

Table 1
Insulin release[a] and aldose reductase inhibitory[b] activities of flavonyl-2,4-TZD compounds



Position	R	Compounds	Insulin release[a]	Aldose reductase inhibition (%)[d]
		Glucose [3.0 mM]	73.00 ± 6.90[c]	
		Glucose [5.6 mM]	100.0	
3′	$CH_2COOCH_2CH_3$	Plus **Va** [1 μg/ml]	103.4 ± 6.82	12.93 ± 4.23
		Plus **Va** [10 μg/ml]	98.43 ± 9.88	
4′	$CH_2COOCH_2CH_3$	Plus **Vb** [1 μg/ml]	106.2 ± 7.44	6.74 ± 2.85
		Plus **Vb** [10 μg/ml]	128.6 ± 9.59[c]	
6	$CH_2COOCH_2CH_3$	Plus **Vc** [1 μg/ml]	93.28 ± 9.24	14.36 ± 0.70
		Plus **Vc** [10 μg/ml]	98.52 ± 7.00	
3′	CH_2COOH	Plus **VIa** [1 μg/ml]	98.10 ± 5.07	86.57 ± 0.55 (IC_{50}: 0.415 ± 0.033)
		Plus **VIa** [10 μg/ml]	84.09 ± 5.01[c]	
4′	CH_2COOH	Plus **VIb** [1 μg/ml]	102.4 ± 6.82	44.64 ± 2.08
		Plus **VIb** [10 μg/ml]	91.88 ± 8.76	
6	CH_2COOH	Plus **VIc** [1 μg/ml]	101.3 ± 6.96	56.31 ± 4.16 (IC_{50}: 0.852 ± 0.081)
		Plus **VIc** [10 μg/ml]	80.92 ± 5.61[c]	
3′	H	Plus **VIIa** [1 μg/ml]	79.76 ± 13.11 (Ref. 23)	22.06 ± 0.19 (Ref. 25)
		Plus **VIIa** [10 μg/ml]	18.95 ± 4.43	
4′	H	Plus **VIIb** [1 μg/ml]	55.70 ± 9.51 (Ref. 22)	88.69 ± 1.46 (IC_{50}: 0.432 ± 0.008) (Ref. 25)
		Plus **VIIb** [10 μg/ml]	23.79 ± 1.34	
6	H	Plus **VIIc** [1 μg/ml]	82.55 ± 13.67 (Ref. 24)	64.86 ± 1.21 (IC_{50}: 0.683 ± 0.006) (Ref. 25)
		Plus **VIIc** [10 μg/ml]	158.9 ± 16.98	
		Plus DMSO	102.8 ± 7.34	
			104.3 ± 5.69	
		Plus glibenclamide [1 μg/ml] (positive control)	179.6 ± 11.23[c]	

[a] Effects of indicated compounds on glucose-mediated insulin release from INS-1 cells. INS-1 cells in multi-wells were washed three times and incubated in KRBH buffer for 90 min at 5.6 mM glucose. The insulin released by 5.6 mM glucose was taken as 100% and the results of others were normalized to this 100% effect. Each value represents the mean ± SEM.

[b] The aldose reductase enzyme activity was assayed by spectrophotometrically monitoring NADPH oxidation which accompanies the reduction of D-L-glyceraldehyde used as substrate.

[c] $p < 0.05$ compared with the effect of 5.6 mM glucose (negative control) and glibenclamide (positive control) was determined by one-way analysis of variance (ANOVA) followed by the Newman Keul's test as the post hoc test. $N = 4–8$.

[d] Values represent the mean ± SD of three individual experiments.

boxylic acid or carboxylic acid ester groups were substituted at N-3 position of the TZD ring instead of imidic hydrogen, the insulin releasing activity in INS-1 cells was increased.

Previously, *N*-methyl, ethyl,[22–24] and benzyl substituted analogs[29] of 2,4-TZD ring of **VIIa–c** series and flavonylsulfonylureas[30] were synthesized, and their antihyperglycemic effects were tested with the same method. When the results of this study are compared with the results of *N*-benzyl substituted analog compounds,[29] it was found that **V–VI** series showed similar insulin releasing effect. On the other hand, it was observed that *N*-methyl, ethyl substituted analogs of 2,4-TZD ring of **VIIa–c** series[22–24] were more potent than **V–VI** series. Besides, novel flavonyl TZD compounds **V–VI** were found to be more effective than flavonylsulfonylureas at lower concentration (1 μg/ml).

