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Original article

Synthesis and aldose reductase inhibitory activity of a new series of 5-[[2-(ω-carboxyalkoxy)aryl]methylene]-4-oxo-2-thioxothiazolidine derivatives

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Abstract – A new series of 5-[[2-(ω-carboxyalkoxy)aryl]methylene]-4-oxo-2-thioxothiazolidine derivatives was synthesized and evaluated for their potency as aldose reductase inhibitors (ARIs). Their activities were examined in terms of their inhibitory effect on rat lens aldose reductase in vitro and in terms of the preventive effect on sorbitol accumulation in the sciatic nerve of streptozotocin (STZ)-induced diabetic rats in vivo. Of these compounds, some of the naphthylmethylene thiazolidine derivatives were comparable to Zenarestat in the inhibitory potency in vitro and in vivo. In particular, compound **30** was 1.5 times more potent than Zenarestat in the in vivo activity, and had an adequate potency for clinical development. © 1999 Éditions scientifiques et médicales Elsevier SAS

5-[[2-(ω-carboxyalkoxy)aryl]methylene]-4-oxo-2-thioxothiazolidine derivatives / aldose reductase inhibitory activity / sorbitol accumulation inhibition

1. Introduction

Aldose reductase (AR), which is the rate limiting enzyme of the polyol pathway, is implicated in diabetic complications. Since AR has a low substrate affinity for glucose, the activity of the polyol pathway is very low at normal physiological glucose concentrations [1]. However, when there is elevated glucose concentration in diabetes, excessive sorbitol production from glucose by AR is thought to cause cellular damage as a result of osmotic imbalance [2]. This effect leads to the development of diabetic complications such as neuropathy, retinopathy, nephropathy, and cataract formation [3–6].

Recently, numerous compounds have been selected as potential aldose reductase inhibitors (ARIs) [7–11], whose representatives are shown in *figure 1*. These compounds possess an acidic proton which is attached to the imidic nitrogen or an acetic acid moiety in the molecule [12, 13]. Thus, we gave our attention to the compounds having a 2-(ω-carboxyalkoxy)aryl moiety and synthesized those compounds. In this paper, we report the synthesis and AR inhibitory activity of the 5-[[2-(ω-carboxyalkoxy)aryl]methylene]-4-oxo-2-thioxo-thiazolidine derivatives **1** shown in *figure 1*.

2. Chemistry

The 4-substitued-2-formylphenoxyacetic acid derivatives were prepared according to the method of Emmott and Livingstone [14] with slight modifications, as shown in *figure 2*. The 5-substitued-salicylaldehydes **2–4** were alkylated with ethyl bromoacetate in the presence of finely ground potassium carbonate, followed by hydrolysis under the alkaline condition to give 4-substitued 2-formylphenoxyacetic acids **5–7**.

The ω-(1-formyl-2-naphthyloxy)alkanoic acid derivatives **9–10** were prepared in a similar manner as described above.

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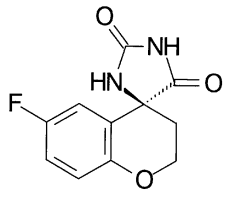

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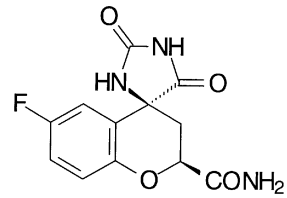

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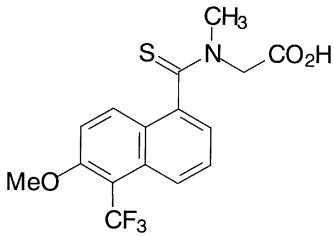

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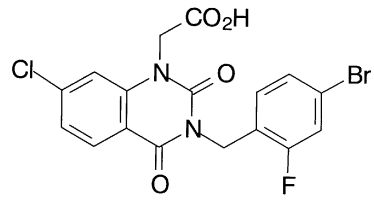

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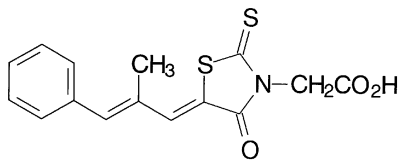

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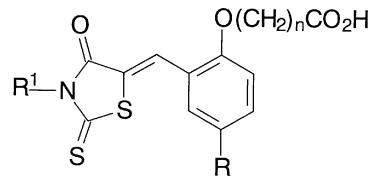

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Figure 1. Potential aldose reductase inhibitors.

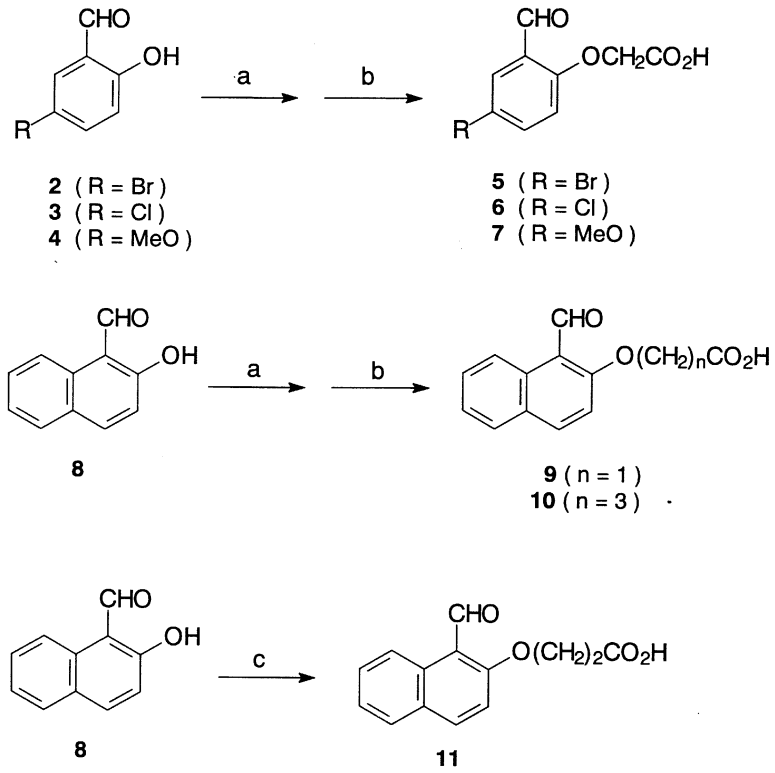
The 3-(1-formyl-2-naphthyloxy)propionic acid **11** was prepared by the alkylation of the aldehyde **8** with β-propiolactone in the presence of sodium hydroxide [15].

