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Original article

Synthesis and aldose reductase inhibitory activity of some new chromonyl-2,4-thiazolidinediones

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Abstract

As it is known that still, there were no any confident ARIs on the market and they have several side effects, we need to approve new ARIs to reduce diabetic complications especially which have effect on the cataract formation. In this study, a new series of chromonyl-2,4-thiazolidinediones (**Ia–e**, **IIa–e**, **IIIa–e**) were prepared by Knoevenagel reaction with substituted 3-formylchromones (**3a–e**) and unsubstituted (**1**) or substituted 2,4-thiazolidinedione (**2**). The synthesized compounds were tested for their ability to inhibit rat kidney AR by an in vitro spectrophotometric assay. Compound **IIIe** showed the highest inhibitory activity (82.43 ± 0.76%). Compounds **Ia–e** and **IIIa–d** also showed significant inhibitory activity (42.40 ± 5.78, 52.71 ± 3.31, 49.69 ± 1.55, 50.80 ± 3.62, 46.70 ± 2.33, 49.44 ± 4.53, 61.17 ± 4.74, 68.58 ± 2.05, 77.28 ± 0.26%, respectively).

Keywords: Chromone derivatives; 2,4-Thiazolidinediones; Chromonyl-2,4-thiazolidinediones; Aldose reductase; Aldose reductase inhibition; Synthesis

1. Introduction

Aldose reductase (AR), the key enzyme of the polyol pathway, has been demonstrated to play important roles in the pathogenesis of diabetic complications such as neuropathy, nephropathy and retinopathy (Fig. 1) [1,2]. Evidence suggests that compounds which inhibit AR could be effective for the prevention of diabetic complications [3–5].

There are around 200 million people with diabetes and the number is expected to rise to almost 350 million by 2025. The global epidemic of diabetes and its impact on ocular complications, particularly diabetic cataract and diabetic retinopathy, is a serious concern. Although strict glycemic control is expected to prevent diabetic complications, perfect glycemic control is not always possible. Hence, agents that can prevent diabetic complications, irrespective of glycemic control, need critical evaluation in the management of secondary complications. Thus inhibition of AR is one of the targets that need to be investigated in the light of diversified physiological functions of AR [6].

Aldose reductase inhibitors (ARIs) have been shown to reduce tissue sorbitol accumulation in diabetic animals [7]. There is some evidence that the blockage of AR can have beneficial effects in some diabetic complications [8,9].

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 is not satisfactory and some 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 [6].

2,4-Thiazolidinediones (2,4-TZDs) are a new class of antidiabetic agents, differ markedly from other antidiabetic agents in that they are effective in normalizing glucose and lipid

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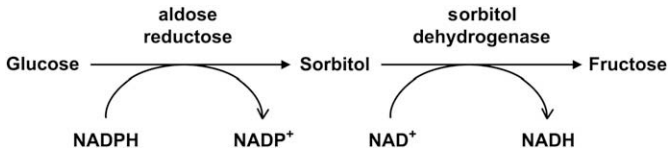


Figure 1. Polyol pathway.

metabolism associated with insulin resistance and are therefore expected to be useful in the treatment of both type 2 diabetes mellitus and obesity [10–12]. Besides, it was reported that some 2,4-TZDs have been patented as antihyperglycemic and AR inhibitory agents with dual activity [13]. There is a great interest in 2,4-TZD derivatives as ARIs [13,14], since they can be viewed as hydantoin bioisosteres potentially free of the hypersensitivity reactions which are linked to the presence of the hydantoin system.

Chromones are a group of naturally occurring compounds that are ubiquitous in nature especially in fruits, vegetables, nuts, seeds, flowers, and barks [15,16]. Due to their abundance in plants and their low mammalian toxicity, chromone derivatives are present in large amounts in the diet of human [17]. Molecules containing the chromone structure (for example chromone and flavonoids) have a wide range of biological activities including antioxidant [18], antiinflammatory [19], antibacterial [20] and antitumor [20,21] properties. Several flavonoid [22,23] and chromone [24,25] derivatives have also found with inhibitory activity against AR enzyme.

In this study, prompted by these investigations, we report the synthesis of some novel 2,4-thiazolidinedione derivatives incorporating with two known bioactive heterocyclic nuclei such as chromone and thiazolidinedione.