The aldose reductase inhibition obtained by newly synthesized flavonyl compounds **Va–c**, **VIa–c** was studied in vitro and the results are represented in Table 1. The enzyme activity was assayed by spectrophotometrically monitoring NADPH oxidation which accompanies the reduction of D-L-glyceraldehyde used as substrate. The inhibition study was performed merely by using one concentration (10^{-4} M) of each drug, and IC_{50} values of compounds **VIa, VIc** were studied. For calculating IC_{50} values full concentration response curves using 10^{-4}, 10^{-5}, and 10^{-6} M concentrations when the compounds had an effect at 10^{-4} M.

As seen in Table 1, acetic acids **VIa–c** which were synthesized by the insertion of an acetic acid chain on N-3 position of 2,4-thiazolidinedione ring were shown significant inhibitory activity. The most active compound **VIa** exerted inhibitory activity 86.57%, **VIc** and **VIb** were shown 56.31% and 44.64% inhibitory activity, respectively. On the other hand, their ester derivatives **Va–c** devoid of any acidic proton have less inhibitory activity compared to *N*-acetic acid analogs (**VIa–c**).

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.[9,31–33] In particular, it is 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.[31–33]

As a result, all novel acetic acid compounds **VIa–c** possessed inhibitory effect on AR enzyme at the given concentration. But, acetic acid ester compounds **Va–c** that had no acidic hydrogen (imidic or acetic acid) on N-3 of the 2,4-TZD possess a slight inhibitory effect of 12.93%, 6.74%, and 14.36%, respectively. Besides, *N*-methyl, ethyl substituted analogs of 2,4-TZD ring of **VIIa–c** series,[25] have shown weak inhibitory effect. Thus, we can say that the potent inhibitory effect of our synthesized compounds might depend on having acidic proton on the TZD ring.

In this study, we aimed to have more effective compounds possessing dual activity as ARIs and insulinotropic by adding acetic acid side chain in N-3 position of the 2,4-TZD ring in flavonyl compounds **VIIa–c** known to have ARI effect.[25] Surprisingly, acetic acid compounds **VIb–c** were less effective on AR than their N–H analogs **VIIb–c** (see Table 1). However, acetic acid derivative **VIa**, almost four times as effective as its counterpart **VIIa**, displayed the most inhibition (86.57%). As for insulinotropic effect, acetic acid ethyl ester analogs **Va–c**, acetic acid analogs **VIa–c** were more potent than **VIIa–c** series except **VIIc** at higher concentration. Finally, it can be

considered that the acidic hydrogen (imidic or acetic acid) instead of lipophilic groups on N-3 position of the 2,4-TZD ring played a noticeable role for increasing the ARI effect, whereas decreasing insulinotropic effect.

3. Conclusions

We report the synthesis and the in vitro insulin releasing and aldose reductase inhibitory activity effects of the flavonyl-2,4-thiazolidinediones **V–VI**. Among newly synthesized compounds, acetic acids **VIa, VIc** with IC_{50} values lower than 1 μM for aldose reductase inhibitory activity were obtained. The importance of the acidic hydrogen (imidic hydrogen or carboxylic acid) function on N-3 was stressed by the findings. But, in this study, it can be concluded that acetic acid as a pharmacophoric group caused an increase for only aldose reductase inhibitory effect as shown for compound **VIa**.

4. Experimental

4.1. Chemistry

Melting points were determined via a Büchi SMP-20 melting point apparatus (Büchi, Flawil, Switzerland) and uncorrected. All instrumental analyses were performed in Central Laboratory of Pharmacy Faculty of Ankara University. IR spectra were recorded on a Jasco FT/IR-420 spectrometer (Jasco, Tokyo, Japan). ^{1}H NMR and ^{13}C NMR spectra were 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. Mass spectra were recorded on Waters Micromass ZQ (Waters Corporation, Milford, MA, USA) by using ESI(+) method. 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. For the chromatographic analysis Merck Silica Gel 60 (230–400 mesh ASTM) was used. The chemical reagents used in synthesis were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA).

