Hydantoin Bioisosteres. In Vivo Active Spiro Hydroxy Acetic Acid Aldose Reductase Inhibitors

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The hypothesis that clinical side effects of the aldose reductase inhibitor (ARI) sorbinil were related to its hydantoin ring led to a bioisosteric analysis and replacement of the hydantoin by a spiro hydroxy acetic acid moiety as in 40. These hydroxy acids, compared to hydantoins, showed a similar potency increase on chroman 2-methyl substitution, a similar orthogonal relationship of acidic to aromatic moieties, and similar ARI enantioselectivity. In this series the six-membered spiro hydroxy acetic acid anion array is a bioisostere for a spiro hydantoin anion and leads to ARIs with excellent in vivo activity. In vitro and in vivo activity was improved over 40 by chroman cis 2-methylation as in 4 and by aromatic 6,7-halogen substitution. Compounds with the best acute in vivo activity in rats were compared for chronic in vivo activity. The highest tissue levels and best chronic in vivo activities were found in the racemic 6,7-dichloro and 6-fluoro-7-chloro analogues 18 and 23. ARI activity was enantioselective for 58 and 60, the 2R,4R-enantiomers of 18 and 23. 7-Chloro-6-fluoro-*cis*-4-hydroxy-2(R)-methyl-chroman-4-acetic acid (60) was selected for phase 1 clinical trials and did not exhibit sorbinil-like hypersensitivity side effects.

Introduction

Previous studies from these laboratories have described the excellent in vitro and in vivo activity of spirohydantoin aldose reductase inhibitors (ARIs).¹ Clinical trials with the spirohydantoin sorbinil suggested efficacy²⁻⁴ but were terminated because of a low incidence of side effects similar to those of the hydantoin anticonvulsant dilantin.⁵ Because both drugs are hydantoins and have qualitatively similar side effects, we decided either to replace the hydantoin moiety entirely or alternatively to explore whether hydantoin side effects might be related to a combination of the hydantoin ring and sorbinil backbone. In this paper we describe the former approach in which we retained the chroman backbone found in sorbinil and focused on the discovery of a spirohydantoin bioisostere.

Sorbinil is a particularly suitable target for bioisosteric analysis since it can be divided into two components, a chroman backbone and an orthogonal spirohydantoin ring (Figure 1). By replacing the hydantoin ring we hoped to prepare an AR inhibitor with the in vivo potency of sorbinil but without sorbinil-like side effects. In our SAR studies the translation of in vitro to in vivo activity was a critical concern because in vitro activity is relatively common among non-hydantoin ARIs, but in vivo activity is rare.

Target Design. Literature precedents, suggesting that a bioisostere for a hydantoin moiety might be found in an appropriately designed carboxylic acid, have been summarized.⁶ Among alicyclic derivatives, a cyclic imide such

- Sarges, R.; Goldstein, S. W.; Welch, W. M.; Swindel, A. C.; Siegel, T. W.; Beyer, T. A. Spiro Hydantoin Aldose Reductase Inhibitors Derived from 8-Aza-4-chromanones. J. Med. Chem. 1990, 33, 1859–1865, and references therein.
- (2) Judzewitsch, R. G.; Jaspan, J. B.; Polonsky, K. S.; Weinberg, C. R.; Halter, J. B.; Halar, E.; Pfeifer, M. A.; Vukadinovic, C.; Bernstein, L.; Schneider, M.; Liang, K-Y.; Gabbay, K. H.; Rubenstein, A. H.; Porte, D. P., Jr. Aldose Reductase Inhibition Improves Nerve Conduction Velocity In Diabetic Patients. N. Engl. J. Med. 1983, 308, 119-125.
- (3) Dyck, P. J.; Zimmerman, B. R.; Vilen, T. H.; Minnerath, S. R.; Karnes, J. L.; Yao, J. K.; Poduslo, J. F. Nerve Glucose, Fructose, Sorbitol, myo-Inositol, And Fiber Degeneration And Regeneration In Diabetic Neuropathy. N. Engl. J. Med. 1988, 319, 542-548.
- (4) Sima, A. A. F.; Bril, V.; Nathaniel, V.; McEwen, T. A. J.; Brown, M. B.; Lattimer, S. A.; Greene, D. A. Regeneration And Repair Of Myelinated Fibers In Sural-Nerve Biopsy Specimens From Patients With Diabetic Neuropathy Treated With Sorbinil. N. Engl. J. Med. 1988, 319, 548-555.
- (5) Pitts, N. E.; Vreeland, F.; Shaw, G. L.; Peterson, M. J.; Mehta, D. J.; Collier, J.; Gunderson, K. Clinical Experience With Sorbinil—An Aldose Reductase Inhibitor. *Metabolism* 1986, 4 (Suppl 1), 96-100.







^aReagents: (a) LiCH₂CO₂C₂H₅, THF, -78 ^aC; (b) CH₃SO₃H, P₂O₅; (c) PCl₅, AlCl₅; (d) HF; (e) H₂SO₄; (f) PPA; (g) CH₃SO₃H, P₂O₅; (h) CH₃SO₃H; (i) CH₃CO₂C₂H₅; NaH; TFA; LiAlH₄; (j) R₁COR₂, pyrrolidine; (k) C₆H₅CHO, NaOH; (l) CH₃CO₂C₂H₅, NaH; TFA; LiAlH₄, (3,4-Cl₂C₆H₃)MgCl, CuBr.

as a hydantoin moiety can be replaced by a carboxylic acid moiety. Imide moieties have been considered as carboxyl bioisosteres in the design of potential anticonvulsants related to GABA and in the evaluation of antifibrinolytic activity in a φ -aminocaproic acid analogue. There are structural similarities between spirohydantoin ARIs derived from N-substituted isatins and acetic acid N-substituted 2-benzimidazolone ARIs. Although the acetic acids are quite active in vitro, they are less potent in vivo than the hydantoins.⁷

The anion is likely the active species in sorbinil ($pK_a = 8.18$) by analogy to ARI carboxylic acids which are anionic at physiological pH. From other studies we also knew that heterocyclic acids less acidic than $pK_a = 8.5$ appended to a sorbinil-like backbone did not have ARI activity.⁸ Al-

- (7) Larson, E. R.; Lipinski, C. A.; Sarges, R. Medicinal Chemistry of Aldose Reductase Inhibitors. Med. Res. Rev. 1988, 8, 159–186.
- (8) Unpublished observations.