The 5-(2-carboxymethoxybenzylidene)thiazolidine derivatives **13–24** were prepared according to the method of Baranov and Komaritsa [16], as shown in *figure 3*. Condensation of the 4-substitued-2-formylphenoxyacetic acids **12**, **5**, **6**, or **7** with rhodanine under refluxing, in the presence of 2 eq. anhydrous sodium acetate in acetic acid, gave compound **13**, **16**, **19**, or **22**. Moreover, condensation of those with N-methylrhodanine gave compound **14**, **17**, **20**, or **23**, and with rhodanine-3-acetic acid likewise gave compound **15**, **18**, **19**, or **24**, respectively.

The 5-[(2-ω-carboxyalkoxy)naphthylmethylene]thiazolidine derivatives **25–30** were prepared in a similar manner as described above.

The configuration of the exocyclic double bond of those thiazolidine derivatives were determined by ^{1}H-NMR and ^{13}C-NMR spectroscopy according to the method of Fresneau et al. and Isida et al. [17, 18].

The 2:3 mixture of compounds **28** and **31** were obtained by the photoirradation (fluorescent lamp) of **28** in methanol, as shown in *figure 4*. However, attempted separation of **28** and **31** failed since smooth reisomerization (**31** to **28**) occured during separation. ^{1}H-NMR and ^{13}C-NMR experiments on the mixture of compound **28** and **31** disclosed the following results. (1) Although the C_5 proton of compound **28** showed a signal at δ 8.26 due to the anisotropic effect by the carbonyl group (C_4=O) of the thiazolidine ring, the same proton signal of **31** appeared at δ 7.91 in ^{1}H-NMR spectroscopy. (2) Although the coupling constant value of the C_4 carbon of compound **28** had J = 6.5 Hz, the same value of **31** had



Reagents: a) K_2CO_3/toluene, tris(dioxa-3,6-heptyl)amine, $BrCH_2CO_2Et$; b) 1 N NaOH/dioxane; c) β-propiolactone, NaOH/H_2O.

Figure 2. Synthesis of compounds **5**–**7**, **9**, **10** and **11**.

J = 12.4 Hz in ^{13}C-NMR spectroscopy. From these results, we confirmed that the exocyclic double bond of compound **28** had Z configuration.

Moreover, we postulated that the configuration of other thiazolidine compounds had Z form on the basis of NMR data.

3. Pharmacology

The AR inhibitory activity of the synthesized compounds was assessed by measuring the inhibition of the enzymatic activity in a partially purified rat lens preparation [1]. The inhibitory activity was expressed as the concentration (nM) of the test compound which inhibited the activity of AR by 50% (IC_{50}). The in vivo AR inhibitory activity of the test compounds were also evaluated by measuring their ability to inhibit the sorbitol accumulation in the sciatic nerve of STZ-induced diabetic rats [19].

4. Results and discussion

The in vitro AR inhibitory activity of the benzylidene thiazolidine derivatives **13–24** are shown in *table I*. When R^1 is hydrogen or a carboxymethyl group, introduction of the substituent R resulted in an increase of AR inhibitory activity by 1 order of the potency as judged from IC_{50} values (i.e. compound **13** vs. **16**, **19** or **22**; or compound **15** vs. **18**, **21** or **24**). When R^1 is a methyl group however, introduction of the substituent R (i.e. compound **14** vs. **17**, **20** or **23**) had no influence on the AR inhibitory activity. Compounds **14** and **19–23**, which inhibited the rat AR in vitro at IC_{50} values of 10 nM order, were equipotent compared with the reference compound Zenarestat. Furthermore, compounds **16**, **17** and **18** were 2 times more potent than Zenarestat. However, these compounds were inactive in inhibiting the sorbitol accumulation in the sciatic nerve of STZ-induced diabetic rats at 100 mg/kg p.o. (*table II*).