4.1.1. Synthesis of 3′ (IIa)-4′ (IIb)-6 (IIc)-bromomethyl flavone

A mixture of *N*-bromosuccinimide (1.2 g, 6.72 mmol) and 3′ (**Ia**)/4′ (**Ib**)/6-methyl flavone (**Ic**) (1.0 g, 4.2 mmol) was dissolved in 70 ml of carbon tetrachloride and benzoyl peroxide (0.1 g) was added. The reaction mixture was refluxed for 7 h and filtered while still hot. The crude product was crystallized from toluene. **IIa** mp: 137 °C (mp: 137 °C[34]), **IIb** mp: 139 °C (mp: 139 °C[34]), **IIc** mp: 174 °C (mp: 175 °C[35]).

4.1.2. Synthesis of 3′ (IIIa)-4′ (IIIb)-6 (IIIc)-flavonecarboxaldehyde

A mixture of bromomethylflavone (1.0 g, 3.17 mmol) and hexamethylenetetramine (4 g, 28.0 mmol) in 20 ml acetic acid (50%, v/v) was refluxed for 4 h. HCl:H_2O (1:1, 10 ml) was added and refluxed for another 30 min. The product was crystallized from ethylacetate:*n*-hexane. **IIIa** mp: 168 °C (mp: 168 °C[34]), **IIIb** mp: 165 °C (mp: 165 °C[34]), **IIIc** mp: 186 °C (mp: 186 °C[35]).

4.1.3. General synthesis of compounds Va–c

A mixture of ethyl 2,4-dioxothiazolidine-3-ylacetate **IV** (0.001 mol) and flavone carboxaldehydes **IIIa–c** (0.001 mol) was heated at 100 °C in the presence of 1 ml glacial acetic acid and sodium acetate (0.001 mol) for 10 h. The residue was crystallized from DMF–ethanol.

4.1.3.1. Ethyl{2,4-dioxo-5-[3-(4-oxo-4H-chromen-2-yl)benzylidene]-1,3-thiazolidine-3yl}acetate (Va). Yield (%): 62.3, mp: 203 °C. Spectroscopic analysis: IR (KBr) υ_{max}/cm^{-1}: 1640 (γ pyrone CO); ^{1}H NMR (CDCl$_3$, 400 MHz, δ, ppm): 1.32 (t, 3H, CH_3), 4.27 (q, 2H, CH_2), 4.52 (s, 2H, CH_2CO), 6.88 (s, 1H, 3-H), 7.47 (td, 1H, 6-H), 7.62 (d, 1H, $j_{8,7}$ = 8.80 Hz, 8-H), 7.65–7.68 (m, 2H, 5′, 6′-H), 7.76 (td, 1H, 7-H), 7.97 (dd, 1H, j_o = 8.40 Hz, j_m = 1.60 Hz, 4′-H), 8.01 (s, 1H, C=C–H), 8.11 (s, 1H, 2-H), 8.25 (dd, 1H, $j_{5,6}$ = 8.00 Hz, $j_{5,7}$ = 1.60 Hz, 5-H); MS (ESI$^+$) *m/z* (rel. intensity): 436 (M+H, 100%); Anal. Calcd for $C_{23}H_{17}NO_6S.0.2H_2O$ C: 62.92, H: 3.97, N: 3.19, S: 7.29%; found: C: 62.91, H: 3.94, N: 3.36, S: 7.18%.

4.1.3.2. Ethyl{2,4-dioxo-5-[4-(4-oxo-4H-chromen-2-yl)benzylidene]-1,3-thiazolidine-3yl}acetate (Vb). Yield (%): 85.1, mp: 248 °C. Spectroscopic analysis: IR (KBr) υ_{max}/cm^{-1}: 1652 (γ pyrone CO); ^{1}H NMR (CDCl$_3$, 400 MHz, δ, ppm): 1.32 (t, 3H, CH_3), 4.27 (q, 2H, CH_2), 4.51 (s, 2H, CH_2CO), 6.90 (s, 1H, 3-H), 7.46 (td, 1H, 6-H), 7.60 (d, 1H, $j_{8,7}$ = 8.40 Hz, 8-H), 7.68 (d, 2H, j_o = 8.40 Hz, 3′,5′-H), 7.74 (td, 1H, 7-H), 7.97 (s, 1H, C=C–H), 8.06 (dd, 2H, j_o = 8.40 Hz, 2′,6′-H), 8.25 (dd, 1H, $j_{5,6}$ = 8.40 Hz, $j_{5,7}$ = 1.60 Hz, 5-H); MS (ESI$^+$) *m/z* (rel. intensity): 436 (M+H, 100%); Anal. Calcd for $C_{23}H_{17}NO_6S$ C: 63.44, H: 3.94, N: 3.22, S: 7.36%; found: C: 63.07, H: 3.95, N: 3.30, S: 7.26%.