2. Results and discussion

2.1. Chemistry

The synthetic protocol of TZD derivatives presented is shown in Scheme 1. 2,4-TZD **1** was synthesized with $ClCH_2COOH$ and thiourea in hot water [26]. N-Alkylation of 2,4-TZD **1** with ethyl bromoacetate furnished ethyl 2,4-dioxothiazolidine-3-ylacetate **2** [27], which on Knoevenagel condensation with 3-formylchromones **3a–e** yielded chromonyl-2,4-TZD derivatives **IIa–e**.

Chromonyl-2,4-thiazolidinediones **Ia–e** were prepared by the condensation of 2,4-TZD with suitable 3-formylchromones in sodium acetate/glacial acetic acid.

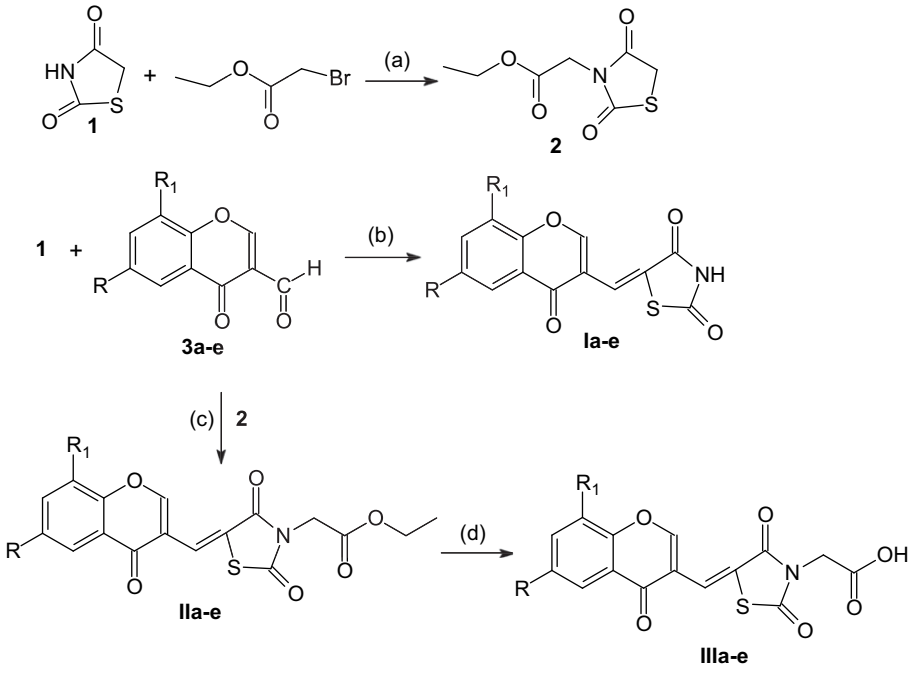
Acidic hydrolysis of **IIa–e** provided the corresponding carboxylic acids **IIIa–e**.

The structure of the synthesized chromonyl compounds was elucidated by elementary analysis, ^1H NMR, mass spectral data and IR findings. All spectral data were in accordance with assumed structures. IR spectra of compounds showed γ pyrone C=O stretching bonds at 1634–1660 cm^{-1}. In ^1H NMR spectra, chromone protons were observed between 7.55 and 9.06 ppm; methylene protons (=CH) for chromonyl-2,4-TZDs were observed at 7.58–7.77 ppm as a singlet. Mass analysis of compounds was performed by using ESI (+)/ESI (−) method. While compounds **Ia–e**, **IIIb**, and **IIIe** have M − H ion peaks, compounds **IIa–e**, **IIIa**, **IIIc**, and **IIId** have M + H ion peaks.

3. Biological studies

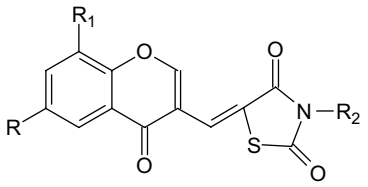
3.1. Aldose reductase enzyme inhibition

The inhibition obtained by chromone derivatives was studied in vitro and the results are presented in Table 1. The



Scheme 1. General synthesis of **Ia–e**, **IIa–e**, **IIIa–e**. (a) NaH/THF; (b) and (c) CH_3COOH/CH_3COONa; (d) CH_3COOH/HCl.