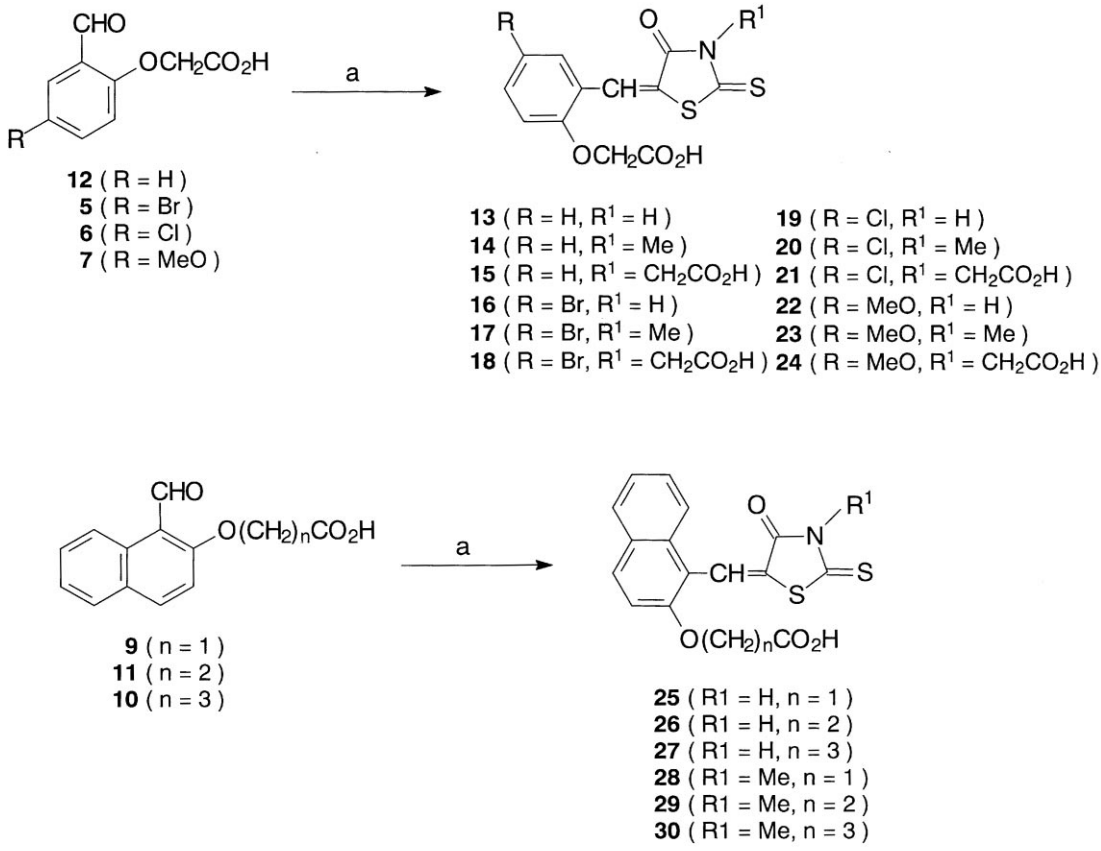


Figure 3. Synthesis of thiazolidine derivatives **13**–**24** and **25**–**30**.

The in vitro AR inhibitory activity of the naphthylmethylene thiazolidine derivatives **25–30** are shown in *table III*. When R^1 is hydrogen, the increase in the number of methylene groups gave a remarkable decrease of the in vitro activity (i.e. compound **25** and **26** vs. **27**). When R^1 is a methyl group, the increase in the number of methylene groups gave a moderate decrease of the in vitro activity (i.e. compound **28** vs. **29** vs. **30**). Of these compounds, compound **25** was equipotent to Zenarestat, and compound **26** was 3 times more potent than Zenarestat.

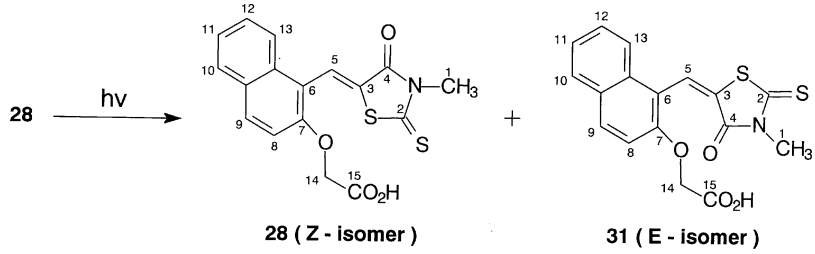


Figure 4. Photoirradiation of derivative **28**.

Table I. Chemical and biological data, in vitro, of benzylidene thiazolidine derivatives.

Compound	M.p. (°C)	Recryst. solvent	Formula	AR inhibition in vitro: rat lens AR[a] IC_{50} (nM)[b]
13	> 250	EtOH-H_2O	$C_{12}H_9NO_4S \cdot 0.2H_2O$	560
14	220–221	AcOH-H_2O	$C_{13}H_{11}NO_4S_2$	27
15	> 250	EtOH-H_2O	$C_{14}H_{10}NNaO_6S_2 \cdot H_2O$	170
16	> 250	$(CH_3)_2CO$-H_2O	$C_{12}H_8BrNO_4S_2$	17
17	243–246	$(CH_3)_2CO$-H_2O	$C_{13}H_{10}BrNO_4S_2$	16
18	204–208	AcOH-H_2O	$C_{14}H_{10}BrNO_6S_2$	18
19	> 250	$(CH_3)_2CO$-H_2O	$C_{12}H_8ClNO_4S_2$	29
20	237–239	$(CH_3)_2CO$-H_2O	$C_{13}H_{10}ClNO_4S_2$	18
21	209–212	AcOH-H_2O	$C_{14}H_{10}ClNO_6S_2 \cdot 0.5H_2O$	21
22	226–228	MeOH-H_2O	$C_{13}H_{11}NO_5S_2 \cdot 0.25H_2O$	38
23	215–216	$(CH_3)_2CO$-H_2O	$C_{14}H_{13}NO_5S_2$	19
24	213–215	AcOH-H_2O	$C_{15}H_{13}NO_7S_2$	86
Zenarestat				36

[a]AR: aldose reductase. [b]The concentration of test compounds required for 50% inhibition of AR.

The in vivo inhibitory activity of the naphthylmethylene thiazolidine derivatives on the sorbitol accumulation in the sciatic nerve of STZ-induced diabetic rats is shown in *table II*. When R^1 is hydrogen, the increase in the number of methylene groups gave a moderate decrease of the in vivo activity (i.e. compound **25** vs. **26**). When R^1 is a methyl group, the increase in the number of methylene groups unexpectedly gave a moderate increase of the in vivo activity. Of these, compound **25** had equipotent in vivo activity to Zenarestat. Of particular interest is that the in vivo activity of compound **30** was more potent than that of Zenarestat, although it was 20 times less potent than Zenarestat in vitro. Therefore, we postulate that compound **30** possessed good oral absorption and efficient tissue penetration properties because of the increased number of methylene groups.