⁽⁶⁾ Lipinski, C. A. Bioisosterism in Drug Design. Annu. Rep. Med. Chem. 1986, 21, 283-291.



Figure 1. Bioisosteric hydantoin and hydroxy acetic acid ARI's.

though there is ambiguity as to which atom in the hydantoin anion is interacting with receptor functional groups, all the negatively charged atoms are orthogonal to the aromatic plane. We wanted to mimic this feature in our bioisostere by designing a carboxylate anion array that would be orthogonal to the backbone aromatic plane.

Analogy to numerous literature examples in which a hydrogen bond can mimic a ring suggested that we might be able to fix the conformation of a carboxylic acid by an internal hydrogen bond. The strong hydrogen bond in salicylic acids suggested that a six-membered hydrogenbonded array between carboxylate anion and a hydroxy moiety should be quite stable. The carboxylate anion might be fixed by an internal hydrogen bond and would be orthogonal to the aromatic plane if hydroxy and acetic acid moieties were appended to the same spiro carbon. Accordingly, our target prototype compound was the spiro hydroxy acetic acid 40, corresponding to the adduct of acetate anion with 6-fluoro-4-chromanone.

Chemistry

The 4-hydroxy-2H-1-benzopyran-c-4-acetic acids⁹ were prepared by a low-temperature addition of ethyl lithio acetate to the requisite 2,3-dihydro-4H-1-benzopyran-4ones (chromanones) followed by ester saponification (Scheme I). Anion addition to 2-monosubstituted chromanones gave the cis-product (alkyl or aryl cis to hydroxy). Trans-addition product was below 2% (the NMR detection limit). The chromanone precursors in Scheme I were prepared from phenols by methods 1-3. Differences in these yields were responsible for almost all of the variation in yields of the hydroxy acetic acid final products.

In method 1, aqueous phenolate anion was reacted with β -butyrolactone to give the intermediate phenoxybutyric acid followed by an acid-catalyzed closure. Methanesulfonic acid and phosphorus pentoxide were used to prepare the precursors to 1, 5–9, 12, 13, 15–19, 24, 27, and 28. The acid from 3,4-dichlorophenol gave a 1:1 mixture of the 5,6- and 6,7-dichlorochromanone precursors to 13 and 18. Changing to phosphorus pentachloride and aluminum chloride gave a 1:9 mixture of precursors to 13 and 18. These cyclization reagents with acids from 3-chloro-and 3-fluorophenol gave predominantly the 7-halo precursors to 24 and 30 while 3-nitro-4-chlorophenol gave only the 5,6-substituted precursor to 14. Hydrofluoric acid with the acid from 3-methoxy-4-chlorophenol gave chromanone 22.

In method 2, phenols were reacted with substituted acrylic acids in sulfuric acid to give the chromanone precursor to 10, in polyphosphoric acid to prepare precursors to 1, 2, 4, 5, and 11, in a methanesulfonic acid and phosphorus pentoxide mixture to prepare precursors to 3, 26, 29, 43, 44, and 48-51, and in methanesulfonic acid to prepare precursors to 4, 22, and 24.

In method 3, chromanones were prepared from the substituted 2-hydroxyacetophenones, which in turn were

Scheme II.^a Chiral Synthesis of 60



^aReagents: (a) DEAD, Ph_3P ; (b) X = OH, $NaBH_4$; (c) X = Br, Ph_3P , Br_2 ; (d) CH_3SO_3H ; (e) Ac_2O , TEA; (f) HCl; (g) $Pb(OAc)_4$; (h) HCl; (i) R = Et, LDA, EtOAc; (j) KOH, 60, R = H.

prepared from phenolic acetates by the Fries reaction. Condensation of hydroxyacetophenones with ethyl acetate in the presence of sodium hydride gave the 4-(2hydroxyphenyl)butan-2,4-diones, which were cyclized with trifluoroacetic acid in methylene chloride to the benzopyranones. Reduction with lithium aluminum hydride gave the chromanone precursor to 18. 2-Hydroxyacetophenones were also reacted directly with ketones in the presence of pyrrolidine to produce 2,2-disubstituted chromanone precursors to 41 and 42. This procedure worked well with longer chain aldehydes to give precursors to 45 and 46 but gave poor results with acetaldehyde. 3-Fluoro-6-hydroxyacetophenone was condensed with benzaldehyde to give the 2-phenyldihydropyranone precursor to 47 and with ethyl acetate was converted to a pyranone intermediate which on 1,4 copper-catalyzed addition of a benzyl Grignard reagent gave the 2-(3,4-dichlorobenzyl)chromanone precursor to 53. Precursors to 20, 25, 31, and 32 were prepared by modification of preformed chromanones.

Brucine resolution of racemic 6,7-dichloro acetic acid 18 gave the positively rotating enantiomer 58 and negative enantiomer 59. Scale-up and resolution of racemic 6fluoro-7-chloro derivative 23 to the positively rotating enantiomer 60 and negative enantiomer 61 was accomplished in 4.8% overall yield by an 11-step chiral synthesis (shown for 60 in Scheme II).

Results and Discussion

SAR Study. In Vitro and Acute in Vivo Activity. The hydantoin in sorbinil can be replaced by a spiro hydroxy acetic acid. The equatorial 2-methyl prototype 4 (Table I) was prepared because 2-methyl substitution increases activity in Eisai M-79175, a 2-methyl analogue of sorbinil.¹⁰ The unmethylated 6-fluoro prototype 40 (Table II) has the same backbone as sorbinil. Both of these compounds were moderately active in vitro and when given

⁽⁹⁾ Lipinski, C. A. Hydroxyacetic Acid Derivatives For The Treatment Of Diabetic Complications. US Patent 4,853,410, 1989; Chem. Abstr. 1988, 108, 75224h.

⁽¹⁰⁾ Kawakami, Y.; Nagai, Y.; Ono, H.; Ueda, K.; Sato, T.; Tanaka, S. Synthesis and Absolute Configuration of 2-Alkyl-chroman-4-spiro-hydantoin Derivatives. *Heterocycles* 1984, 21, 583.

