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Synthesis and Aldose Reductase Inhibitory Activity of 2-Substituted 6-Fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-diones

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Optically active and racemic 2-substituted 6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-diones were synthesized stereoselectively from (+)-, (−)-, and (±)-6-fluoro-3,4-dihydro-4-oxo-2*H*-1-benzopyran-2-carboxylic acid [(+)-1, (−)-1, and (±)-1], respectively, for evaluation as new aldose reductase inhibitors. Among these compounds, the 2*S*,4*S* compounds were found to be more potent inhibitors of aldose reductase *in vitro* and *in vivo* than the corresponding 2*R*,4*R* enantiomers. The chloromethyl compound [(+)-5] showed highly potent activities in inhibiting cataract formation in the lenses and polyol accumulation in the sciatic nerve of rats.

Keywords aldose reductase inhibitor; spirohydantoin; polyol accumulation; cataract formation; diastereoselective hydantoin formation

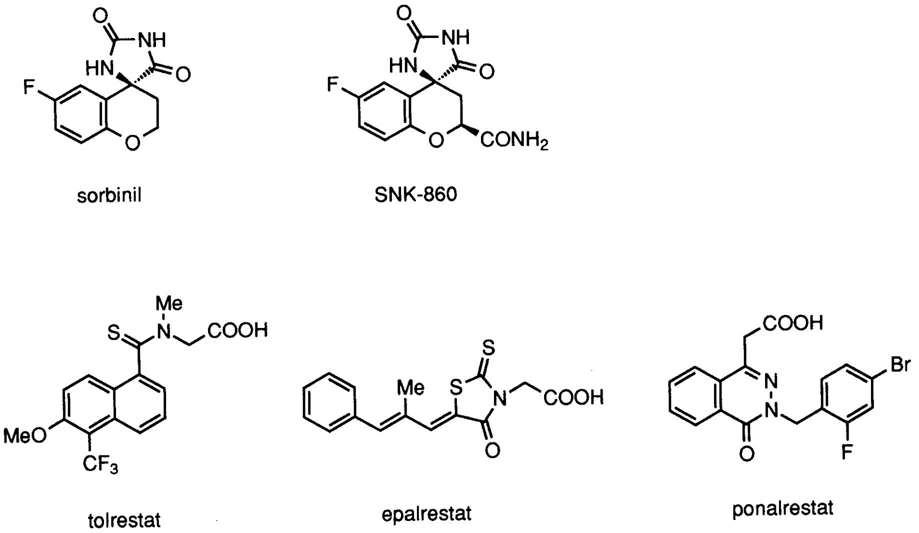
The pathogenesis of diabetic cataracts has been shown to involve intracellular hyperosmolality, which is due to elevated sorbitol levels in the lens.[1] Aldose reductase (AR, EC 1.1.1.21) converts glucose to sorbitol and is the rate-limiting enzyme in the sorbitol pathway. The elevated sorbitol pathway flux has been implicated as the cause of other diabetic complications.[2] A number of aldose reductase inhibitors (ARIs) have been shown to be effective in treating diabetic cataracts and neuropathy in rodent models.[3]

Recent potent ARIs can be classified, as shown in Fig. 1, into two main types: spirohydantoins including sorbinil,[4] and acetic acids such as tolrestat,[5] epalrestat,[6] and ponalrestat.[7] Our attention has been focussed on structural variations of the sorbinil molecule, and we found a new ARI, (2*S*,4*S*)-6-fluoro-2,3-dihydro-2′,5′-dioxospiro-[4*H*-1-benzopyran-4,4′-imidazolidine]-2-carboxamide (SNK-860), which shows highly potent *in vitro* and *in vivo* activities.[8] Further, the 2*S*,4*S* compound (SNK-860) was the most potent among the four stereoisomers at the C-2 and C-4 positions. Therefore, in the course of studies on 6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione derivatives possessing the 2*S*,4*S* configuration, we introduced various substituents other than a carboxamide group at the C-2 position.

In this paper, we report our studies on the synthesis and AR inhibitory activity of optically active and racemic 2-substituted 6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-diones (**4**—**8**).

Chemistry

The synthetic routes to the optically active key intermediates (+)- and (−)-**2a** are shown in Chart 1. Diastereoselective hydantoin formation of the chiral (+)-**1**[9] by treatment with potassium cyanide and ammonium carbonate under the Bucherer–Bergs condition[10] afforded a 3 : 1 mixture of spirohydantoins [(+)-**2a** and (+)-**2b**] in 96.7% yield. Recrystallization of the mixture from water afforded optically pure (+)-**2a**. The absolute configuration of (+)-**2a** was determined to be 2*S*,4*S* by single-crystal X-ray analysis.[11] The enantiomer [(−)-**2a**] was obtained from the chiral (−)-**1**[9] by the same pro-