4.1.3.3. Ethyl{2,4-dioxo-5-[(4-oxo-2-phenyl-4H-chromen-6-yl)-methylene]-1,3-thiazolidine-3yl}acetate (Vc). Yield (%): 60.02, mp: 223.5 °C. Spectroscopic analysis: IR (KBr) υ_{max}/cm^{-1}: 1658 (γ pyrone CO); ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 1.22 (t, 3H, CH_3), 4.18 (q, 2H, CH_2), 4.52 (s, 2H, CH_2CO), 7.16 (s, 1H, 3-H), 7.60–7.64 (m, 3H, 3′, 4′, 5′-H), 7.97 (d, 1H, $j_{8,7}$ = 8.80 Hz, 8-H), 8.10–8.15 (m, 3H, 7, 2′, 6′-H), 8.17 (s, 1H, C=C–H), 8.27 (d, 1H, $j_{5,7}$ = 2.40 Hz, 5-H); MS (ESI$^+$) *m/z* (rel. intensity): 436 (M+H, 100%); Anal. Calcd for $C_{23}H_{17}NO_6S$ C: 63.44, H: 3.94, N: 3.22, S: 7.36%; found: C: 63.32, H: 4.13, N: 3.32, S: 7.28%.

4.1.4. General synthesis of compounds VIa–c

A mixture of acetate **Va–c** (10 mmol), glacial acetic acid (40 ml), and HCl 12 N (10 ml) was refluxed for 2 h. After evaporation in vacuo, the residue was refluxed again with glacial acetic acid (40 ml) and HCl 12 N (10 ml) for 2 h. After evaporation to dryness in vacuo, the crude solid was washed with water and recrystallized from DMF–ethanol providing pure carboxylic acids **VIa–c**.

4.1.4.1. {2,4-Dioxo-5-[3-(4-oxo-4H-chromen-2-yl)benzylidene]-1,3-thiazolidine-3yl}acetic acid (VIa). Yield (%): 82.9, mp: 286 °C. Spectroscopic analysis: IR (KBr) υ_{max}/cm^{-1}: 1645 (γ pyrone CO); ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 4.40 (s, 2H, CH_2CO), 7.16 (s, 1H, 3-H), 7.54 (td, 1H, 6-H), 7.76–7.88 (m, 4H, 7, 8, 5′, 6′-H), 8.07 (d, 1H, j_o = 8.00 Hz, 4′-H), 8.14 (s, 1H, C=C–H), 8.25 (d, 1H, $j_{5,6}$ = 8.00 Hz, 5-H), 8.44 (s, 1H, 2-H); MS (ESI$^+$) *m/z* (rel. intensity): 408 (M+H, 100%); Anal. Calcd for $C_{21}H_{13}NO_6S$. H_2O C: 59.29, H: 3.53, N: 3.29, S: 7.53%; found: C: 59.31, H: 3.65, N: 3.44, S: 7.38%.

4.1.4.2. {2,4-Dioxo-5-[4-(4-oxo-4H-chromen-2-yl)benzylidene]-1,3-thiazolidine-3yl}acetic acid (VIb). Yield (%): 85.5, mp: 335.5 °C. Spectroscopic analysis: IR (KBr) υ_{max}/cm^{-1}: 1611 (γ pyrone CO); ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 4.39 (s, 2H, CH_2CO), 7.19 (s, 1H, 3-H), 7.53 (td, 1H, 6-H), 7.81–7.89 (m, 4H, 7, 8, 3′, 5′-H), 8.07 (d, 1H, $j_{5,6}$ = 8.40 Hz, $j_{5,7}$ = 1.20 Hz, 5-H), 8.09 (s, 1H, C=C–H), 8.30 (dd, 2H, j_o = 8.40 Hz, 2′, 6′-H); MS (ESI$^-$) *m/z* (rel. intensity): 408 (M+H, 100%); Anal. Calcd for $C_{21}H_{13}NO_6S.0.3H_2O$ C: 61.11, H: 3.29, N: 3.39, S: 7.76%; found: C: 60.93, H: 3.19, N: 3.53, S: 7.60%.