Table I. Aromatic Ring Substitution Pattern and ARI Activity



no.	X	R	mp, °C	IC ₅₀ , ^{<i>a</i>} μM	% inhibn ^b	ED ₅₀ , ^c mg/kg
1	Н	Н	99-101	14.8 (1.65-133)	9 ^d	
2	6-CH ₃	н	143-144	11.3 (2.39-53.7)	-16	
3	$6-OCH_3$	н	84-87	281 (30.3-2600)	nte	
4	6-F	н	122 - 125	0.20 (0.12-0.34)	56'	23.0 (19.2-27.5)
5	6-Cl	н	119-121	0.82 (0.23-2.90)	67	14.9 (10.2-21.6)
6	6-Br	н	135-137	1.67 (0.36-7.84)	21	60.8 (40.9-90.2)
7	$6-NO_2$	н	135-138	0.08 (0.03-0.25)	74	12.0 (8.12-17.7)
8	$6-SO_2CH_3$	н	164-167	8.22 (0.92-73.5)	nt	
9	6-COCH ₃	н	159–163	0.65 (0.19-2.31)	5	
10	6,8-Cl ₂	н	143-145	1.00 (0.28-3.53)	48	26.6 (18.6-38.0)
11	6-Cl, 8-CH ₃	н	118 - 120	1.18 (0.33 - 4.16)	28	49.0 (33.6-71.6)
12	6-Br, 8-Cl	н	168 - 171	5.16 (1.10-24.3)	nt	
13	$5,6-Cl_2$	н	144-147	1.63 (0.46-5.77)	50	25.0 (17.5-35.8)
14	5-NO ₂ , 6-Cl	н	180-182	95.5 (10.4-872)	nt	
15	5-C1	H	176-180	43.5 (4.81-395)	nt	
16	5-CH ₃ , 5-NO ₂	н	155 - 158	1.63 (0.46-5.77)	-31	
17	5,7-Cl ₂	н	141-143	29.4 (3.26-266)	nt	
18	$6,7-Cl_2$	н	159–162	0.18 (0.09-0.37)	91	8.06 (5.72-11.4)
19	6-Cl, 7-CH ₃	н	134-136	0.24 (0.08-0.70)	72	12.7 (8.67-18.7)
20	6-NO ₂ , 7-Cl	н	133-137	0.11 (0.04-0.32)	67	14.9 (10.2-21.6)
21	6-NO ₂ , 7-CH ₃	н	95-100	0.09 (0.03-0.28)	39	35.0 (24.4-50.3)
22	6-Cl, 7-OCH ₃	н	178 - 180	0.19 (0.07-0.51)	53	36.5 (25.3-52.5)
23	6-F, 7-Cl	н	134 - 136	0.06 (0.03-0.12)	76	5.10 (3.15-8.24)
24	7-Cl	н	147 - 150	3.32 (0.71-15.6)	0	
25	$7-CO_2H$	н	221-223	0.07 (0.03-0.23)	48	26.6 (18.6-38.0)
26	6-Cl, 7,8-(CH) ₄	н	143-145	0.40 (0.11-1.41)	45	29.1 (20.3-41.7)
27	5,8-Cl ₂	н	104-110	13.1 (2.76-62.3)	nt	
28	8-C1	н	84-86	5.70 (1.21-26.9)	nt	
29	$7,8-Cl_2$	н	126 - 131	0.78 (0.26-2.33)	nt	
30	7-F	н	116-119	5.42 (1.15 - 25.6)	nt	
31	6-NO ₂ , 7-F	н	133–137	0.07 (0.02-0.21)	5 ^e	
32	6-Cl, 7-F	н	133-134	0.39 (0.16-0.95)	29	19.0 (13.2-27.4)
33	6,7-F ₂	н	128 - 131	0.25 (0.10-0.60)	67 ^g	5.94 (3.75-9.42)
34	6-CN, 7-F	н	142 - 144	0.13 (0.04-0.39)	15^{g}	29.2 (20.4-41.8)
35	6-Cl, 7-Br	н	154 - 157	0.07 (0.03-0.15)	46 ^g	11.3 (7.62-16.8)
36	6-F, 7-Br	н	140-143	0.09 (0.04-0.21)	73 ^g	4.94 (3.05-8.03)
37	5-Br, 6-F	н	121 - 124	4.50 (1.27-16.0)	nt	
38	6-Cl, $7-C_2H_5$	н	99– 103	0.10 (0.03-0.28)	40^{s}	13.6 (9.28-19.9)
39	6-Cl, 7-OCH $_2C_6H_5$	н	192-194	0.06 (0.02-0.18)	12^{g}	32.0 (22.3-45.9)
41	6-F	CH_3	108-111	3.46 (0.98-12.3)	56 ^h	83.2 (54.4-127)
55	$6-NO_2$	CH_3	113-114	0.40 (0.11-1.41)	37	37.2 (25.8-53.6)
56	6,7-Cl ₂	CH_3	147–149	1.86 (0.53-6.58)	6	
57	$5,6-Cl_2$	CH_3	152 - 155	14.8 (1.65-133)	nt	

 a IC₅₀ (μ M) against human placental aldose reductase. Lower and upper bounds of the 95% confidence interval in parentheses. b Percent inhibition of sorbitol accumulation in sciatic nerves from streptozotocin-diabetic rats dosed at 3 × 25 mg/kg over 27 h. c Calculated ED₅₀. Lower and upper bounds of the 95% confidence interval in parentheses. d Tested at 3 × 50 mg/kg over 27 h. e Not tested. f Other percent inhibitions and doses; 92% (3 × 100 mg/kg) and 81% (3 × 50 mg/kg). g Tested at 3 × 10 mg/kg over 27 h. h Tested at 3 × 100 mg/kg over 27 h.

orally to diabetic rats decreased sorbitol accumulation in sciatic nerves. Methylated hydroxy acetic acid 4 was three times more active than the parent 40 in vitro and in vivo. Most of the aromatic SAR was explored with the 2-methyl substitution as in 4. Equatorial 2-methyl substitution improves activity in spiro hydroxy acetic acids just as it does in sorbinil-related hydantoins. This activity improvement may in part be due to a conformational effect. An equatorial 2-methyl group in both spiro hydroxy acetic acid and hydantoin ARIs fixes the chroman ring in a pseudochair. The trans-relationship between the 2-methyl and acetic acid group in the hydroxy acids orients the acetic acid group axially. This relationship is similar to the axial acidic NH in the 2-methylsorbinil analogue M-79175, which has the trans relationship between the 2-methyl and hydantoin imide moieties.