sorbinil

SNK-860

tolrestat

epalrestat

ponalrestat

Fig. 1. Some Potent Aldose Reductase Inhibitors

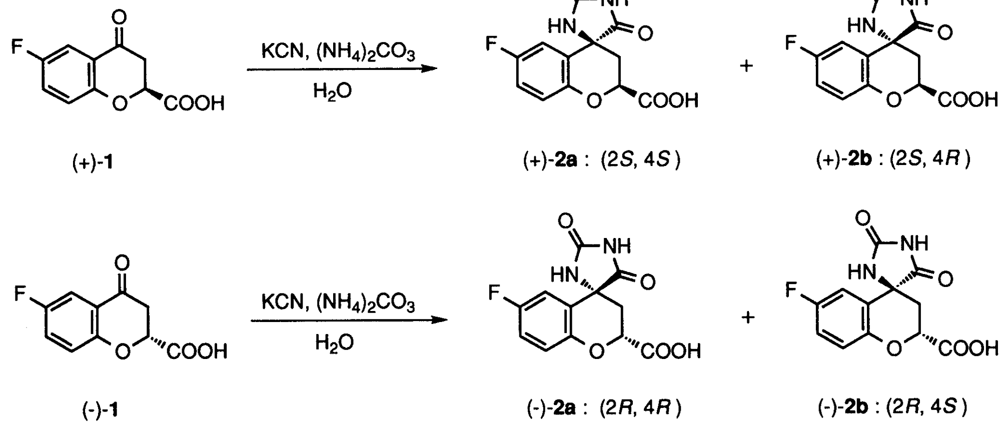


Chart 1

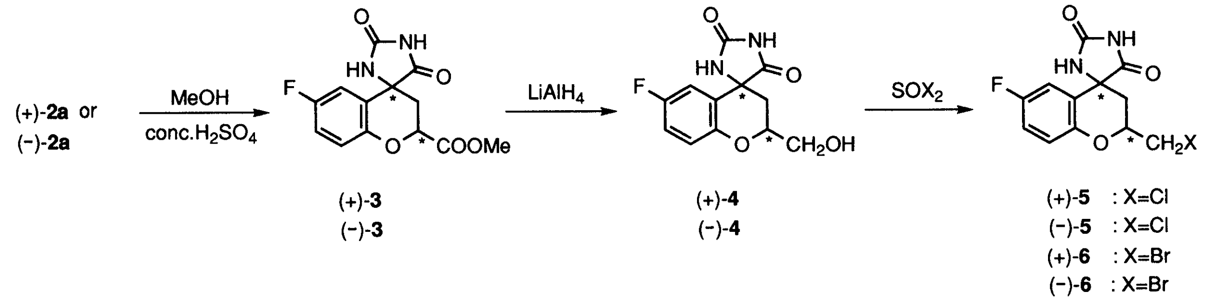


Chart 2

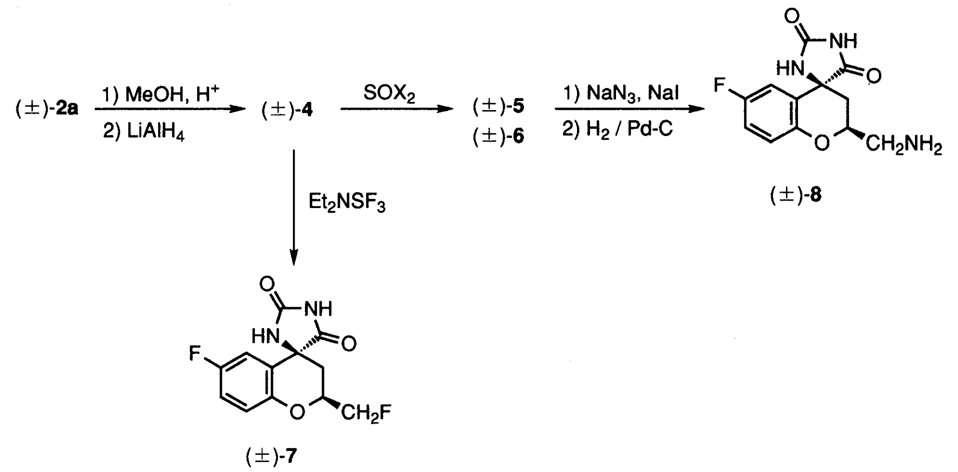


Chart 3

cedure.

(2*S*,4*S*)-2-Substituted 6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-diones and their enantiomers were synthesized as shown in Chart 2. Esterification of (+)-**2a** by treatment with methanol in the presence of a catalytic amount of concentrated sulfuric acid afforded the methyl ester [(+)-**3**] in a quantitative yield. The hydroxymethyl compound [(+)-**4**] was obtained by reduction of (+)-**3** with lithium aluminum hydride, and the halogenomethyl compounds [(+)-**5** and (+)-**6**] were obtained by halogenation with thionyl chloride and thionyl bromide, respectively. The enantiomers [(−)-**3**, (−)-**4**, (−)-**5**, and (−)-**6**] were obtained similarly from compound (−)-**2**.

Racemic spirohydantoins [(±)-**4**, (±)-**5**, and (±)-**6**] were prepared from the racemic (±)-**1**$^{9)}$ by a procedure similar to that described for the optically active spirohydantoins (Chart 3). The fluoromethyl compound [(±)-**7**] was obtained from (±)-**4** by fluorination with diethylaminosulfur trifluoride (DAST).$^{12)}$ The aminomethyl compound [(±)-**8**] was obtained from (±)-**5** by azidation following hydrogenation.

Results and Discussion

These compounds were evaluated for *in vitro* inhibitory activity against AR isolated from rat lens and for the

Table I. Aldose Reductase Inhibitory Activity of Racemic Spirohydantoins

Compd. No.	*In vitro*	*In vivo*[a]	
	IC_{50}[b] ($\times 10^{-7}$ M)	Lens inhibit. (%)	Nerve inhibit. (%)
(±)-**4**	6.9	12	23
(±)-**5**	0.9	100	87
(±)-**6**	1.3	100	63
(±)-**7**	3.8	Death	Death
(±)-**8**	>100	Inactive	Inactive
(±)-Sorbinil	3.9	100	33

a) Inhibitory percentage at the dose of 10 mg/kg in the case of 8-day administration. *b*) The 50% inhibitory concentration on aldose reductase extracted from rat lens.