Table 1
Aldose reductase inhibition of chromonyl-2,4-TZD compounds[a]



Code	R	R_1	R_2	Inhibition (%)[b]
Ia	H	H	H	42.40 ± 5.78
Ib	CH_3	H	H	52.71 ± 3.31 (IC_{50}: 0.944 ± 0.073)
Ic	CH_3	CH_3	H	49.69 ± 1.55
Id	$(CH_3)_2CH$	H	H	50.80 ± 3.62 (IC_{50}: 0.955 ± 0.084)
Ie	NO_2	H	H	46.70 ± 2.33
IIa	H	H	$CH_2COOCH_2CH_3$	8.18 ± 0.77
IIb	CH_3	H	$CH_2COOCH_2CH_3$	0.0 ± 0.0
IIc	CH_3	CH_3	$CH_2COOCH_2CH_3$	0.0 ± 0.0
IId	$(CH_3)_2CH$	H	$CH_2COOCH_2CH_3$	9.46 ± 3.66
IIe	NO_2	H	$CH_2COOCH_2CH_3$	[c]
IIIa	H	H	CH_2COOH	49.44 ± 4.53
IIIb	CH_3	H	CH_2COOH	61.17 ± 4.74 (IC_{50}: 0.805 ± 0.066)
IIIc	CH_3	CH_3	CH_2COOH	68.58 ± 2.05 (IC_{50}: 0.678 ± 0. 021)
IIId	$(CH_3)_2CH$	H	CH_2COOH	77.28 ± 0.26 (IC_{50}: 0.522 ± 0.005)
IIIe	NO_2	H	CH_2COOH	82.43 ± 0.76 (IC_{50}: 0.261 ± 0.021)

[a] Values represent the mean ± S.D. of three individual experiments.
[b] IC_{50} (μM) or % inhibition at the given concentration.
[c] poor solubility – not determined.

enzyme activity was assayed by spectrophotometrically monitoring NADPH oxidation which accompanies the reduction of DL-glyceraldehyde that was used as substrate. The inhibition study was performed merely by using a 10^{-4} M concentration of each drug, and IC_{50} values of compounds **Ib**, **Id**, and **IIIb–IIIe** were studied.

Chromonyl-2,4-thiazolidinediones with various substitution pattern on the chromone ring were tested and displayed IC_{50} values ranging from 0.261 to 0.955 μM (Table 1). The most active of them (**IIIe**) exerted inhibitory activity (82.43%), followed by **IIId**, **IIIc**, **IIIb**, **Ib**, **Id**, **Ic**, **IIIa**, **Ie** and **Ia** with 77.28, 68.58, 61.17, 52.71, 50.80, 49.69, 49.44, 46.70 and 42.40%, respectively. Interestingly, unsubstituted chromone compound **Ia** showed to be less effective than other substituted chromone compounds. In particular, methyl substituted chromone compound **Ib** proved to be the most active among these substituted compounds, the positive influence was exerted by 6-methyl substitution at chromone ring on activity.

As seen in Table 1, the insertion on N-3 of an acetic chain led to 2,4-thiazolidinedione-3-acetic acids **IIIa–e**, which were significantly more potent than their *N*-unsubstituted analogs **Ia–e**. The nitro substituted derivative **IIIe**, almost two times as effective as its counterpart **Ie**, displayed the most significant inhibitory properties (82.43%). Structure–activity relationships revealed that attaching an electron withdrawing group such as nitro on position 6 of chromone ring of 2,4-thiazolidinedione-3-acetic acids **IIIa–e** played very important role to increase the activity.

On the other hand, esters **IIa–e**, devoid of any acidic proton, have slight inhibitory activity compared to unsubstituted-2,4-thiazolidinediones **Ia–e** and 2,4-thiazolidinedione-3-acetic acids **IIIa–e**. Surprisingly, compounds **IIb** and **IIc** having methyl and dimethyl substituents on chromone did not inhibit AR, respectively. Since the nitro chromonyl TZD ester compound **IIe** was poorly soluble in DMF/methanol (50%), its AR inhibitory activity was not determined.

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 the known pharmacophoric requirements [13,28,29]. An aromatic ring with the hydrogen-bonding ability also required to afford AR inhibitory activity.

As a result, all our tested compounds except esters **IIa–e** that had no acidic hydrogen (imidic or acetic acid) on N-3 of the 2,4-TZD possessed inhibitory effect on AR enzyme inhibition at the given concentration. We may conclude that the increasing inhibitory effect of compounds **IIIa–e** might depend on the acetic acid side chain of 2,4-TZD and these compounds especially **IIIe** could display therapeutic potential in the prevention and the treatment of diabetic complications as promising ARIs.

4. Experimental

Melting points were determined with a Büchi SMP-20 melting point apparatus (Büchi, Flawil, Switzerland) and are 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) as potassium bromide discs. ^{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 (+)/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 the synthesis were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA).