Aromatic derivatives with an equatorial 2-methyl group are shown in Table I. Among 6-substituted compounds (1-9), good in vitro activity is found in fluoro (4), chloro (5), nitro (7), and benzoyl (9) derivatives. Activity is low for hydrogen (1), methyl (2), methylsulfonyl (8), and methoxy (3) analogues. All possible combinations of mono and dichloro derivatives were prepared. The in vitro potency order is 6-chloro (5) > 7-chloro (24) = 8-chloro (28)> 5-chloro (15). Among dichloro derivatives the 6,7-dichloro analogue 18 is by far the most active in vitro followed by the 6,8-dichloro (10) and 7,8-dichloro (29) compounds, which are equipotent. Relative to the 6,7-dichloro analogue, derivatives with chloro at the 5-position are all less active, with 5,6-dichloro (13) being more active than 5,8-dichloro (27) and 5,7-dichloro (17). Among halogen substituents, fluoro and chloro were generally equiactive in vitro and superior to bromo. The potency order was 6-fluoro (4) > 6-chloro (5) > 6-bromo (6) and 6,8-dichloro (10) > 6-bromo-8-chloro (12). The excellent activity of the 6,7-dichloro derivative 18 prompted examination of other

Table II. Effect of Chromane Ring Substituents on ARI Activity



no.	R_1	R_3	R_2	R_4	mp, °C	IC_{50} , ^{<i>a</i>} $\mu \mathbf{M}$	% inhibn ^ø	ED_{50} , c mg/kg
40	Н	Н	Н	Н	oil	0.60 (0.13-2.79)	61 ^d	71.4 (47.4–108)
4	CH_3	н	Н	Н	122 - 125	0.20(0.12 - 0.34)	54^{e}	23.0 (19.2-27.5)
41	CH_3	Н	CH_3	н	108 - 111	3.46(0.98 - 12.3)	56^d	83.2 (54.4-127)
42	$(CH_2)_3$		Н	Н	110 - 112	1.92(0.54 - 6.80)	43^d	124 (77.3-199)
43	C_2H_5	Н	Н	Н	89-91	0.62 (0.21 - 1.85)	27	50.6(34.5-74.0)
44	$n - C_3 H_7$	Н	Н	н	97 - 100	0.97(0.27 - 3.41)	16	70.8 (47.1-107)
45	$i - C_3 H_7$	Н	Н	Н	119-122	0.57 (0.16 - 2.02)	-7	
46	$t - C_4 H_9$	Н	Н	Н	123 - 125	0.36 (0.10 - 1.28)	29	47.6 (32.6-69.4)
47	Ph	н	Н	Н	140 - 143	2.67(0.75 - 9.44)	3⁄	
48	Н	Н	Н	CH_3	86-103	11.0 (1.23-98.8)	nt^g	
49	CH_3	Н	Н	CH_3	147 - 150	29.4 (3.26-266)	nt ^g	
50	CH_3	CH_3	Н	Н	149 - 151	0.91 (0.26 - 3.20)	29	47.6 (32.6-69.4)
51	$(CH_2)_4$		Н	Н	150 - 154	11.0 (1.23-98.8)	nt^{g}	
52	$(CH_2)_2Ph$	Н	Н	Н	oil	1.14(0.32 - 4.02)	5	
53^h	$3,4-(ClBz)_2$	Н	H	Н	168 - 170	7.65(1.62 - 36.1)	-27	
54^h	Bz	Н	н	Н	166 - 167	1.39 (0.39-4.90)	1	

 a IC₅₀ (μ M) against human placental aldose reductase. Lower and upper bounds of the 95% confidence interval in parentheses. b Percent inhibition of sorbitol accumulation in sciatic nerves from streptozotocin-diabetic rats dosed at 3 × 25 mg/kg over 27 h. c Calculated ED₅₀. Lower and upper bounds of the 95% confidence interval in parentheses. d Tested at 3 × 100 mg/kg over 27 h. e Other percent inhibitions and doses; 92% (3 × 100 mg/kg) and 81% (3 × 50 mg/kg). f Tested at 3 × 50 mg/kg over 27 h. e Not tested. h Dicyclohexylamine salt.

6,7-disubstituents (19-23, 31-33). These were all very active in vitro (IC₅₀'s of 0.06-0.39 μ M).

In vivo activity is found in 6-substituted fluoro (4), chloro (5), and nitro (6) but not benzoyl (9) derivatives. The 6,7-dichloro derivative 18 is more active in vivo than the 6-chloro (5) or other dichloro derivatives. Combinations of fluoro and chloro at the 6,7-positions were all active in vivo. After 10 mg/kg doses the acute in vivo¹¹ potency order was 6-fluoro-7-chloro (23) = 6,7-difluoro (33) > 6,7-dichloro (18) \gg 6-chloro-7-fluoro (32).

Changing substitution on the 2- and 3-carbons of the chroman ring (Table II) had variable effects on in vitro activity, but all of the compounds with good in vitro activity had reduced or no in vivo activity. In vitro activity decreased with 2-dialkyl substitution, as in 41 (Table I). A variety of monoalkyl substituents at the 2-position (43–46, Table I) were almost as active in vitro as the monomethyl derivative 4 but had significantly lower in vivo activity. Appending a 2-aryl group (47) or constructing a 2,3-cis-tetramethylene bridge (51) led to decreased in vitro activity, as did the addition of a 3-monomethyl substituent (49). Marked differences were observed in vitro between trans- and cis-2,3-dimethyl derivatives 49 and 50. The trans derivative 49, in which the 3-methyl group is equatorial and relatively close to the axial acetic acid group, was only weakly active while cis-derivative 50, in which the 3-methyl is trans diaxial and remote from the 4-acetic acid moiety, was quite active. Compounds with aralkyl groups at the 2-position, such as 52-54, were active in vitro but showed little or no in vivo activity. This contrasts with the in vivo potency increase found with pendant benzyl or heteroalkyl substituents in ARI acetic acids such as ponalrestat and zopolrestat 12 (Figure 2).



Figure 2. Acetic acid ARI's.

By NMR analysis, the 6-fluoro-2,2-dimethyl derivative 41, unlike the 2-monomethyl derivatives, has the acetic acid in an equatorial conformation. Moreover, 41, with modest in vitro activity, had surprisingly good in vivo activity. The 2,2-dimethyl derivatives 55 and 56 (Table I), corresponding to in vivo active 2-monomethyl derivatives 7 and 18, were prepared to test the hypothesis that acetic acid side chain metabolism might differ between an equatorial and axial acetic acid. These compounds, while active in vitro, were only weakly active or inactive in vivo at the dose tested. A 5,6-dichloro-2,2-dimethyl derivative, 57, probed the effect of a possible peri-interaction between a 5-chloro group and the equatorial acetic acid side chain. It had low in vitro activity.