Table II. Aldose Reductase Inhibitory Activity of Optically Active Spirohydantoins

Compd. No.	*In vitro*	*In vivo*	
	IC_{50}[a] ($\times 10^{-7}$ M)	Lens[b] inhibit. (%)	Nerve[c] ED_{50} (mg/kg)
(+)-**4**	2.9	59	39.5
(−)-**4**	145	Inactive	Inactive
(+)-**5**	0.47	100	0.6
(−)-**5**	298	Inactive	Inactive
(+)-**6**	0.68	100	2.2
(−)-**6**	272	Inactive	Inactive
(+)-Sorbinil	2.5	100	4—16

a) The 50% inhibitory concentration on aldose reductase extracted from rat lens. *b*) Inhibitory percentage at the dose of 10 mg/kg in the case of 8-day administration. *c*) The 50% effective dose in the case of 8-day administration.

ability to inhibit polyol accumulation in the sciatic nerve and cataract formation in the lens of galactosemic rats after oral administration.

The biological data are summarized in Tables I and II. The chloromethyl compound [(+)-**5**] showed the most potent activity among the compounds. Although the halogenomethyl compounds [(+)-**5** and (+)-**6**] are less active *in vitro* than SNK-860 (IC_{50} = 35 nM), they are more effective *in vivo* than SNK-860. It is particularly noteworthy that the halogenomethyl compounds [(+)-**5** and (+)-**6**] showed highly potent inhibition of both polyol accumulation and cataract formation. The optically active 2*S*,4*S* compounds are far more active *in vitro* and *in vivo* than the corresponding 2*R*,4*R* enantiomers. Thus, it is apparent that the absolute configuration at the C-2 and C-4 positions is very important. Further, it is suggested that the AR inhibitory activity of spirohydantoins increases as the C-2 substituent becomes more lipophilic.

Experimental

All melting points were measured on a Yanaco MP-1 apparatus and are uncorrected. Optical rotations were obtained with a Jasco DIP-310 polarimeter. Infrared (IR) spectra were recorded on a Jasco IR-810 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM GSX-270 (270 MHz) spectrometer in dimethyl sulfoxide-d_6 (DMSO-d_6) with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in ppm, and the following abbreviations are used; s = singlet, d = doublet, t = triplet, dd = double doublet, dt = double triplet, m = multiplet, br = broad. Mass spectra (MS) were obtained on a JEOL JMS-DX300 spectrometer. Elemental analyses were performed with a Yanaco CHN CORDER MT-3. Column chromatography was performed on silica gel (Kieselgel 60, 70—230 mesh, Merck).

(2*S*,4*S*)-(+)-6-Fluoro-2,3-dihydro-2′,5′-dioxospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2-carboxylic Acid [(+)-2a] and (2*S*,4*R*)-(+)-6-Fluoro-2,3-dihydro-2′,5′-dioxospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2-carboxylic Acid [(+)-2b] A mixture of (+)-**1** (54.8 g, 0.261 mol), potassium cyanide (34.0 g, 0.522 mol) and ammonium carbonate (150 g, 1.56 mol) in water (450 ml) was stirred at 65—70 °C for 24 h and then at 80—90 °C for 1 h. After cooling to room temperature, the reaction mixture was acidified to pH 1 with concentrated hydrochloric acid. The resulting crystals were collected by filtration, washed with water and dried to afford 70.6 g (96.7%) of a diastereomeric mixture (3 : 1) of (+)-**2a** and (+)-**2b**. The diastereomeric mixture was recrystallized from water to afford 45.8 g (62.7%) of (+)-**2a**, mp 146 °C, $[\alpha]_D^{27}$ +194° (c = 1.0, MeOH). IR (KBr): 1787 and 1718 (C=O of hydantoin), 1735 (COOH) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.12 (1H, dd, J = 13.7, 12.2 Hz, $C_{3\beta}$-H), 2.54 (1H, dd, J = 13.7, 2.0 Hz, $C_{3\alpha}$-H), 5.19 (1H, dd, J = 12.2, 2.0 Hz, C_2-H), 6.97 (1H, dd, J = 8.8, 2.9 Hz, C_5-H), 6.99 (1H, dd, J = 8.8, 4.4 Hz, C_8-H), 7.14 (1H, dt, J = 8.8, 2.9 Hz, C_7-H), 8.34 (1H, br s, 3′-NH), 10.99 (1H, br s, 1′-NH). MS m/z: 280 (M^+). *Anal.* Calcd for $C_{12}H_9FN_2O_5\cdot H_2O$: C, 48.33; H, 3.72; N, 9.39. Found: C, 48.58; H, 3.76; N, 9.39.