In conclusion, we have reported in this article 5-[[2-(ω-carboxyalkoxy)aryl]methylene]-4-oxo-2-thioxothiazolidine derivatives having biological activities which were comparable to the in vitro and in vivo inhibitory activities of Zenarestat. Of these, compound **30** was 1.5 times more potent than Zenarestat in the in vivo activity, and had an adequate potency for clinical development.

Table II. Biological data, in vivo, of benzylidene thiazolidine and naphthylmethylene thiazolidine derivatives.

Compound	Dose (mg/kg)	Aldose reductase inhibition: in vivo % inhibition[a]
13	100	5.8 ± 3.1
16	100	NS
17	100	NS
18	100	NS
25	100	20.1 ± 4.8
26	100	8.0 ± 4.8
28	30	11.7 ± 5.8
29	100	8.6 ± 7.1
30	100	34.6 ± 6.4
Zenarestat	100	21.8 ± 7.2

[a]Percent inhibition of sorbitol accumulation in the sciatic nerve of streptozotocin-induced diabetic rats. Test compounds were orally given at the single dose indicated. Values are mean ± SEM; mean of 4-6 rats. NS, no significant inhibition.

Table III. Chemical and biological data, in vitro, of naphthylmethylene thiazolidine derivatives.

Compound	M.p. (°C)	Recryst. solvent	Formula	AR inhibition in vitro: rat lens AR[a] IC_{50} (nM)[b]
25	231–234	AcOH-H_2O	$C_{16}H_{11}NO_4S_2$	33
26	212–216	AcOH-H_2O	$C_{17}H_{13}NO_4S_2$	11
27	195–198	AcOEt-hexane	$C_{18}H_{15}NO_4S_2$	1 100
28	206–209	$(CH_3)_2CO$-H_2O	$C_{17}H_{13}NO_4S_2$	180
29	165–168	$(CH_3)_2CO$-H_2O	$C_{18}H_{15}NO_4S_2$	630
30	93–95	AcOEt-hexane	$C_{19}H_{17}NO_4S_2 \cdot 0.25H_2O$	700
Zenarestat				36

[a]AR: aldose reductase. [b]The concentration of test compounds required for 50% inhibition of AR.

5. Experimental protocols

5.1. Chemistry

Melting points (m.p.) were determined on a Yanagimoto micro-melting apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a Varian Gemini-200 spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in δ values from internal tetramethylsilane. Splitting patterns are designated as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; br s, broad singlet; m, multiplet. Coupling constants are reported in hertz (Hz). Infrared (IR) spectra were recorded on a Shimazu FTIR-8200PC spectrophotometer. Elemental analyses were carried out on a Perkin-Elmer 2400 element analyzer and results obtained for specified elements were within ± 0.4% of the theoretical values. Visualization was accomplished with UV light. Unless otherwise noted, all commercially available materials were used without further purification.

5.1.1. Typical procedure for the preparation of 2-formylphenoxy acetic acids **5–7**

5.1.1.1. (4-Chloro-2-formylphenoxy)acetic acid **6**

5-Chlorosalicylaldehyde (**3**; 6.3 g, 40 mmol) was dissolved in dry toluene (90 mL) and finely ground potassium carbonate (5.6 g, 40 mmol) were added. After the mixture was heated at 100 °C for 4 h, tris(dioxa-3,6-heptyl)amine (1.3 mL, 4.1 mmol) and ethyl bromoacetate (5.6 mL, 50 mmol) were added. The mixture was heated at 100 °C for 6 h, filtered through Celite and washed with toluene. The filtrate was washed with water, and brine, dried (anhydrous $MgSO_4$), and evaporated. The residue was dissolved in dioxane (50 mL), and 1 N NaOH (50 mL) was added. The mixture was heated at 100 °C for 1 h. After cooling to room temperature, the reaction mixture was diluted with water (150 mL) and then acidified with concentrated HCl on an ice bath, and the precipitated solid was collected by filtration. The solid was recrystallized from acetone/H_2O to give the title compound as a solid (5.3 g, 62%). M.p. 174–175 °C (lit. 173–174 °C) [20].

The following compounds were prepared in the same way as described above:

(4-Bromo-2-formylphenoxy)acetic acid **5** (prepared from 5-bromosalicylaldehyde **2**; recrystallized from acetone/H_2O). M.p. 172–174 °C (lit. 174–176 °C) [20]. (2-Formyl-4-methoxyphenoxy)acetic acid **7** (prepared from 5-methoxysalicylaldehyde **4**; recrystallized from acetone/H_2O). M.p. 156–159 °C (lit. 157–159 °C) [20].

5.1.2. (1-Formyl-2-naphthyloxy)acetic acid **9**

2-Hydroxy-1-naphthylaldehyde (**8**; 32 g, 0.2 mol) was dissolved in dry toluene (200 mL) and finely ground potassium carbonate (15 g, 0.11 mol) was added. After the mixture was heated at 100 °C for 4 h, tris(dioxa-3,6-heptyl)amine (4 mL, 12.5 mmol) and ethyl bromoacetate (28 mL, 0.25 mmol) were added. The mixture was heated at 100 °C for 4 h, filtered through Celite and washed with toluene. The filtrate was washed with water and brine, dried (anhydrous $MgSO_4$), and evaporated. The residue was dissolved in dioxane (250 mL) and 1 N NaOH (250 mL) was added. The mixture was heated at 100 °C for 1 h. After cooling to room temperature, the reaction mixture was diluted with water (700 mL) and then acidified with concentrated HCl on an ice bath, and the precipitated solid was collected by filtration. The solid was recrystallized from AcOEt/n-hexane to give the title compound as a solid (15 g, 65%): M.p. 176–179 °C (lit. 176–177 °C) [14].