4.1. Chemistry

4.1.1. General synthesis of compounds *Ia–e*

A mixture of 2,4-TZD **1** (0.001 mol) and 3-formylchromones **3a–e** (0.001 mol) was heated at 100 °C in the presence of 1 mL glacial acetic acid and sodium acetate (0.001 mol) for 5 h. The crude product was crystallized from DMF/ethanol.

*4.1.2. 5-[(4-Oxo-4H-chromen-3-yl)methylene]thiazolidine-2,4-dione (**Ia**)*

Yield (%): 66.5, m.p.: 271 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1637 (γ pyrone CO); ^1H NMR (δ ppm): 7.58 (ddd, 1H, 6-H), 7.61 (s, 1H, C=C–H), 7.74 (d, 1H, $J_{8,7} = 8.40$ Hz, 8-H), 7.88 (ddd, 1H, 7-H), 8.13 (dd, 1H, $J_{5,6} = 8.40$ Hz, $J_{5,7} = 1.60$ Hz, 5-H), 8.85 (s, 1H, 2-H), 12.48 (s, 1H, NH); MS (ESI^-) *m/z* (rel. intensity): 272 (M − H, 100%). Anal. Calcd. for $C_{13}H_7NO_4S$: C 57.14, H 2.58, N 5.13, S 11.73%; found: C56.84, H 2.74, N 5.25, S 11.46%.

*4.1.3. 5-[(6-Methyl-4-oxo-4H-chromen-3-yl)methylene] thiazolidine-2,4-dione (**Ib**)*

Yield (%): 62.3, m.p.: 257 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1646 (γ pyrone CO); ^1H NMR (δ ppm): 2.42 (s, 3H, CH_3), 7.58 (s, 1H, C=C–H), 7.61 (d, 1H, $J_{8,7} = 8.80$ Hz, 8-H), 7.67 (dd, 1H, $J_{7,8} = 8.40$ Hz, $J_{7,5} = 2.40$ Hz, 7-H), 7.90 (d, 1H, $J_{5,7} = 1.60$ Hz, 5-H), 8.79 (s, 1H, 2-H), 12.42 (s, 1H, NH); MS (ESI^-) *m/z* (rel. intensity): 286 (M − H, 100%). Anal. Calcd. for $C_{14}H_9NO_4S$: C 58.53, H 3.16, N 4.88, S 11.16%; found: C 58.26, H 3.30, N 5.00, S 10.85%.

*4.1.4. 5-[(6,8-Dimethyl-4-oxo-4H-chromen-3-yl)methylene] thiazolidine-2,4-dione (**Ic**)*

Yield (%): 66.4, m.p.: 298 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1654 (γ pyrone CO); ^1H NMR (δ ppm): 2.40 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 7.57 (s, 1H, 7-H), 7.60 (s, 1H, C=C–H), 7.75 (s, 1H, 5-H), 8.83 (s, 1H, 2-H), 12.44 (s, 1H, NH); MS (ESI^-) *m/z* (rel. intensity): 300 (M − H, 100%). Anal. Calcd. for $C_{15}H_{11}NO_4S \cdot 0.1H_2O$: C 59.43, H 3.69, N 4.62, S 10.56%; found: C 59.30, H 3.60, N 4.79, S 10.34%.

*4.1.5. 5-[(6-Isopropyl-4-oxo-4H-chromen-3-yl)methylene] thiazolidine-2,4-dione (**Id**)*

Yield (%): 54.0, m.p.: 242 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1650 (γ pyrone CO); ^1H NMR (δ ppm): 1.26 (d, 6H, CH_3), 3.07–3.10 (m, 1H, CH), 7.61 (s, 1H, C=C–H), 7.67 (d, 1H, $J_{8,7} = 8.80$ Hz, 8-H), 7.80 (dd, 1H, $J_{7,8} = 8.80$ Hz, $J_{7,5} = 2.40$ Hz, 7-H), 7.95 (d, 1H, $J_{5,7} = 2.00$ Hz, 5-H), 8.83 (s, 1H, 2-H), 12.42 (s, 1H, NH); MS (ESI^-) *m/z* (rel. intensity): 314 (M − H, 100%). Anal. Calcd. for $C_{16}H_{13}NO_4S \cdot 0.1H_2O$: C 60.60, H 4.17, N 4.42, S 10.14%; found: C 60.80, H 4.41, N 4.49, S 9.70%.