The filtrate was extracted with ethyl acetate (200 ml × 3) and the organic layer was washed with water, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residue was recrystallized twice from ethyl acetate to afford 6.85 g (8.0%) of (+)-**2b**, mp 215 °C, $[\alpha]_D^{26}$ +144° (c = 1.0, MeOH). IR (KBr): 1762 (C=O of hydantoin), 1726 (C=O of hydantoin and COOH) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (1H, dd, J = 13.7, 12.2 Hz, $C_{3\beta}$-H), 2.43 (1H, dd, J = 13.7, 2.4 Hz, $C_{3\alpha}$-H), 5.19 (1H, dd, J = 12.2, 2.4 Hz, C_2-H), 6.87 (1H, dd, J = 8.8, 2.9 Hz, C_5-H), 6.99 (1H, dd, J = 8.8, 4.9 Hz, C_8-H), 7.16 (1H, dt, J = 8.8, 2.9 Hz, C_7-H), 8.81 (1H, br s, 3′-NH), 11.12 (1H, br s, 1′-NH). MS m/z: 280 (M^+). *Anal.* Calcd for $C_{12}H_9FN_2O_5$: C, 51.43; H, 3.24; N, 10.00. Found: C, 51.60; H, 2.99; N, 10.21.

(2*R*,4*R*)-(−)-6-Fluoro-2,3-dihydro-2′,5′-dioxospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2-carboxylic Acid [(−)-2a] and (2*R*,4*S*)-(−)-6-Fluoro-2,3-dihydro-2′,5′-dioxospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2-carboxylic Acid [(−)-2b] Compounds (−)-**2a** and (−)-**2b** were obtained from (−)-**1** by a procedure similar to that described for the (+)-isomers, in yield of 56.7% and 9.0%, respectively. (−)-**2a**: mp 145 °C, $[\alpha]_D^{27}$ −193° (c = 1.0, MeOH). IR (KBr): 1787 and 1718 (C=O of hydantoin), 1735 (COOH) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.12 (1H, dd, J = 13.7, 12.2 Hz, $C_{3\beta}$-H), 2.54 (1H, dd, J = 13.7, 2.0 Hz, $C_{3\alpha}$-H), 5.19 (1H, dd, J = 12.2, 2.0 Hz, C_2-H), 6.97 (1H, dd, J = 8.8, 2.9 Hz, C_5-H), 6.99 (1H, dd, J = 8.8, 4.4 Hz, C_8-H), 7.14 (1H, dt, J = 8.8, 2.9 Hz, C_7-H), 8.34 (1H, br s, 3′-NH), 10.99 (1H, br s, 1′-NH). MS m/z: 280 (M^+). *Anal.* Calcd for $C_{12}H_9FN_2O_5\cdot H_2O$: C, 48.33; H, 3.72; N, 9.39. Found: C, 48.22; H, 3.89; N, 9.59. (−)-**2b**: mp 214 °C, $[\alpha]_D^{27}$ −143° (c = 1.0, MeOH). IR (KBr): 1762 (C=O of hydantoin), 1726 (C=O of hydantoin and COOH) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.27 (1H, dd, J = 13.7, 12.2 Hz, $C_{3\beta}$-H), 2.43 (1H, dd, J = 13.7, 2.4 Hz, $C_{3\alpha}$-H), 5.19 (1H, dd, J = 12.2, 2.4 Hz, C_2-H), 6.87 (1H, dd, J = 8.8, 2.9 Hz, C_5-H), 6.99 (1H, dd, J = 8.8, 4.9 Hz, C_8-H), 7.16 (1H, dt, J = 8.8, 2.9 Hz, C_7-H), 8.81 (1H, br s, 3′-NH), 11.12 (1H, br s, 1′-NH). MS m/z: 280 (M^+). *Anal.* Calcd for $C_{12}H_9FN_2O_5$: C, 51.43; H, 3.24; N, 10.00. Found: C, 51.56; H, 3.45; N, 10.11.

(2*S*,4*S*)-(+)-6-Fluoro-2,3-dihydro-2′,5′-dioxospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2-carboxylic Acid Methyl Ester [(+)-3] Concentrated sulfuric acid (30.0 g, 0.306 mol) was added to a solution of (+)-**2a** (35.0 g, 0.125 mol) in methanol (1300 ml), and the mixture was refluxed for 1.5 h. After cooling to room temperature, the resulting crystals were collected by filtration, washed with methanol and dried to afford 33.6 g (91.6%) of (+)-**3**, mp 340 °C, $[\alpha]_D^{20}$ +186° (c = 1.0, *N*,*N*-dimethylformamide (DMF)). IR (KBr): 1790 (C=O of hydantoin), 1740 (C=O of hydantoin and ester) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.15 (1H, dd, J = 13.7, 11.7 Hz, $C_{3\beta}$-H), 2.56 (1H, dd, J = 13.7, 2.4 Hz, $C_{3\alpha}$-H), 3.75 (3H, s, COOMe), 5.29 (1H, dd, J = 11.7, 2.4 Hz, C_2-H), 6.97 (1H, dd, J = 8.8, 2.9 Hz, C_5-H), 7.00 (1H, dd, J = 8.8, 4.4 Hz, C_8-H), 7.14 (1H, dt, J = 8.8, 2.9 Hz, C_7-H), 8.35 (1H, s, 3′-NH), 11.01 (1H, br s, 1′-NH). MS m/z: 294 (M^+). *Anal.* Calcd for $C_{13}H_{11}FN_2O_5$: C, 53.06; H, 3.77; N, 9.52. Found: C, 53.29; H, 3.85; N, 9.34.