In summary SAR studies indicated that high in vivo activity following acute (a single days) dosing could be attained in 6,7-disubstituted derivatives having an equatorial chroman 2-methyl group.

Acute and Chronic in Vivo Activities. Clinically, sorbinil was dosed once a day. To better model this regimen, chronic in vivo activity was measured by dosing streptozotocin-diabetic rats once daily with 10 mg/kg doses for 5 days. Because of the longer dosing interval, the chronic screen is much more sensitive to drug half-life than is the acute in vivo screen, in which doses are given three times in a 27-hour period.

Acute and chronic in vivo activities of the racemic 6fluoro analogue 4 and six of the most active racemic 6,7dihalo analogues are compared in Table III. In vitro inhibitory activities against human placental aldose reductase were determined in a side-by-side dose response comparison. Four compounds (18, 23, 35, 36) displayed

⁽¹¹⁾ Dose in mg/kg at each of three dosing times. See Experimental Section.

⁽¹²⁾ Mylari, B. L.; Larson, E. R.; Beyer, T. A.; Zembrowski, W. J.; Aldinger, C. E.; Dee, M. F.; Siegel, T. W.; Singleton, D. H. Novel, Potent Aldose Reductase Inhibitors: 3,4-Dihydro-4oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1phthalazine-acetic Acid (Zopolrestat) and Congeners. J. Med. Chem. 1991, 34, 108-122.

Table III. In Vitro and Acute and Chronic in Vivo Activity and Plasma and Nerve Levels



			in vivo % inhibn		tissue levels, ^b μ g/mL		
no.	х	Y	in vitro IC ₅₀ ª	acute ^c	chronic ^d	plasma	nerve
4	F	Н	$3.1 \pm 2.6 \times 10^{-7}$ (4)	22	13	<0.50	NA
18	Cl	Cl	$1.4 \pm 0.8 \times 10^{-7}$ (4)	53	39	19.7 ± 11.0	6.01
23	F	C1	0.12×10^{-7} (2)	72	55	14.2 ± 4.7	1.85
32	Cl	F	4.1×10^{-7}	29	-30	13.4 ± 1.6	2.35
33	F	F	3.5×10^{-7}	67	-20	2.19 ± 1.88	< 0.10
35	Cl	Br	0.5×10^{-7}	46	33	12.7 ± 2.4	3.11
36	F	Br	1.2×10^{-7}	73	44	8.8 ± 6.4	1.31

^a Molar IC₅₀ \pm standard deviation in (n) experiments against human placental aldose reductase. ^bTissue concentration in $\mu g/mL$ at 3 h after the last dose of 25 mg/kg for 5 days. ^cPercent inhibition of sorbitol accumulation in sciatic nerves from streptozotocin-diabetic rats dosed at 3 × 25 mg/kg over 27 h. ^dPercent inhibition of sorbitol accumulation in sciatic nerves from streptozotocin-diabetic rats dosed at 1 × 25 mg/kg over 5 days. Negative numbers correspond to sorbitol accumulation.

significant chronic in vivo activity. All showed appreciable plasma levels (8.8–19.7 μ g/mL) and had similar in vitro activity (IC₅₀ × 10⁻⁷ M = 0.5 (35) to 0.12 (23)). Three compounds (4, 32, 33) lacked chronic in vivo activity. Compounds 4 and 33 had very low plasma levels. Compound 32 had plasma levels very similar to the active 23 but was 30-fold less active than 23 in vitro and gave much lower nerve levels.

Differences in plasma level could be related to increased compound lipophilicity since log P's calculated from the MedChem CLOGP program¹³ were lowest for those compounds in Table III (4 and 33) which had the lowest plasma levels. pK_a 's in water for the seven racemic analogues in Table III as well as the parent 1 and 6-NO₂ analogue 7 were insensitive to aromatic substituent effects with pK_a 's in the range 3.96-4.02.¹⁴ Since aromatic substituents do not affect acidity, tissue-level differences are likely not related to changes in hydrogen-bonding properties of the carboxylate anions.

ARI Enantiospecificity. The high nerve levels of 18 and high chronic activity of 23 led to preparation and testing of their enantiomers. Compound 58, the positively rotating 2R, 4R-enantiomer of 18, and compound 60, the positively rotating 2R, 4R-enantiomer of 23, are respectively 100 times as active in vitro as the 2S, 4S-enantiomers 59 and 61. Similar high enantioselectivities for the AR receptor site have been observed for 2-methylchromane and tetralone hydantoins.^{10,15} The absolute configurations of the methylated backbone rings of active enantiomers 58 and 60 are identical at the chiral centers at C2 and C4 to those reported for the more active enantiomers of methylated dihydrobenzopyran and tetralone hydantoins, if one overlays the OH moiety of the hydroxy acetic acid with the hydantoin ring amide NH. The C4 center of the more active enantiomer of the hydroxy acetic acids is R, rather than S as in the hydantoins, because of a difference in sequence rule nomenclature.

- Wiley-Interscience: New York, 1979; pp 18-43.
 (14) For comparison the pK_a of β-hydroxybutyric acid is 4.39. Cannan, R. K.; Kibrick, A. Complex Formation between Carboxylic Acids and Divalent Metal Cations. J. Am. Chem. Soc. 1938, 60, 2314-2320.
- (15) Lipinski, C. A. Spiro-3-Heteroazolones For Treatment Of Diabetic Complications. US Patent 4,556,670, 1985. Chem. Abstr. 1984, 101, 230523f.

Table IV. Enantiospecificity and in Vitro ARI Activity^a

v	H O	, СО₂н
×́√	\checkmark	\mathbf{i}
	\nearrow	СН

compd	х	$enant^b$	ARI IC ₅₀ °
18	Cl	±	$1.56 \pm 0.84 \times 10^{-7}$ (3)
58	Cl	+	$5.09 \pm 3.93 \times 10^{-8}$ (5)
59	Cl	-	$5.62 \pm 2.45 \times 10^{-6}$ (3)
23	F	±	$4.93 \pm 3.23 \times 10^{-8}$ (3)
60	F	+	$4.55 \pm 2.80 \times 10^{-8}$ (3)
61	F	-	59% at 1 × 10 ⁻⁵ (1)

^aThe structure shown depicts the absolute configuration of the more active, positively rotating, 2R,4R enantiomers. ^bEnantiomeric status: $+ = 2R,4R; - = 2S,4S; \pm =$ racemate. ^cMolar IC₅₀ \pm standard deviation in (*n*) experiments against human placental aldose reductase.