*4.1.6. 5-[(6-Nitro-4-oxo-4H-chromen-3-yl)methylene] thiazolidine-2,4-dione (**Ie**)*

Yield (%): 55.0, m.p.: 277 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1654 (γ pyrone CO); ^1H NMR (δ ppm): 7.61 (s, 1H, C=C–H), 8.01 (d, 1H, $J_{8,7} = 9.60$ Hz, 8-H), 8.63 (dd, 1H, $J_{7,8} = 9.20$ Hz, $J_{7,5} = 2.80$ Hz, 7-H), 8.78 (d, 1H, $J_{5,7} = 2.80$ Hz, 5-H), 8.93 (s, 1H, 2-H), 12.54 (s, 1H, NH); MS (ESI^-) *m/z* (rel. intensity): 317 (M − H, 100%). Anal. Calcd. for $C_{13}H_6N_2O_6S \cdot 0.5H_2O$: C 47.71, H 2.14, N 8.56, S 9.78%; found: C 47.96, H 2.41, N 8.50, S 9.47%.

*4.2. General synthesis of compounds **IIa–e***

A mixture of ethyl 2,4-dioxothiazolidine-3-ylacetate **2** (0.001 mol) and 3-formylchromones **3a–e** (0.001 mol) was heated at 100 °C in the presence of 1 mL glacial acetic acid and sodium acetate (0.001 mol) for 5 h. The residue was purified by column chromatography on silica gel 60 (230–400 mesh ASTM) using hexane/dichloromethane (1:2) as eluant.

*4.2.1. Ethyl{2,4-dioxo-5-[(4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl}acetate (**IIa**)*

Yield (%): 60.1, m.p.: 206 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1636 (γ pyrone CO); ^1H NMR (δ ppm): 1.22 (t, 3H, CH_3), 4.17 (q, 2H, CH_2), 4.47 (s, 2H, CH_2CO), 7.59 (ddd, 1H, 6-H), 7.76 (d, 1H, $J_{8,7} = 8.40$ Hz, 8-H), 7.77 (s, 1H, C=C–H), 7.85 (ddd, 1H, 7-H), 8.15 (dd, 1H, $J_{5,6} = 8.00$ Hz, $J_{5,7} = 1.60$ Hz, 5-H), 8.99 (s, 1H, 2-H); MS (ESI^+) *m/z* (rel. intensity) : 360 (M + H, 100%). Anal. Calcd. for $C_{17}H_{13}NO_6S$: C 56.82, H 3.65, N 3.90, S 8.92%; found: C 56.67, H 3.81, N 3.93, S 8.87%.

*4.2.2. Ethyl{2,4-dioxo-5-[(6-methyl-4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl}acetate (**IIb**)*

Yield (%): 63.1, m.p.: 185–186 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1653 (γ pyrone CO); ^1H NMR (δ ppm): 1.19 (t, 3H, CH_3), 2.43 (s, 3H, CH_3), 4.15 (q, 2H, CH_2), 4.44 (s, 2H, CH_2CO), 7.63 (d, 1H, $J_{8,7} = 8.40$ Hz, 8-H), 7.68(dd, 1H, $J_{7,8} = 8.40$ Hz, $J_{7,5} = 2.40$ Hz, 7-H), 7.73 (s, 1H, C=C–H), 7.91 (d, 1H, $J_{5,7} = 1.60$ Hz, 5-H), 8.93 (s, 1H, 2-H); MS (ESI^+) *m/z* (rel. intensity): 396 (M + 22, 100%). Anal. Calcd. for $C_{18}H_{15}NO_6S \cdot 0.1H_2O$: C 57.62, H 4.05, N 3.73, S 8.54%; found: C 57.28, H 4.17, N 3.73, S 8.46%.

*4.2.3. Ethyl{2,4-dioxo-5-[(6,8-dimethyl-4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl}acetate (**IIc**)*

Yield (%): 45.2, m.p.: 206 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1652 (γ pyrone CO); ^1H NMR (δ ppm): 1.19 (t, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 4.15 (q, 2H, CH_2), 4.44 (s, 2H, CH_2CO), 7.55 (s, 1H, 7-H), 7.73 (s, 2H, C=C–H, 5-H), 8.95 (s, 1H, 2-H); MS (ESI^+) *m/z* (rel. intensity): 388 (M + H, 100%). Anal. Calcd. for $C_{19}H_{17}NO_6S$: C 58.90, H 4.42, N 3.62, S 8.28%; found: C 58.57, H 4.37, N 3.63, S 8.28%.