(2*R*,4*R*)-(−)-6-Fluoro-2,3-dihydro-2′,5′-dioxospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2-carboxylic Acid Methyl Ester [(−)-3] Compound (−)-**3** was obtained from (−)-**2a** by a procedure similar to that described for (+)-**3**, in 90.0% yield, mp 340 °C, $[\alpha]_D^{20}$ −185° (*c*=1.0, DMF). IR (KBr): 1790 (C=O of hydantoin), 1740 (C=O of hydantoin and ester) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.15 (1H, dd, *J*=13.7, 11.7 Hz, $C_{3\beta}$-H), 2.56 (1H, dd, *J*=13.7, 2.4 Hz, $C_{3\alpha}$-H), 3.75 (3H, s, COOMe), 5.29 (1H, dd, *J*=11.7, 2.4 Hz, C_2-H), 6.97 (1H, dd, *J*=8.8, 2.9 Hz, C_5-H), 7.00 (1H, dd, *J*=8.8, 4.4 Hz, C_8-H), 7.14 (1H, dt, *J*=8.8, 2.9 Hz, C_7-H), 8.35 (1H, s, 3′-NH), 11.01 (1H, br s, 1′-NH). MS *m/z*: 294 (M$^+$). *Anal.* Calcd for $C_{13}H_{11}FN_2O_5$: C, 53.06; H, 3.77; N, 9.52. Found: C, 53.07; H, 3.62; N, 9.56.

(±)-6-Fluoro-2,3-dihydro-2′,5′-dioxospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2-carboxylic Acid Methyl Ester [(±)-3] Compound (±)-**3** was obtained from (±)-**2a** by a procedure similar to that described for (+)-**3**, in 93.4% yield, mp 291 °C. IR (KBr): 1790 and 1730 (C=O of hydantoin), 1745 (C=O of ester) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.16 (1H, dd, *J*=13.7, 11.7 Hz, $C_{3\beta}$-H), 2.57 (1H, dd, *J*=13.7, 2.4 Hz, $C_{3\alpha}$-H), 3.75 (3H, s, COOMe), 5.30 (1H, dd, *J*=11.7, 2.4 Hz, C_2-H), 6.98 (1H, dd, *J*=8.8, 2.9 Hz, C_5-H), 7.01 (1H, dd, *J*=8.8, 4.4 Hz, C_8-H), 7.15 (1H, dt, *J*=8.8, 2.9 Hz, C_7-H), 8.36 (1H, s, 3′-NH), 11.02 (1H, br s, 1′-NH). MS *m/z*: 294 (M$^+$). *Anal.* Calcd for $C_{13}H_{11}FN_2O_5$: C, 53.06; H, 3.77; N, 9.52. Found: C, 53.07; H,3.62; N, 9.56.

(2*S*,4*S*)-(+)-6-Fluoro-2,3-dihydro-2-hydroxymethylspiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(+)-4] A solution of (+)-**3** (11.7 g, 40.0 mmol) in absolute tetrahydrofuran (THF) (100 ml) was added dropwise to a suspension of lithium aluminum hydride (2.30 g, 60.0 mmol) in absolute THF (100 ml) with cooling at 5—10 °C, and the mixture was stirred at 15—20 °C for 20 h. The reaction mixture was poured into ice water (100 ml) and acidified to pH 1 with concentrated hydrochloric acid. The solution was extracted with ethyl acetate (400 ml) and the organic layer was washed with water, dried over Na$_2$SO$_4$, filtered and evaporated *in vacuo*. The residue was recrystallized from ethanol to afford 8.70 g (82.0%) of (+)-**4**, mp 188—189 °C, $[\alpha]_D^{20}$ +222° (*c*=0.60, MeOH). IR (KBr): 1750 and 1720 (C=O of hydantoin) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.97 (1H, dd, *J*=14.2, 11.7 Hz, $C_{3\beta}$-H), 2.19 (1H, dd, *J*=14.2, 2.0 Hz, $C_{3\alpha}$-H), 3.61 (2H, m, CH$_2$O), 4.67 (1H, m, C_2-H), 4.90 (1H, br s, OH), 6.89 (1H, dd, *J*=8.8, 4.9 Hz, C_8-H), 6.92 (1H, dd, *J*=8.8, 2.9 Hz, C_5-H), 7.08 (1H, dt, *J*=8.8, 2.9 Hz, C_7-H), 8.32 (1H, s, 3′-NH), 10.87 (1H, br s, 1′-NH). MS *m/z*: 266 (M$^+$). *Anal.* Calcd for $C_{12}H_{11}FN_2O_4$: C, 54.14; H, 4.16; N, 10.52. Found: C, 54.29; H, 4.25; N, 10.53.

(2*R*,4*R*)-(−)-6-Fluoro-2,3-dihydro-2-hydroxymethylspiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(−)-4] Compound (−)-**4** was obtained from (−)-**3** by a procedure similar to that described for (+)-**4**, in 81.0% yield, mp 188—189 °C, $[\alpha]_D^{20}$ −231° (*c*=0.60, MeOH). IR (KBr): 1750 and 1720 (C=O of hydantoin) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.97 (1H, dd, *J*=14.2, 11.7 Hz, $C_{3\beta}$-H), 2.19 (1H, dd, *J*=14.2, 2.0 Hz, $C_{3\alpha}$-H), 3.60 (2H, m, CH$_2$O), 4.67 (1H, m, C_2-H), 4.90 (1H, br s, OH), 6.89 (1H, dd, *J*=8.8, 4.9 Hz, C_8-H), 6.92 (1H, dd, *J*=8.8, 2.9 Hz, C_5-H), 7.08 (1H, dt, *J*=8.8, 2.9 Hz, C_7-H), 8.32 (1H, s, 3′-NH), 10.87 (1H, br s, 1′-NH). MS *m/z*: 266 (M$^+$). *Anal.* Calcd for $C_{12}H_{11}FN_2O_4$: C, 54.14; H, 4.16; N, 10.52. Found: C, 54.31; H, 4.15; N, 10.54.