4.1.4.3. {2,4-Dioxo-5-[(4-oxo-2-phenyl-4H-chromen-6-yl)-methylene]-1,3-thiazolidine-3yl}acetic acid (VIc). Yield (%): 93.1, mp: 350 °C. Spectroscopic analysis: IR (KBr) υ_{max}/cm^{-1}: 1614 (γ pyrone CO); ^{1}H NMR (DMSO-d_6, 400 MHz, δ, ppm): 4.41 (s, 2H, CH_2CO), 7.17 (s, 1H, 3-H), 7.60–7.65 (m, 3H, 3′, 4′, 5′-H),

7.98 (d, 1H, $j_{8,7}$ = 8.40 Hz, 8-H), 8.12-8.16 (m, 3H, 7, 2′, 6′-H), 8.17 (s, 1H, C=C–H), 8.28 (d, 1H, $j_{5,7}$ = 1.60 Hz, 5-H); MS (ESI^+) *m/z* (rel. intensity): 408 (M+H, 100%); Anal. Calcd for $C_{21}H_{13}NO_6S$ C: 61.91, H: 3.22, N: 3.44, S: 7.87%; found: C: 61.62, H: 3.25, N: 3.44, S: 7.81%.

4.2. Biological activity

4.2.1. Measurement of insulin release from INS-1 cells

4.2.1.1. Cell culture of INS-1 cells. INS-1 cells, generously provided by Dr. C. Wollheim, Geneva, Switzerland,[36] were grown in plastic culture bottles or micro-wells for 4–6 days (half confluence:1–2 × 10^6 cells per ml) in RPMI medium supplemented with 10% (v/v) fetal calf serum, 100 U of penicillin per ml, and 0.1 mg of streptomycin per ml. Cells were seeded at a density of 5 × 10^5 cells/ml. The medium was changed every 5 days, and the cells were detached from the culture flask with trypsin 1 week after seeding, centrifuged and reseeded as described above. Prior to the experiment cells, were washed 2 times and then incubated in Krebs-Ringer buffer containing 10 mM HEPES and 0.5% bovine serum albumin (KRBH).

4.2.1.2. Insulin release. To measure insulin secretion, half-confluent cells in micro-wells were incubated for 90 min at 37 °C in the aforementioned KRBH buffer. Insulin released into the medium was assayed with a radioimmunoassay using rat insulin (Novo Nordisk, Bagsvaerd, Denmark) as a standard, (mono ^{125}I-Tyr A^{14})-porcine insulin as the labeled compound (Sanofi-Aventis, Germany) and anti-insulin antibodies from Linco (St. Louis, USA). Each compound had been checked for non-interference with the insulin radioimmunoassay. The data were corrected for the effects of solubilizing compounds (ethanol, DMSO).

4.2.2. Measurement of aldose reductase inhibitory activity

4.2.2.1. Animals. Male Albino rats weighing 200–250 g were used for experiments. They received standard diet. Thirty rats were sacrificed, and kidney tissues were obtained. Aldose reductase inhibitory activities were determined after isolation of the enzyme from kidney tissue. All the enzyme experiments were performed in triplicates. 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.

4.2.2.2. Isolation of the aldose reductase enzyme. The aldose reductase enzyme was isolated by a method described below. Thirty pooled kidneys which were obtained from 200 to 250 g albino rats, were thawed on ice and homogenized with 3 volumes of distilled water, followed by centrifugation at 10,000g for 20 min. Saturated ammonium sulfate was added to the supernatant to 40% saturation. The thick suspension was stirred for 15 min, followed by centrifugation at 10,000g 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 10,000g 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 10,000g for 20 min. Protein concentration was measured by the method of Bradford[37] using bovine serum albumin as a standard. Protein concentration was 7.18 ± 0.08 mg/ml.

4.2.2.3. 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 NaCl 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 addition 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. Readings were taken when the changes in absorbance were linear.[38] For calculating IC_{50}values, experiments using concentrations of 10^{-4}, 10^{-5}, and 10^{-6} M were used. The results represent three individual experiments.

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