Figure 3. Brucine salt of ARI active 2R,4R-enantiomer 58.



hydrogen bonded to the CO₂H (see arrows).

Figure 4. X-ray of ARI inactive 2S, 4S-free acid 59. Neither OH of the two molecules in the unit cell is hydrogen bonded to the CO_2H (see arrows).

In dose–response comparisons, the ARI IC₅₀'s (M) of 58 and 60 were 6.9×10^{-8} and 2.8×10^{-8} in comparison with 3.5×10^{-8} for the acetic acid inhibitor ponalrestat and 4.8×10^{-7} for the hydantoin sorbinil. The acute and chronic in vivo activities of 58 and 60 (Table V) are very comparable to those of ponalrestat. In particular, 58 and 60, like their racemic counterparts 18 and 23, retain good in vivo activity following once-a-day dosing.

⁽¹³⁾ Pomona Medicinal Chemistry Project Med Chem V3.54 program to calculate CLOGP. Hansch, C.; Leo, A. Substituent Constants for Correlation Analysis in Chemistry and Biology; Wiley-Interscience: New York, 1979; pp 18-43.

Table V. Acute and Chronic in Vivo Activity of 58, 60, and Ponalrestat in the Streptozotocin-Diabetic Rat

		activity		
compd	dose, mg/kg	acutea	chronic	
58	2.5	49	31	
	5	60	34	
	10	70	68	
	25	89	78	
60	2.5	39	19	
	5	61	52	
	10	88	69	
	25	98	97	
ponalrestat	2.5	12	0	
-	5	57	60	
	10	81	81	
	25	85	88	

^aPercent inhibition of sorbitol accumulation in sciatic nerves from streptozotocin-diabetic rats given three doses over 27 h. ^bPercent inhibition of sorbitol accumulation in sciatic nerves from streptozotocin-diabetic rats given one dose per day over 5 days.

Spiro Hydroxy Acetic Acids as Hydantoin Bioisosteres. The spiro hydroxy acetic acids were designed on the hypothesis that a hydrogen bond would form between a β -hydroxy and carboxylate anion when the drug was in a receptor-like environment. The interaction of the hydroxyl with the carboxylate anion, which is the only significant species at a pH of 7.4, would be expected to be 2.5-3.5 kcal mol⁻¹ more stable than the interaction with the neutral acid.¹⁶ We were interested to see if this prediction could be observed by X-ray. Accordingly, we examined the X-ray structure of the brucine salt of the 2R,4R-enantiomer 58 and the X-ray of the 2S,4S-free acid 59. These are shown in Figures 3 and 4.

The X-ray of the brucine salt (Figure 3) shows a strong hydrogen bond between the hydroxy group hydrogen (see arrow in Figure 3) and the carboxylate anion, with a hydroxyl hydrogen carboxylate oxygen distance of 1.67 Å and OHO angle of 143°. In contrast, in the single crystal X-ray of the free acid (Figure 4), neither of the dimeric forms contained in the unit cell forms an internal hydrogen bond. This is consistent with the supposition that the internal hydrogen bond in the free acid may only be worth 0.5-1.5 kcal mol⁻¹. In terms of crystal packing this effect could be outweighed by either chance nucleation of a minor conformation or crystal lattice forces, which in the case of nucleic acid bases have been estimated in the range 0.35-0.7 kcal mol^{-1.17} The X-rays of the salt and both dimeric forms of the free acid show a pseudochair conformation for the chroman ring with methyl group equatorial and acetic acid group axial. The same chroman conformation is seen in two very different crystalline environments and a similar conformation is also observed in solution NMR studies, suggesting that a similar conformation may be presented to the enzyme receptor site.

Figure 5 presents an overlay of the X-ray structures of the active enantiomer 58 (as found in the brucine salt) and those of sorbinil and a chiral 2-methyltetralone hydantoin (4,1'(S)-3'(R)-methyl-7'-fluorospiro[imidazolidine-4,1'-(2'H)-naphthalene]-4'(3'H),2,5-trione).¹⁵ The similarity in chroman ring conformation and orthogonal relationship of acidic moieties to the aromatic backbone is striking.





Figure 5. Stereo overlay of 58, sorbinil, and a methylated tetralone hydantoin.

Atoms bearing possible negative charge do not exactly superimpose, but this need not be a requirement for similar receptor binding since hydrogen bonding to a common receptor site can occur through a cone of directionality.

In conclusion, in this series a spiro hydroxy acetic acid is a hydantoin bioisostere. The backbones are similar between hydantoin and the hydroxy acid series, the orthogonal relationship of acidic to aromatic backbones is similar, the effect of 2-methyl substitution is similar, and the high aldose reductase activity enantioselectivity is similar. Compound **60**, the positively rotating 2R,4R-enantiomer of racemate **23** was advanced to phase 1 clinical studies and was well-tolerated with no evidence of dilantin-like hypersensitivity side effects.

Experimental Section

Chemistry. Melting points are uncorrected and were taken with a Thomas-Hoover capillary apparatus. NMR spectra were recorded on Varian T-60, EM-390, or XL-300 or Bruker WM-250 instruments with TMS as internal standard or referenced to residual protosolvent peaks as appropriate for the instrument. All J values are reported in hertz. Mass spectra were obtained with a Perkin-Elmer RMU-6E mass spectrometer. Microanalyses were performed by the Analytical Department of Pfizer Central Research or by Schwarzkopf Microanalytical Laboratory, Inc. (Woodside, NY).

Scheme I. Ethyl 6-Chloro-c-4-hydroxy-2-methylchroman-4-acetate. To 13.2 mL (94 mmol) of diisopropylamine in 190 mL of dry THF at -20 °C was added 44.8 mL (94 mmol) of 2.4 M *n*-butyllithium in hexane. After 15 min the reaction was cooled to -78 °C and 9.17 mL (94 mmol) of EtOAc was added at below -70 °C. After 1.5 h at -78 °C, a solution of 2.3 g (12 mmol) of 6-chloro-2-methylchroman-4-one in 10 mL of THF was added at -70 °C. After 1.5 h at -78 °C, the reaction was quenched with H₂O. Workup gave the product as a pale yellow oil (3.5 g, 100%): ¹H NMR (CDCl₃) δ 7.35 (m, 1 H), 7.00 (m, 1 H), 6.40 (m, 1 H), 4.13 (m, 1 H), 4.13 (q, 2 H), 2.77 (s, 2 H), 2.53-1.63 (m, 2 H), 1.37 (d, 3 H), 1.27 (t, 3 H).