5.1.3. 4-(1-Formyl-2-naphthyloxy)butyric acid **10**

Compound **10** was synthesized in the same way as in 5.1.2. Yield 34%. M.p. 179–180 °C (recrystallized from AcOEt/n-hexane); ^{1}H-NMR (DMSO-d_6) δ 2.00–2.18 (2H, m), 2.47 (2H, t, J = 7.5 Hz), 4.35 (2H, t, J = 7.5 Hz), 7.40–7.70 (3H, m), 7.90–8.00 (1H, m), 8.27 (1H, d, J = 9 Hz), 9.07–9.17 (1H, m), 10.7 (1H, s), 12.18 (1H, br s); IR (KBr, cm^{-1}) 1 705 (CO), 1 672 (CO). Anal. calcd. for $C_{15}H_{14}O_4$: C 69.76; H 5.46; found: C 69.65; H 5.40.

5.1.4. 3-(1-Formyl-2-naphthyloxy)propionic acid **11**

A solution of β-propiolactone (7.6 g, 0.1 mol) in water (10 mL) was added dropwise to a stirred solution of 2-hydroxy-1-naphthylaldehyde (**8**; 17.2 g, 0.1 mol) and NaOH (4 g, 0.1 mol) in water (50 mL) at 100 °C, and the mixture was stirred for a further 30 min at 100 °C. After cooling to room temperature, the reaction mixture was acidified with concentrated HCl on an ice bath, and then extracted three times with diethyl ether. The combined ether extracts were shaken three times with saturated $NaHCO_3$. The solid, which was precipitated on acidification of the alkaline solution, was filtered, washed with water, dried, and recrystallized from AcOEt/n-hexane to give the title compound as a solid (4.2 g, 17%). M.p. 157–159 °C (recrystallized from AcOEt/n-hexane); ^{1}H-NMR (DMSO-d_6) δ 2.84 (2H, t, J = 6 Hz), 4.52 (2H, t, J = 6 Hz), 7.42–7.52 (1H, m), 7.58–7.72 (2H, m), 7.92–8.02 (1H, m), 8.30 (1H, d, J = 9 Hz), 9.07–9.17 (1H, m), 10.72 (1H, s), 12.48 (1H, br s); IR (KBr, cm^{-1}) 1 705 (CO), 1 672 (CO). Anal. calcd. for $C_{14}H_{12}O_4$: C 68.85; H 4.95; found: C 68.74; H 4.88.

5.1.5. 5-(5-Bromo-2-carboxymethoxybenzylidene)-4-oxo-2-thioxothiazolidine **16**

A mixture of 4-Bromo-2-formylphenoxyacetic acid (**5**; 6.0 g, 23.2 mmol), rhodanine (3.7 g, 27.8 mmol) and anhydrous sodium acetate (3.8 g, 46.3 mmol) in acetic acid (80 mL) was refluxed for 17 h. Upon cooling, the reaction mixture was diluted with water and stirred at room temperature for a further 1 h. The solid was filtrated, and added to dilute HCl, then the mixture was stirred at room temperature for 1 h. After filtration, the solid was recrystallized from acetone/H_2O to give the title compound as a yellow solid (5.9 g, 68%). M.p. > 250 °C; ^{1}H-NMR (DMSO-d_6) δ 4.90 (2H, S), 7.06 (1H, d, J = 9 Hz), 7.49 (1H, d, J = 2.5 Hz), 7.63 (1H, dd, J = 2.5, 9 Hz), 7.76 (1H, s), 13.20 (1H, br s), 13.88 (1H, br s); IR (KBr, cm^{-1}) 1 714 (CO). Anal. calcd. for $C_{12}H_8BrNO_4S_2$: C 38.51; H 2.15; N 3.74; S 17.14; Br 21.35; found: C 38.57; H 1.92; N 3.73; S 17.29; Br; 21.50.

5.1.6. 5-(2-Carboxymethoxybenzylidene)-4-oxo-2-thioxothiazolidine **13**

Compound **13** was synthesized in the same way as in 5.1.5. Yield 32%. M.p. > 250 °C (recrystallized from aq. EtOH); ^{1}H-NMR (DMSO-d_6) δ 4.88 (2H, S), 7.03–7.18 (2H, m), 7.38–7.52 (2H, m), 7.90 (1H, s), 13.20 (1H, br s), 13.74 (1H, br s); IR (KBr, cm^{-1}) 1 705 (CO), 1 672 (CO). Anal. calcd. for $C_{12}H_9NO_4S_2{\cdot}0.2H_2O$: C 48.21; H 3.17; N 3.74; S 21.45; found: C 48.49; H 2.93; N 4.74; S 21.27.

5.1.7. 5-(2-Carboxymethoxybenzylidene)-3-methyl-4-oxo-2-thioxothiazolidine **14**

Compound **14** was synthesized in the same way as in 5.1.5. Yield 77%. M.p. 220–221 °C (recrystallized from AcOH/H_2O); ^{1}H-NMR (DMSO-d_6) δ 3.43 (3H, s), 4.90 (2H, s), 7.04–7.20 (2H, m), 7.43–7.55 (2H, m), 8.07 (1H, s), 13.18 (1H, br s); IR (KBr, cm^{-1}) 1 753 (CO), 1 689 (CO). Anal. calcd. for $C_{13}H_{11}NO_4S_2$: C 50.47; H 3.58; N 4.53; S 20.73; found: C 50.17; H 3.56; N 4.62; S 21.00.

5.1.8. [5-(2-Carboxymethoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]-acetic acid mono sodium salt **15**

Compound **15** was synthesized in the same way as in 5.1.5. Yield 11%. M.p. > 250 °C (recrystallized from aq. EtOH); ^{1}H-NMR (DMSO-d_6) δ 4.50 (2H, s), 4.69 (2H, s), 6.95–7.15 (2H, m), 7.41–7.51 (2H, m), 8.06 (1H, s); IR (KBr, cm^{-1}) 1 716 (CO). Anal. calcd. for $C_{14}H_{10}NNaO_6S_2{\cdot}H_2O$: C 42.75; H 3.07; N 3.56; S 16.30; Na 5.84; found: C 42.82; H 3.07; N 3.59; S 16.01; Na 5.54.