(±)-6-Fluoro-2,3-dihydro-2-hydroxymethylspiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(±)-4] Compound (±)-**4** was obtained from (±)-**3** by a procedure similar to that described for (+)-**4**, in 82.0% yield, mp 224—225 °C. IR (KBr): 1760 and 1720 (C=O of hydantoin) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.97 (1H, dd, *J*=14.2, 11.7 Hz, $C_{3\beta}$-H), 2.19 (1H, dd, *J*=14.2, 2.0 Hz, $C_{3\alpha}$-H), 3.60 (2H, m, CH$_2$O), 4.67 (1H, m, C_2-H), 4.90 (1H, t, *J*=5.9 Hz, OH), 6.89 (1H, dd, *J*=8.8, 4.9 Hz, C_8-H), 6.92 (1H, dd, *J*=8.8, 2.9 Hz, C_5-H), 7.08 (1H, dt, *J*=8.8, 2.9 Hz, C_7-H), 8.32 (1H, s, 3′-NH), 10.87 (1H, br s, 1′-NH). MS *m/z*: 266 (M$^+$). *Anal.* Calcd for $C_{12}H_{11}FN_2O_4$: C, 54.14; H, 4.16; N, 10.52. Found: C, 53.98; H, 4.34; N, 10.35.

(2*S*,4*S*)-(+)-2-Chloromethyl-6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(+)-5] Thionyl chloride (280 mg, 2.34 mmol) was added dropwise to a solution of (+)-**4** (600 mg, 2.25 mmol) in DMF (3 ml) and the mixture was stirred at room temperature for 3 h then further at 80 °C for 3 h. After cooling, the reaction mixture was poured into ice water (100 ml) and extracted with ethyl acetate (70 ml). The organic layer was washed with water, dried over Na$_2$SO$_4$, filtered and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 460 mg (72.0%) of (+)-**5**, mp 239—240 °C, $[\alpha]_D^{20}$ +216° (*c*=0.60, MeOH). IR (KBr): 1775 and 1710 (C=O of hydantoin) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.12 (1H, dd, *J*=13.7, 11.7 Hz, $C_{3\beta}$-H), 2.31 (1H, dd, *J*=13.7, 2.0 Hz, $C_{3\alpha}$-H), 3.88 (1H, dd, *J*=11.7, 4.9 Hz, CHCl), 4.00 (1H, dd, *J*=11.7, 3.4 Hz, CHCl), 4.95 (1H, m, C_2-H), 6.94 (1H, dd, *J*=8.8, 3.4 Hz, C_8-H), 6.96 (1H, dd, *J*=8.8, 2.0 Hz, C_5-H), 7.11 (1H, dt, *J*=8.8, 2.0 Hz, C_7-H), 8.37 (1H, s, 3′-NH), 10.95 (1H, br s, 1′-NH). MS *m/z*: 284(M$^+$). *Anal.* Calcd for $C_{12}H_{10}ClFN_2O_3$: C, 50.63; H, 3.54; N, 9.84. Found: C, 50.72; H,3.49; N, 9.94.

(2*R*,4*R*)-(−)-2-Chloromethyl-6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(−)-5] Compound (−)-**5** was obtained from (−)-**4** by a procedure similar to that described for (+)-**5**, in 76.6% yield, mp 239—240 °C, $[\alpha]_D^{20}$ −217° (*c*=0.60, MeOH). IR (KBr): 1775 and 1710 (C=O of hydantoin) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.12 (1H, dd, *J*=13.7, 11.7 Hz, $C_{3\beta}$-H), 2.31 (1H, dd, *J*=13.7, 2.0 Hz, $C_{3\alpha}$-H), 3.88 (1H, dd, *J*=11.7, 4.9 Hz, CHCl), 4.00 (1H, dd, *J*=11.7, 3.4 Hz, CHCl), 4.95 (1H, m, C_2-H), 6.94 (1H, dd, *J*=8.8, 3.4 Hz, C_8-H), 6.96 (1H, dd, *J*=8.8, 2.0 Hz, C_5-H), 7.11 (1H, dt, *J*=8.8, 2.0 Hz, C_7-H), 8.37 (1H, s, 3′-NH), 10.95 (1H, br s, 1′-NH). MS *m/z*: 284(M$^+$). *Anal.* Calcd for $C_{12}H_{10}ClFN_2O_3$: C, 50.63; H, 3.54; N, 9.84. Found: C, 50.46; H, 3.34; N, 9.86.

(±)-2-Chloromethyl-6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(±)-5] Compound (±)-**5** was obtained from (±)-**4** by a procedure similar to that described for (+)-**5**, in 85.1% yield, mp 212—214 °C. IR (KBr): 1780 and 1740 (C=O of hydantoin) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.08 (1H, dd, *J*=13.7, 12.2 Hz, $C_{3\beta}$-H), 2.31 (1H, dd, *J*=13.7, 2.0 Hz, $C_{3\alpha}$-H), 3.88 (1H, dd, *J*=12.2, 4.9 Hz, CHCl), 4.00 (1H, dd, *J*=12.2, 3.4 Hz, CHCl), 4.94 (1H, m, C_2-H), 6.94 (1H, dd, *J*=8.8, 3.4 Hz, C_8-H), 6.96 (1H, dd, *J*=8.8, 2.0 Hz, C_5-H), 7.11 (1H, dt, *J*=8.8, 2.0 Hz, C_7-H), 8.37 (1H, s, 3′-NH), 10.95 (1H, br s, 1′-NH). MS *m/z*: 284 (M$^+$). *Anal.* Calcd for $C_{12}H_{10}ClFN_2O_3$: C, 50.63; H, 3.54; N, 9.84. Found: C, 50.77; H, 3.40; N, 9.71.