*4.2.4. Ethyl{2,4-dioxo-5-[(6-isopropyl-4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl}acetate (**IId**)*

Yield (%): 74.1, m.p.: 158 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1651 (γ pyrone CO); ^1H NMR (δ ppm): 1.22 (t, 3H, CH_3), 1.26 (d, 6H, CH_3), 3.08–3.11 (m, 1H, CH), 4.17 (q, 2H, CH_2), 4.46 (s, 2H, CH_2CO), 7.69 (d, 1H, $J_{8,7} = 8.40$ Hz, 8-H), 7.76 (s, 1H, C=C–H), 7.81(dd, 1H, $J_{7,8} = 8.40$ Hz, $J_{7,5} = 2.40$ Hz, 7-H), 7.97 (d, 1H, $J_{5,7} = 2.40$ Hz, 5-H), 8.97 (s, 1H, 2-H); MS (ESI^+) *m/z* (rel. intensity): 402 (M + H, 100%). Anal. Calcd. for $C_{20}H_{19}NO_6S$: C 59.84, H 4.77, N 3.49, S 7.99%; found: C 59.61, H 5.05, N 3.52, S 7.96%.

*4.2.5. Ethyl{2,4-dioxo-5-[(6-nitro-4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl}acetate (**IIe**)*

Yield (%): 46.9, m.p.: 205 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1658 (γ pyrone CO); ^1H NMR (δ ppm): 1.19 (t, 3H, CH_3), 4.15 (q, 2H, CH_2), 4.46 (s, 2H, CH_2CO), 7.76 (s, 1H, C═C−H), 8.00 (d, 1H, $J_{8,7} = 8.80$ Hz, 8-H), 8.62 (dd, 1H, $J_{7,8} = 8.80$ Hz, $J_{7,5} = 2.40$ Hz, 7-H), 8.78 (d, 1H, $J_{5,7} = 2.80$ Hz, 5-H), 9.05 (s, 1H, 2-H); MS (ESI^+) *m/z* (rel. intensity): 405 (M + H, 100%). Anal. Calcd. for $C_{17}H_{12}N_2O_8S$: C 50.50, H 2.99, N 6.93, S 7.93%; found: C 50.19, H 3.14, N 6.87, S 7.80%.

*4.2.6. General synthesis of compounds **IIIa–e***

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

*4.2.7. {2,4-Dioxo-5-[(4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl}acetic acid (**IIIa**)*

Yield (%): 70.5, m.p.: 285 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1634 (γ pyrone CO); ^1H NMR (δ ppm): 4.33 (s, 2H, CH_2CO), 7.56 (ddd, 1H, 6-H), 7.73 (s, 1H, C═C−H), 7.74 (d, 1H, $J_{8,7} = 8.40$ Hz, 8-H), 7.87 (ddd, 1H, 7-H), 8.12 (dd, 1H, $J_{5,6} = 8.00$ Hz, $J_{5,7} = 1.60$ Hz, 5-H), 8.96 (s, 1H, 2-H); MS (ESI^+) *m/z* (rel. intensity): 332 (M + H, 100%). Anal. Calcd. for $C_{15}H_9NO_6S$: C 54.38, H 2.74, N 4.23, S 9.68%; found: C 54.06, H 3.05, N 4.18, S 9.36%.

*4.2.8. {2,4-Dioxo-5-[(6-methyl-4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl}acetic acid (**IIIb**)*

Yield (%): 70.3, m.p.: 268 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1652 (γ pyrone CO); ^1H NMR (δ ppm): 2.46 (s, 3H, CH_3), 4.35 (s, 2H, CH_2CO), 7.66 (d, 1H, $J_{8,7} = 8.80$ Hz, 8-H), 7.72(dd, 1H, $J_{7,8} = 8.80$ Hz, $J_{7,5} = 2.00$ Hz, 7-H), 7.76 (s, 1H, C═C−H), 7.94 (d, 1H, 5-H), 8.95 (s, 1H, 2-H); MS (ESI^-) *m/z* (rel. intensity): 344 (M − H, 100%). Anal. Calcd. for $C_{16}H_{11}NO_6S \cdot 0.2H_2O$: C 55.07, H 3.27, N 4.02, S 9.18%; found: C 54.94, H 3.38, N 4.04, S 9.17%.