5.1.9. 5-(5-Bromo-2-carboxymethoxybenzylidene)-3-methyl-4-oxo-2-thioxothiazolidine **17**

Compound **17** was synthesized in the same way as in 5.1.5. Yield 67%. M.p. 243–246 °C (recrystallized from acetone/H_2O); ^{1}H-NMR (DMSO-d_6) δ 3.41 (3H, s), 4.93 (2H, s), 7.07 (1H, d, J = 9 Hz), 7.53 (1H, d, J = 2.5 Hz), 7.65 (1H, dd, J = 2.5, 9 Hz) 7.91 (1H, s), 13.24 (1H, br s); IR (KBr, cm^{-1}) 1 705 (CO). Anal. calcd. for $C_{13}H_{10}BrNO_4S_2$: C 40.22; H 2.60; N 3.61; S 16.52; Br 20.58; found: C 40.15; H 2.61; N 3.49; S 16.58; Br 20.55.

5.1.10. [5-(5-Bromo-2-carboxymethoxybenzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid **18**

Compound **18** was synthesized in the same way as in 5.1.5. Yield 55%. M.p. 204–208 °C (recrystallized from AcOH/H_2O); ^{1}H-NMR (DMSO-d_6) δ 4.75 (2H, s), 4.95 (2H, s), 7.09 (1H, d, J = 9 Hz), 7.60 (1H, d, J = 2.5 Hz), 7.96 (1H, dd, J = 2.5, 9 Hz) 7.96 (1H, s), 13.36 (2H, br s); IR (KBr, cm^{-1}) 1 712 (CO). Anal. calcd. for $C_{14}H_{10}BrNO_6S_2$: C 38.90; H 2.33; N 3.24; S 14.84; Br 18.49; found: C 38.73; H 2.39; N 3.15; S 14.59; Br 18.26.

5.1.11. 5-(5-Chloro-2-carboxymethoxybenzylidene)-4-oxo-2-thioxothiazolidine **19**

Compound **19** was synthesized in the same way as in 5.1.5. Yield 62%. M.p. > 250 °C (recrystallized from acetone/H_2O); ^{1}H-NMR (DMSO-d_6) δ 4.90 (2H, s), 7.14 (1H, d, *J* = 9 Hz), 7.44 (1H, d, *J* = 2.5 Hz), 7.52 (1H, dd, *J* = 2.5, 9 Hz) 7.84 (1H, s), 13.24 (1H, br s), 13.88 (1H, br s); IR (KBr, cm^{-1}) 1 733 (CO), 1 683 (CO). Anal. calcd. for $C_{12}H_8ClNO_4S_2$: C 43.71; H 2.45; N 4.25; S 19.45; Cl 10.75; found: C 43.79; H 2.42; N 4.19; S 19.12; Cl 10.75.

5.1.12. 5-(5-Chloro-2-carboxymethoxybenzylidene)-3-methyl-4-oxo-2-thioxothiazolidine **20**

Compound **20** was synthesized in the same way as in 5.1.5. Yield 58%. M.p. 237–239 °C (recrystallized from acetone/H_2O); ^{1}H-NMR (DMSO-d_6) δ 3.43 (3H, s), 4.93 (2H, s), 7.14 (1H, d, *J* = 9 Hz), 7.42 (1H, d, *J* = 2.5 Hz), 7.54 (1H, dd, *J* = 2.5, 9 Hz) 7.93 (1H, s), 13.24 (1H, br s); IR (KBr, cm^{-1}) 1 705 (CO). Anal. calcd. for $C_{13}H_{10}ClNO_4S_2$: C 45.42; H 2.93; N 4.07; S 18.65; Cl 10.31; found: C 45.43; H 2.89; N 4.02; S 18.92; Cl 10.11.

5.1.13. [5-(5-Chloro-2-carboxymethoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]-acetic acid **21**

Compound **21** was synthesized in the same way as in 5.1.5. Yield 64%. M.p. 209–212 °C (recrystallized from AcOH/H_2O); ^{1}H-NMR (DMSO-d_6) δ 4.75 (2H, s), 4.95 (2H, s), 7.15 (1H, d, *J* = 9 Hz), 7.49 (1H, d, *J* = 2.5 Hz), 7.55 (1H, dd, *J* = 2.5, 9 Hz) 7.97 (1H, s), 13.36 (1H, br s); IR (KBr, cm^{-1}) 1 716 (CO). Anal. calcd. for $C_{14}H_{10}ClNO_6S_2 \cdot 0.5H_2O$: C 42.38; H 2.79; N 3.53; S 16.16; Cl 8.93; found: C 42.66; H 2.65; N 3.45; S 15.83; Cl 8.76.

5.1.14. 5-(2-Carboxymethoxy-5-methoxybenzylidene)-4-oxo-2-thioxothiazolidine **22**

Compound **22** was synthesized in the same way as in 5.1.5. Yield 8%. M.p. 226–228 °C (recrystallized from MeOH/H_2O); ^{1}H-NMR (DMSO-d_6) δ 3.78 (3H, s), 4.83 (2H, s), 6.88 (1H, d, *J* = 2.5 Hz), 7.03 (1H, d, *J* = 9 Hz), 7.08 (1H, dd, *J* = 2.5, 9 Hz), 7.87 (1H, s), 13.10 (1H, br s), 13.84 (1H, br s); IR (KBr, cm^{-1}) 1 751 (CO), 1 695 (CO). Anal. calcd. for $C_{13}H_{11}NO_5S_2 \cdot 0.25H_2O$: C 47.34; H 3.51; N 4.25; S 19.44; found: C 47.39; H 3.19; N 4.38; S 19.22.