6-Chloro-c-4-hydroxy-2-methylchroman-4-acetic Acid (5). To a solution of 0.672 g (12 mmol) of KOH dissolved in 20 mL of ethanol was added 3.41 g (12 mmol) of ethyl 6-chloro-c-4-hydroxy-2-methylchroman-4-acetate dissolved in 14 mL of ethanol. After stirring at 23 °C for 4 h, the reaction was concentrated in vacuo and the residue was taken up in 40 mL of H₂O. Workup gave the product as a white solid (1.9 g, 61%): ¹H NMR δ 7.17 (br s, 2 H), 7.01 (m, 1 H), 6.62 (m, 1 H), 4.17 (m, 1 H), 2.87 (s,

⁽¹⁶⁾ Andrews, P. R.; Craik, D. J.; Martin, J. L. Functional Group Contributions to Drug-Receptor Interactions. J. Med. Chem. 1984, 27, 1648–1657.

⁽¹⁷⁾ Ornstein, R. L.; Fresco, J. R. Correlation of crystallographically determined and computationally predicted hydrogen-bonded pairing configurations of nucleic acid bases. *Proc. Natl. Acad. Sci. U.S.A.* 1983, 80, 5171–5175.

3 H), 2.50–1.63 (m, 2 H), 1.37 (d, 3 H). Anal. $(C_{10}H_9O_2Cl) C$, H.

Method 1. Chromanone Synthesis. 3-(4-Chlorophenoxy)butyric Acid. A solution of 40 g (1 mol) of NaOH in 400 mL of H₂O and 128.6 g (1 mol) of 4-chlorophenol was heated at reflux for 15 min. β -Butyrolactone (81.5 g, 1 mol) was added to the refluxing solution over 1 h. The reaction was cooled to 23 °C. After workup the product was isolated as a white solid (24 g, 11%): ¹H NMR (CDCl₃) δ 10.5 (br s, 1 H), 7.12 (d, 2 H), 6.73 (d, 2 H), 4.67 (m, 1 H), 3.0-2.27 (m, 2 H), 1.33 (d, 3 H).

Method 1b. 6-Chloro-2-methylchroman-4-one. 3-(4-Chlorophenoxy)butyric acid (30.5 g, 142.1 mmol) was combined with 142 g of CH_3SO_3H and 7.1 g of P_2O_5 and the slurry was warmed for 15 min. The reaction was cooled to 23 °C. The product was isolated as a yellow solid (22 g, 79%): mp 99–100 °C; ¹H NMR (CDCl₃) δ 7.63 (m, 1 H), 7.20 (m, 1 H), 6.73 (m, 1 H), 4.47 (m, 1 H), 2.57 (m, 2 H), 1.47 (d, 3 H). Anal. ($C_{10}H_9O_2Cl$) C, H.

Method 1c. 7-Chloro-6-fluoro-2-methylchroman-4-one. PCl₅ (8.96 g, 43 mmol) was added in portions to a solution of 10.0 g (43 mmol) of 3-(3-chloro-4-fluorophenoxy)butyric acid in 21 mL of 1,2-dichloroethane. After addition, the reaction was stirred for 20 min at 23 °C and then added dropwise to a stirred slurry of 17.2 g (129 mmol) of AlCl₃ in 21 mL of 1,2-dichloroethane. After workup the product was isolated as 7.75 g (84%) of a tan solid, mp 75–76 °C. An analytical sample was obtained by recrystallization of 500 mg from hexane to give 410 mg, mp 79–81 °C. Anal. (C₁₀H₈O₂ClF) C, H.

Method 1d. 6-Chloro-7-methoxy-2-methylchroman-4-one. In a polyethylene bottle were combined 3.3 g (13 mmol) of 3-(4-chloro-3-methoxyphenoxy)butyric acid and 30 mL of liquid HF. The reaction mixture was allowed to stand at 23 °C for 72 h. On workup 2.7 g (93%) of product was isolated as a yellow solid: ¹H NMR (CDCl₃) δ 7.82 (s, 1 H), 6.47 (s, 1 H), 4.53 (m, 1 H), 3.93 (s, 3 H), 2.62 (m, 2 H), 1.52 (d, 3 H). Anal. (C₁₁H₁₁O₃Cl) C, H.

Method 2f. 6,8-Dichloro-2-methylchroman-4-one. To a mechanically stirred paste of 580 mL of PPA was added 94.6 g (0.58 mol) of 2,4-dichlorophenol and 100.0 g (1.16 mol) of crotonic acid. The reaction was heated at 120 °C for 3 h and then at 140 °C for 5 h. After workup product was obtained as a white solid (5 g, 3.7%): mp 98–102 °C; ¹H NMR (CDCl₃) δ 7.63 (d, 1 H), 7.40 (d, 1 H), 4.60 (m, 1 H), 2.65 (m, 2 H), 1.55 (d, 3 H). An analytical sample was prepared by recrystallization from hexane, mp 105–108 °C. Anal. (C₁₀H₈O₂Cl₂) C, H.

Method 2g. 6-Chloro-3,4-dihydro-2-methyl-2H-benzo-[h]chroman-4-one. A mixture of 185 mL (2.85 mol) of CH₃SO₃H, 5.56 g (39 mmol) of P₂O₅, 24.1 g (280 mmol) of crotonic acid, and 50 g (280 mmol) of 4-chloro-1-naphthol was stirred at 23 °C for 1 h and then at 55 °C for 4 h. The reaction was cooled to 23 °C. Workup gave the product (4.58 g, 67%): mp 134–136 °C; ¹H NMR (CDCl₃) δ 8.33–7.27 (m, 4 H), 7.80 (s, 1 H), 4.70 (m, 1 H), 2.70 (d, 2 H), 1.63 (d, 3 H). An analytical sample was crystallized from isopropyl ether and CH₂Cl₂; mp 135–137 °C. Anal. (C₁₄H₁₁O₂Cl) C, H.

Method 2h. 6-Chloro-7-methoxy-2-methylchroman-4-one. A solution of 1.0 g (6.3 mmol) of 4-chloro-3-methoxyphenol and 5.4 g (6.3 mmol) of crotonic acid in 15 mL of CH_3SO_3H was heated at 95 °C under N₂ for 20 h. The reaction was cooled to 23 °C. Workup gave the product as a yellow crystalline solid (1.1 g, 78%): mp 96-100 °C; ¹H NMR (CDCl₃) δ 7.65 (s, 1 H), 6.32 (s, 1 H), 4.45 (m, 1 H), 3.80 (s, 3 H), 2.48 (d, 2 H), 1.42 (d, 3 H). Anal. (C₁₁H₁₁O₃Cl) C, H.