*4.2.9. {2,4-Dioxo-5-[(6,8-dimethyl-4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl} acetic acid (**IIIc**)*

Yield (%): 75.5, m.p.: 337 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1652 (γ pyrone CO); ^1H NMR (δ ppm): 2.41 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.35 (s, 2H, CH_2CO), 7.59 (s, 1H, 7-H), 7.76 (s, 1H, C═C−H), 7.77 (s, 1H, 5-H), 8.98 (s, 1H, 2-H); MS (ESI^+) *m/z* (rel. intensity): 360 (M + H, 100%). Anal. Calcd. for $C_{17}H_{13}NO_6S$: C 56.82, H 3.65, N 3.90, S 8.92%; found: C 56.58, H 3.81, N 3.92, S 8.93%.

*4.2.10. {2,4-Dioxo-5-[(6-isopropyl-4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl} acetic acid (**IIId**)*

Yield (%): 82.2, m.p.: 230 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1653 (γ pyrone CO); ^1H NMR (δ ppm): 1.26 (d, 6H, CH_3), 3.08–3.11 (m, 1H, CH), 4.35 (s, 2H, CH_2CO), 7.69 (d, 1H, $J_{8,7} = 8.80$ Hz, 8-H), 7.76 (s, 1H, C═C−H), 7.81(dd, 1H, $J_{7,8} = 8.40$ Hz, $J_{7,5} = 2.40$ Hz, 7-H), 7.97 (d, 1H, $J_{5,7} = 2.40$ Hz, 5-H), 8.97 (s, 1H, 2-H); MS (ESI^+) *m/z* (rel. intensity): 374 (M + H, 100%). Anal. Calcd. for $C_{18}H_{15}NO_6S \cdot 0.1H_2O$: C 57.62, H 4.05, N 3.73, S 8.56%; found: C 57.48, H 4.28, N 3.77, S 8.47%.

*4.2.11. {2,4-Dioxo-5-[(6-nitro-4-oxo-4H-chromen-3-yl)methylene]-1,3-thiazolidine-3yl}acetic acid (**IIIe**)*

Yield (%): 86.4, m.p.: 238 °C. *Spectroscopic analysis*. IR (cm^{-1}): 1660 (γ pyrone CO); ^1H NMR (δ ppm): 4.37 (s, 2H, CH_2CO), 7.77 (s, 1H, C═C−H), 8.02 (d, 1H, $J_{8,7} = 9.20$ Hz, 8-H), 8.64 (dd, 1H, $J_{7,8} = 8.80$ Hz, $J_{7,5} = 2.40$ Hz, 7-H), 8.80 (d, 1H, $J_{5,7} = 3.20$ Hz, 5-H), 9.06 (s, 1H, 2-H), 13.42 (s, 1H, COOH); MS (ESI^-) *m/z* (rel. intensity): 375 (M − H, 100%). Anal. Calcd. for $C_{15}H_8N_2O_8S$: C 47.88, H 2.14, N 7.44, S 8.52%; found: C 47.92, H 2.30, N 7.46, S 8.48%.

4.3. Biological activity studies

4.3.1. 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 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.

4.3.2. Isolation of aldose reductase enzyme

The aldose reductase enzyme was isolated by a method described below. Thirty pooled kidneys, which were obtained from 200–250 g albino rats, were thawed on ice and homogenized with three volume of distilled water, followed by centrifugation at 10 000 × 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 10 000 × 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 10 000 × 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 10 000 × g for 20 min. Protein concentration was measured by the method of Bradford [30] using bovine serum albumin as the standard. Protein concentration is 7.18 ± 0.08 mg/mL.

4.3.3. 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 an 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 of it 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. Reading were taken at intervals in the periods when the changes in absorbance were linear [31].