(2*S*,4*S*)-(+)-2-Bromomethyl-6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(+)-6] Thionyl bromide (640 mg, 8.27 mmol) was added dropwise to a solution of (+)-**4** (2.00 g, 7.51 mmol) in DMF (15 ml) and the mixture was stirred at room temperature for 2 h and then refluxed for 1.5 h. After cooling, the reaction mixture was poured into ice water (100 ml) and extracted with ethyl acetate (150 ml). The organic layer was washed with water, dried over Na$_2$SO$_4$, filtered and evaporated *in vacuo*. The residue was recrystallized from ethyl acetate to afford 1.74 g (70.4%) of (+)-**6**, mp 226—227 °C, $[\alpha]_D^{20}$ +193° (*c*=0.60, MeOH). IR (KBr): 1780 and 1710 (C=O of hydantoin) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.06 (1H, dd, *J*=13.7, 11.7 Hz, $C_{3\beta}$-H), 2.32 (1H, dd, *J*=13.7, 2.0 Hz, $C_{3\alpha}$-H), 3.76 (1H, dd, *J*=11.2, 4.9 Hz, CHBr), 3.88 (1H, dd, *J*=11.2, 3.4 Hz, CHBr), 4.90 (1H, m, C_2-H), 6.93 (1H, dd, *J*=8.8, 4.4 Hz, C_8-H), 6.96 (1H, dd, *J*=8.8, 2.9 Hz, C_5-H), 7.11 (1H, dt, *J*=8.8, 2.9 Hz, C_7-H), 8.37 (1H, s, 3′-NH), 10.95 (1H, br s, 1′-NH). MS *m/z*: 328 (M$^+$). *Anal.* Calcd for $C_{12}H_{10}BrFN_2O_3$: C, 43.79; H, 3.06; N, 8.51. Found: C, 43.75; H, 2.80; N, 8.63.

(2*R*,4*R*)-(−)-2-Bromomethyl-6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(−)-6] Compound (−)-**6** was obtained from (−)-**4** by a procedure similar to that described for (+)-**6**, in 73.3% yield, mp 226—227 °C, $[\alpha]_D^{20}$ −193° (*c*=0.60, MeOH). IR (KBr): 1780 and 1710 (C=O of hydantoin) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.06 (1H, dd, *J*=13.7, 11.7 Hz, $C_{3\beta}$-H), 2.32 (1H, dd, *J*=13.7, 2.0 Hz, $C_{3\alpha}$-H), 3.76 (1H, dd, *J*=11.2, 4.9 Hz, CHBr), 3.88 (1H, dd, *J*=11.2, 3.4 Hz, CHBr), 4.90 (1H, m, C_2-H), 6.93 (1H, dd, *J*=8.8, 4.4 Hz, C_8-H), 6.96 (1H, dd, *J*=8.8, 2.9 Hz, C_5-H), 7.11 (1H, dt, *J*=8.8, 2.9 Hz, C_7-H), 8.37 (1H, s, 3′-NH), 10.95 (1H, br s, 1′-NH). MS *m/z*: 328 (M$^+$). *Anal.* Calcd for $C_{12}H_{10}BrFN_2O_3$: C, 43.79; H, 3.06; N, 8.51. Found: C, 43.50; H, 2.81; N, 8.53.

(±)-2-Bromomethyl-6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(±)-6] Compound (±)-**6** was obtained from (±)-**4** by a procedure similar to that described for (+)-**6**, in 77.3% yield, mp 209—211 °C. IR (KBr): 1780 and 1740 (C=O of hydantoin) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.07 (1H, dd, *J*=13.7, 11.7 Hz, $C_{3\beta}$-H), 2.33 (1H, dd, *J*=13.7, 2.0 Hz, $C_{3\alpha}$-H), 3.77 (1H, dd, *J*=11.2, 4.9 Hz, CHBr), 3.88 (1H, dd, *J*=11.2, 3.4 Hz, CHBr), 4.90 (1H, m, C_2-H), 6.93 (1H, dd, *J*=8.8, 4.4 Hz, C_8-H), 6.96 (1H, dd, *J*=8.8, 2.9 Hz, C_5-H), 7.11 (1H, dt, *J*=8.8, 2.9 Hz, C_7-H), 8.37 (1H, s, 3′-NH), 10.95 (1H, br s, 1′-NH). MS *m/z*: 328 (M$^+$). *Anal.* Calcd for $C_{12}H_{10}BrFN_2O_3$: C, 43.79; H, 3.06; N, 8.51. Found: C, 43.67; H, 3.02; N, 8.48.