5.1.15. 5-(2-Carboxymethoxy-5-methoxybenzylidene)-4-oxo-2-thioxothiazolidine **23**

Compound **23** was synthesized in the same way as in 5.1.5. Yield 35%. M.p. 215–216 °C (recrystallized from acetone/H_2O); ^{1}H-NMR (DMSO-d_6) δ 3.43 (3H, s), 3.80 (3H, s), 4.83 (2H, s), 6.92 (1H, d, *J* = 2.5 Hz), 7.05 (1H, d, *J* = 9 Hz), 7.10 (1H, dd, *J* = 2.5, 9 Hz) 8.03 (1H, s), 13.12 (1H, br s); IR (KBr, cm^{-1}) 1 712 (CO). Anal. calcd. for $C_{14}H_{13}NO_5S_2$: C 49.55; H 3.86; N 4.13; S 18.90; found: C 49.36; H 3.59; N 4.03; S 18.73.

5.1.16. [5-(2-Carboxymethoxy-5-methoxybenzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid **24**

Compound **24** was synthesized in the same way as in 5.1.5. Yield 40%. M.p. 213–215 °C (recrystallized from acetone/H_2O); ^{1}H-NMR (DMSO-d_6) δ 3.80 (3H, s), 4.75 (2H, s), 4.84 (2H, s), 6.97 (1H, d, *J* = 2.5 Hz), 7.06 (1H, d, *J* = 9 Hz), 7.12 (1H, dd, *J* = 2.5, 9 Hz), 8.07 (1H, s), 13.30 (2H, br s); IR (KBr, cm^{-1}) 1 716 (CO). Anal. calcd. for $C_{15}H_{13}NO_7S_2$: C 46.99; H 3.42; N 3.65; S 16.73; found: C 47.09; H 3.26; N 3.75; S 16.67.

5.1.17. 5-(2-Carboxymethoxy-1-naphthylmethylene)-4-oxo-2-thioxothiazolidine **25**

Compound **25** was synthesized in the same way as in 5.1.5. Yield 50%. M.p. 231–234 °C; ^{1}H-NMR (DMSO-d_6) δ 5.03 (2H, s), 7.40 (1H, d, *J* = 9 Hz), 7.43–7.53 (1H, m), 7.57–7.67 (1H, m), 7.82–7.90 (1H, m), 7.92–8.00 (1H, m), 8.08 (1H, d, *J* = 9 Hz), 8.09 (1H, s), 13.20 (1H, br s), 13.70 (1H, br s); IR (KBr, cm^{-1}) 1 712 (CO), 1 662 (CO). Anal. calcd. for $C_{16}H_{11}NO_4S_2$: C 55.64; H 3.21; N 4.06; S 18.57; found: C 55.74; H 3.49; N 3.97; S 18.49.

5.1.18. 5-[2-(2-carboxyethoxy)-1-naphthylmethylene]-4-oxo-2-thioxothiazolidine **26**

Compound **26** was synthesized in the same way as in 5.1.5. Yield 31%. M.p. 212–216 °C (recrystallized from AcOH/H_2O); ^{1}H-NMR (DMSO-d_6) δ 2.78 (2H, t, *J* = 6 Hz), 4.48 (2H, t, *J* = 6 Hz), 7.42–7.66 (2H, m), 7.57 (1H, d, *J* = 9 Hz), 7.78–7.86 (1H, m), 7.94–8.02 (1H, m), 8.00 (1H, s), 8.11 (1H, d, *J* = 9 Hz), 12.38 (1H, br s), 13.66 (1H, br s); IR (KBr, cm^{-1}) 1 716 (CO), 1 683 (CO). Anal. calcd. for $C_{17}H_{13}NO_4S_2$: C 56.81; H 3.65; N 3.90; S 17.84; found: C 56.55; H 3.82; N 3.71; S 17.49.

5.1.19. 5-[2-(3-carboxypropoxy)-1-naphthylmethylene]-4-oxo-2-thioxothiazolidine **27**

Compound **27** was synthesized in the same way as in 5.1.5. Yield 58%. M.p. 195–198 °C (recrystallized from AcOEt/n-hexane); ^{1}H-NMR (DMSO-d_6) δ 1.93–2.10 (2H, m), 2.40 (2H, t, *J* = 7.5 Hz), 4.31 (2H, t, *J* = 7.5 Hz), 7.41–7.66 (2H, m), 7.55 (1H, d, *J* = 9 Hz) 7.79–7.87 (1H, m), 7.93–8.00 (1H, m), 8.05 (1H, s), 8.10 (1H, d, *J* = 9 Hz), 12.15 (1H, br s), 13.68 (1H, br s); IR (KBr, cm^{-1}) 1 705 (CO). Anal. calcd. for $C_{18}H_{15}NO_4S_2$: C 57.89; H 4.05; N 3.75; S 17.17; found: C 57.94; H 4.11; N 3.71; S 16.88.

5.1.20. 5-(2-carboxymethoxy-1-naphthylmethylene)-3-methyl-4-oxo-2-thioxothiazolidine **28**

Compound **28** was synthesized in the same way as in 5.1.5. Yield 60%. M.p. 206–209 °C; ^{1}H-NMR (DMSO-d_6) δ 3.43 (3H, s), 5.02 (2H, s), 7.42 (1H, d, J = 9 Hz), 7.43–7.53 (1H, m), 7.57–7.67 (1H, m), 7.82–7.91 (1H, m), 7.93–8.01 (1H, m), 8.10 (1H, d, J = 9 Hz), 8.26 (1H, s), 13.22 (1H, br s); IR (KBr, cm^{-1}) 1 757 (CO), 1 668 (CO). Anal. calcd. for $C_{17}H_{13}NO_4S_2$: C 56.81; H 3.65; N 3.90; S 17.84; found: C 56.67; H 3.54; N 3.76; S 18.14.