References

[1] J.Á. de la Fuente, S. Manzanaro, Nat. Prod. Rep. 20 (2003) 243–251.
[2] R. Maccari, R. Ottanà, R. Ciurleo, M.G. Vigorita, D. Rakowitz, T. Steindl, T. Langer, Bioorg. Med. Chem. Lett. 14 (2007) 3886–3893.
[3] E.C. Leal, A.R. Santiago, A.F. Ambrosio, Curr. Drug Target. CNS Neurol. Disord. 4 (2005) 421–434.
[4] S.K. Srivastava, K.V. Ramana, A. Bhatnagar, Endocr. Rev. 26 (2005) 380–392.
[5] S. Yamagishi, T. Imaizumi, Curr. Pharm. Des. 11 (2005) 2279–2299.
[6] P.A. Kumar, G.B. Reddy, Exp. Eye Res. (2006) 1–2.
[7] M.J. Peterson, R. Sarges, C.E. Aldinger, D.P. McDonald (4.Suppl 1), Metabolism 28 (1979) 456–461.
[8] F.P. Kador, J.H. Kinoshita, N.E. Sharpless, J. Med. Chem. 28 (1985) 841–849.
[9] V.F. Carvalho, E.O. Barreto, M.F. Serra, R.S.B. Cordeiro, M.A. Martins, Z.B. Fortes, P.M.R. Silva, Eur. J. Pharm. 549 (2006) 173–178.
[10] T. Sohda, Y. Momose, K. Meguro, Y. Kawamatsu, Y. Sugiyama, H. Ikeda, Arzneim. Forsch. 40 (1990) 37–42.
[11] Y. Iwamoto, T. Kuzuya, A. Matsuda, T. Awata, S. Kumakura, G. Inooka, I. Shiraishi, Diabetes Care 14 (1991) 1083–1086.
[12] S.L. Suter, J.J. Nolan, P. Wallace, B. Gumbiner, J.M. Olefsky, Diabetes Care 15 (1992) 193–203.
[13] L. Costantino, G. Rastelli, M.C. Gamberoni, D. Barlocco, Exp. Opin. Ther. Pat. 10 (2000) 1245–1262.
[14] P. Fresneau, M. Cussac, J.M. Morand, B. Szymonski, D. Tranqui, G. Leclerc, J. Med. Chem. 41 (1998) 4706–4715.
[15] V.M. Malikov, M.P. Yuldashev, Chem. Nat. Compd. 38 (2002) 358–406.
[16] T. Nagao, F. Abe, J. Kinjo, H. Okabe, Biol. Pharm. Bull. 7 (2002) 875–879.
[17] G.R. Beecher, J. Nutr. 133 (2003) 3248S–3253S.
[18] Z.Q. Liu, X.Y. Luo, Y.X. Sun, W. Wu, C.M. Liu, Z.Q. Liu, S.Y. Liu, J. Pharm. Pharmacol. 56 (12) (2004) 1557–1562.
[19] M. Mazzei, R. Dondero, E. Sottofattori, E. Melloni, R. Minafra, Eur. J. Med. Chem. 36 (11–12) (2001) 851–861.
[20] J. Nawrot-Modranka, E. Nawrot, J. Graczyk, Eur. J. Med. Chem. 41 (11) (2006) 1301–1309.
[21] F.A. Jimenez-Orozco, J.S. Lopez-Gonzalez, A. Nieto-Rodriquez, M.A. Velasco-Velazquez, J.A. Molina-Guarneros, N. Mendoza-Patino, M.J. Garcia-Mondragon, P. Elizalde-Galvan, F. Leon-Cedeno, J.J. Mandoki, Lung Cancer 34 (2001) 185–194.
[22] H. Matsuda, T. Morikawa, I. Toguchida, M. Yoshikawa, Chem. Pharm. Bull. 50 (2002) 788–795.
[23] H. Xie, T. Wang, H. Matsuda, T. Morikawa, M. Yoshikawa, T. Tani, Chem. Pharm. Bull. 53 (2005) 1416–1422.
[24] J.M. Petrash, S.K. Srivastava, Biochimica et Biophysica Acta 707 (1982) 105–114.
[25] A. Bhatnagar, S. Liu, B. Das, N.H. Ansari, S.K. Srivastava, Biochem. Pharmacol. 39 (1990) 1115–1124.
[26] M.C.A. DeLima, D.L.B. Costa, A.J.S. Goes, S.L. Galdino, I.R. Pitta, C. Luu-Duc, Pharmazie 4 (1992) 182–184.
[27] S.M. Rida, H.M. Salama, I.M. Labouta, Y.S.A. Ghany, Pharmazie 40 (1985) 727–728.
[28] A. Urzhumtsev, F. Tete-Favier, A. Mitschler, J. Barbanton, P. Barth, L. Urzhumtseva, J.F. Biellmann, A. Podjarny, D.A. Moras, Structure 5 (1997) 601–612.
[29] Y.S. Lee, Z. Chen, P.F. Kador, Bioorg. Med. Chem. 6 (1998) 1811–1819.
[30] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[31] K.J. Cerelli, D.L. Curtis, J.P. Dunn, P.H. Nelson, T.M. Peak, L.D. Waterbury, J. Med. Chem. 29 (1986) 2347–2351.