(±)-6-Fluoro-2-fluoromethyl-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(±)-7] A solution of (±)-**4** (7.98 g, 30.0 mmol) in absolute THF (180 ml) was added dropwise to a stirred solution of diethylaminosulfur trifluoride (8.18 g, 50.0 mmol) in absolute tetrahydrofuran (30 ml) at below −50 °C and the mixture was stirred at room temperature for 4.5 h. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in ethyl acetate (200 ml) and water (30 ml). The organic layer was washed with water, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) and then recrystallized from EtOAc–*n*-hexane (1 : 2) to afford 2.10 g (26.3%) of (±)-**7**, mp 183—185 °C. IR (KBr): 1780 and 1730 (C=O of hydantoin) cm^{-1}. ^1H-NMR (DMSO-d_6) δ: 2.02 (1H, dd, J=13.7, 12.2 Hz, $C_{3\beta}$-H), 2.31 (1H, dd, J=13.7, 2.0 Hz, $C_{3\alpha}$-H), 4.47—4.85 (2H, m, CH_2F), 4.92 (1H, m, C_2-H), 6.89—6.98 (2H, m, C_5-H and C_8-H), 7.11 (1H, dt, J=8.8, 2.9 Hz, C_7-H), 8.34 (1H, s, 3′-NH), 10.95 (1H, br s, 1′-NH). MS *m/z*: 268 (M^+). *Anal.* Calcd for $C_{12}H_{10}F_2N_2O_3$: C, 53.73; H, 3.76; N, 10.44. Found: C, 54.29; H, 4.09; N, 9.94.

(±)-2-Aminomethyl-6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione [(±)-8] A mixture of (±)-**5** (4.54 g, 16.0 mmol), sodium iodide (600 mg, 4.00 mmol) and sodium azide (1.47 g, 22.6 mmol) in DMF (20 ml) was refluxed for 1.5 h. After cooling, the reaction mixture was poured into ice water (50 ml) and extracted with ethyl acetate (150 ml). The organic layer was washed with water, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc) to afford 3.06 g (70.1%) of (±)-2-azidomethyl-6-fluoro-2,3-dihydrospiro-[4*H*-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione. A suspension of 20% Pd–C (0.6 g) in 50% aqueous ethanol (20 ml) was treated with a solution of the azidomethyl compound (3.00 g, 10.3 mmol) in ethanol (160 ml) and the mixture was hydrogenated for 16 h at room temperature under atmospheric pressure. After filtration, the filtrate was evaporated *in vacuo*. The residue was recrystallized from ethanol to afford 2.30 g (84.0%) of (±)-**8**, mp 231—233 °C (dec.). IR (KBr): 1775 and 1725 (C=O of hydantoin) cm^{-1}. ^1H-NMR (DMSO-d_6) δ: 1.90 (1H, dd, J=14.2, 11.7 Hz, $C_{3\beta}$-H), 2.25 (1H, dd, J=14.2, 2.0 Hz, $C_{3\alpha}$-H), 2.78 (2H, d, J=5.4 Hz, CH_2N), 4.58 (1H, m, C_2-H), 6.89 (1H, dd, J=8.8, 4.4 Hz, C_8-H), 6.91 (1H, dd, J=8.8, 2.9 Hz, C_5-H), 7.07 (1H, dt, J=8.8, 2.9 Hz, C_7-H), 8.29 (1H, br s, 3′-NH). MS *m/z*: 265 (M^+). *Anal.* Calcd for $C_{12}H_{12}FN_3O_3$: C, 54.34; H,4.56; N, 15.84. Found: C, 54.03; H, 4.52; N, 15.45.

Biological Assays 1. Inhibition of Aldose Reductase Aldose reductase was partially purified from rat lens and its activity was measured by the method of Kador and Sharpless.[13] Assays were performed at 37 °C in 0.1 M sodium phosphate buffer (pH 6.2) containing 1.04 mM nicotinamide adenine diphosphate (NADPH), 15 mM DL-glyceraldehyde and an appropriate amount of enzyme (supernatant of rat lens homogenate) in a total volume of 2 ml. The effect of an inhibitor on the enzyme activity was determined by adding 0.2 ml of 0.4% dimethyl sulfoxide solution of a test compound to the reaction mixture. The concentration required to reduce the enzyme activity by 50% (IC_{50}) was determined from the least-squares regression line in the plot of the logarithm of concentration *vs.* enzyme activity.

2. Inhibition of Galactosemic Cataract Formation and Polyol Accumulation Sprague–Dawley rats were fed 30% galactose diet *ad libitum* throughout the experimental periods using the method of Peterson *et al.*[4a)] A suspension of test compound in 5% arabic gum was administered orally once a day for 8 d. Cataract formation was observed with a slit lamp biomicroscope and the severity was expressed as opacity index. Normal appearance, spotted equatorial vacuolation or ringed equatorial vacuolation, and formation of opaque plaques were assigned opacity index values of 0, 1, and 2, respectively. Sciatic nerves were quickly removed from anesthetized rats 24 h after final administration, weighed, and stored at −80 °C. The frozen tissues were homogenized with 1 ml of 5% (w/v) zinc sulfate containing 10 μg of D-(+)-arabitol, and then neutralized with 1 ml of 0.3 N barium hydroxide. The supernatants obtained by centrifugation at 2500 *g* for 10 min at 4 °C were lyophilized overnight and silylated by the method of Sweeley *et al.*[14] After incubation for 60 min at 80 °C, 1 ml of chloroform and 2 ml of distilled water were added and mixed. The samples were centrifuged at 2500 *g* for 5 min at 4 °C. The lower phases were dried under a stream of nitrogen gas and dissolved in 0.1 ml of carbon disulfide for polyol determination by gas chromatography. Percent inhibition of polyol accumulation or cataract formation was based on mean polyol level or opacity of animals fed normal diet as 0%. ED_{50} values of compounds were determined from a least-squares regression line in the plot of the logarithm of concentration *vs.* activity.

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