5.1.21. 5-[2-(2-carboxyethoxy)-1-naphthylmethylene]-3-methyl-4-oxo-2-thioxothiazolidine **29**

Compound **29** was synthesized in the same way as in 5.1.5. Yield 28%. M.p. 165–168 °C (recrystallized from acetone/H_2O); ^{1}H-NMR (DMSO-d_6) δ 2.78 (2H, t, J = 6 Hz), 3.43 (3H, s), 4.48 (2H, t, J = 6 Hz), 7.43–7.66 (2H, m), 7.59 (1H, d, J = 9 Hz), 7.79–7.87 (1H, m), 7.94–8.02 (1H, m), 8.13 (1H, d, J = 9 Hz), 8.18 (1H, s), 12.38 (1H, br s); IR (KBr, cm^{-1}) 1 716 (CO). Anal. calcd. for $C_{18}H_{15}NO_4S_2$: C 57.89; H 4.05; N 3.75; S 17.17; found: C 57.77; H 4.21; N 3.56; S 16.70.

5.1.22. 5-[2-(3-carboxypropoxy)-1-naphthylmethylene]-3-methyl-4-oxo-2-thioxothiazolidine **30**

Compound **30** was synthesized in the same way as in 5.1.5. Yield 43%. M.p. 93–95 °C (recrystallized from AcOEt/n-hexane); ^{1}H-NMR (DMSO-d_6) δ 1.80–2.20 (2H, m), 2.40 (2H, t, J = 7.5 Hz), 3.44 (3H, s), 4.30 (2H, t, J = 7.5 Hz), 7.42–7.66 (2H, m), 7.56 (1H, d, J = 9 Hz), 7.80–7.88 (1H, m), 7.94–8.01 (1H, m), 8.12 (1H, d, J = 9 Hz), 8.23 (1H, s), 12.14 (1H, br s); IR (KBr, cm^{-1}) 1 716 (CO), 1 695 (CO). Anal. calcd. for $C_{19}H_7NO_4S_2 \cdot 0.25H_2O$: C 58.22; H 4.50; N 3.57; S 16.36; found: C 58.31; H 4.35; N 3.60; S 16.67.

5.2. Pharmacology

5.2.1. Preparation of aldose reductase

Rat lens AR was prepared according to the method of Hayman and Kinoshita [1] with slight modifications. The lenses were homogenized in 250 mM phosphate buffer (pH 7.4) containing 2 mM mercaptoethanol at 0–4 °C, and the homogenate was centrifuged at 20 000 *g* for 30 min. The supernatant was subjected to a 40–60% ammonium sulfate fractionation. The resultant precipitate was dissolved in 5 mM phosphate buffer (pH 7.4) containing 2 mM mercaptoethanol and used for enzyme assay. One unit (U) of AR was defined as the enzyme activity which oxidizes 1 μmol of NADPH in 1 min under the assay conditions described below.

5.2.2. Inhibition of aldose reductase in vitro

The reaction mixture consisted of 100 mM phosphate buffer (pH 6.5), 0.2 mM NADPH, 1.5 mM D, L-glyceraldehyde, 0.4 M lithium sulfate, 7.0 mU/mL of enzyme and test compounds at various concentrations. The reaction mixture was incubated at 37 °C, and the absorbance at 340 nm was measured with a spectrophotometer (Model 150-20, Hitachi Ltd., Japan). The enzyme activity was estimated on the basis of its decrease in the absorbance over a period of 1 min. The concentration of compounds required for 50% inhibition of enzyme activity (IC_{50}) was estimated graphically from the log concentration-inhibition curve.

5.2.3. Inhibition of sorbitol accumulation in vivo

Male Wistar rats (200–250 g) were rendered diabetic by an intravenous injection of streptozotocin (40 mg/kg), which had been freshly dissolved in physiological saline. After 7 days, the rats were divided into various groups with 4–6 animals/group, and orally given test compounds suspended in 0.5% tragacanth, or an equivalent volume of 0.5% tragacanth. The rats were sacrificed 4 h after the administration of the test compounds. Tissue sorbitol levels were determined according to the method of Clements et al. [17] with slight modifications.

The sciatic nerve sample (30–60 mg) was quickly dissected from the hind limb, placed into water (1.0 mL/40 mg of tissue), heated in a boiling bath for 2 min, and then homogenized with a Polytron instrument in 6% perchloric acid (1 mL/10 mg of tissue). The homogenate was centrifuged at 1 050 *g* for 15 min at 4 °C. The supernatant was neutralized with 2 M K_2CO_3 and used as tissue extract for the assaying of sorbitol. Sorbitol was assayed by an enzymatic method in which sorbitol dehydrogenase catalyses the stoichiometric conversion of NAD by sorbitol to a fluorogenic product, NADH. The reaction mixture contained 30 mM glycine buffer (pH 9.4), 1.3 mM NAD, 1.3 U/mL sorbitol dehydrogenase and 1.0 mL of the tissue extract in a total volume of 3 mL. After the mixture was allowed to stand for 60 min at room temperature, the fluorescence intensity was measured at 365 nm excitation wavelength and 430 nm emission wavelength using a fluorospectrophotometer (F3000, Hitachi Ltd., Japan). The sorbitol concentration was quantitated by comparison with standards of sorbitol. The sorbitol content in the sciatic nerve of each animal was expressed as nmole/wet weight.

The activity of test compounds was expressed as the percent inhibition of sorbitol accumulation at a given dose, which was calculated according to the following equation:

$$\% \text{ inhibition} = (S - T) / (S - N) \times 100$$

where S is the sorbitol content in the sciatic nerve of untreated diabetic control rats, T is the sorbitol content in the sciatic nerve of diabetic rats given test compounds and N is the sorbitol content in the sciatic nerve of age-matched non-diabetic control rats.

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