6,7-Dichloro-2-methyl-4H-1-benzopyran-4-one. To 316 mL (4.1 mol) of trifluoroacetic acid in 1.5 L of CH_2Cl_2 was added 240 g (0.971 mol) of 1-(2-hydroxy-4,5-dichlorophenyl)-1,3-butandione. The reaction was stirred at 23 °C for 60 h. Workup gave the product as a tan solid (168.5 g, 76%): mp 143-147 °C; ¹H NMR (CDCl₃) δ 8.07 (s, 1 H), 7.10 (s, 1 H), 6.08 (s, 1 H), 2.37 (s, 3 H). Anal. ($C_{10}H_6O_2Cl_2$) C, H.

6,7-Dichloro-2-methylchroman-4-one. LiAlH₄ (12.94 g, 0.341 mol) was suspended in 1.3 L of THF. To the stirred slurry at -78 °C was added in one portion 76.1 g (0.332 mol) of 6,7-dichloro-2-methyl-4*H*-1-benzopyran-4-one. The reaction was quenched after 1 h by addition of 82 mL (1.43 mol) of glacial HOAc. Workup gave the product as a tan solid (64.4 g, 84%): mp 59-62 °C; ¹H NMR (CDCl₃) δ 7.80 (s, 1 H), 7.03 (s, 1 H), 4.55 (m, 1 H), 2.36 (m, 2 H), 1.50 (d, 3 H). Anal. $(C_{10}H_8O_2Cl_2)$ C, H. **Method 3j. 6-Fluoro-2-methylchroman-4-one**. A solution of 0.91 mL (16.3 mmol) of acetaldehyde in 5 mL of benzene was added over 30 min to a refluxing solution of 2.71 mL (32.5 mmol) of pyrrolidine in 20 mL of benzene while H₂O was removed with a Dean-Stark trap. To the refluxing solution was added 0.5 g (3.25 mmol) of 5-fluoro-2-hydroxyacetophenone in 5 mL of benzene in one portion. Following 30-min reflux the reaction was cooled to 23 °C. Workup gave 287 mg (49%) of product; ¹H NMR (CDCl₃) δ 7.57-6.73 (m, 3 H), 4.57 (m, 1 H), 2.67 (m, 2 H), 1.50 (d, 3 H).

Method 3k. 6-Fluoro-2-phenylchroman-4-one. In a shaker bottle were combined 2.5 g (16.2 mmol) of 5-fluoro-2-hydroxyacetophenone, 1.65 mL (16.2 mmol) of benzaldehyde, 20 mL of 96% ethanol, and 5.83 g (145.8 mmol) of NaOH. The yellow slurry was shaken vigorously for 30 min, during which time the reaction solidified. After standing for 3 h, the reaction was triturated with Et₂O and after filtration the resulting orange solid was added to 200 mL of 1 N HCl, and the resulting yellow solid was collected by filtration and dried at 60 °C in vacuo for 20 h to give 2.4 g of the intermediate 2-hydroxy-5-fluorochalcone. This material was added to a solution of 0.396 g (9.91 mmol) of NaOH in 99 mL of a $3:1 H_2O$ /ethanol solution. The resultant orange slurry was stirred for 5 h at 23 °C and then filtered and the solid was washed well with H_2O and dried to give the title compound (1.92 g, 80%); ¹H NMR (CDCl₃) δ 7.67–6.77 (m, 8 H), 5.40 (m, 1 H), 2.93 (m, 2 H).

Method 31. 6-Fluoro-2-(3,4-dichlorobenzyl)chroman-4-one. To a dried 125-mL three-neck flask containing 0.243 g (10 mmol) of Mg under N_2 was added 5 mL of dry Et₂O, followed by 1.38 mL (10 mmol) of 3,4-dichlorobenzyl chloride in 20 mL of Et₂O to form the Grignard reagent. After stirring for 30 min, 60 mg of CuI was added to the reaction at -20 °C and during stirring for 15 min at this temperature a tan-green suspension was observed in the reaction. To the stirred reaction was added 1.64 g (10 mmol) of 6-fluoro-4H-1-benzopyran-4-one dissolved in 25 mL of Et₂O in portions over 2 min. A momentary, localized red color was observed during the addition. The reaction was stirred at -20 °C and allowed to warm over 20 h to give a red-orange solid suspension. This was diluted with 50 mL of CHCl₃ and 3 N HCl was added with stirring until the reaction was acidic and the organic layer was a bright yellow color. Further workup gave the product (1.1 g, 34%) as a yellow oil which crystallized on standing: mp 101–103 °C; ¹H NMR (CDCl₃) δ 7.62–6.96 (m, 6 H), 4.68 (m, 1 H), 3.1 (m, 2 H), 2.7 (d, 2 H).

Chiral Synthesis. Ethyl (R)-2-(3-Chloro-4-fluorophenoxy)propionate. To a solution of 50 g (0.341 mol) of 3-chloro-4-fluorophenol, 38.6 mL (0.341 mol) of ethyl L-(+)-(S)-lactate [Aldrich; $[\alpha]^{20}_{D} = -11.51^{\circ}$ (neat)], and 89.44 g (0.341 mol) of triphenylphosphine in 665 mL of THF was added a solution of 53.7 mL (0.341 mol) of diethyl azodicarboxylate over 30 min while the reaction temperature was maintained below 10 °C. The reaction was allowed to warm to 23 °C, stirred for 18 h, and then concentrated in vacuo to a slury. Workup gave 67.2 g of a pale yellow oil: ¹H NMR (CDCl₃) δ 7.23-6.47 (m, 3 H), 4.63 (q, 1 H), 4.20 (q, 2 H), 1.60 (d, 3 H), 1.27 (t, 3 H).

(*R*)-2-(3-Chloro-4-fluorophenoxy)propanol. To a solution of 307 g (1.24 mol) of ethyl (*R*)-2-(3-chloro-4-fluorophenoxy)propionate in 3 L of THF and 300 mL of H₂O was added 131.3 g (3.47 mol) of NaBH₄. The reaction was stirred at 23 °C for 20 h and then cooled to 10 °C. Acetone (455 mL, 6.2 mol) was added dropwise with cooling as required to maintain the temperature below 25 °C. Workup gave 252.2 g of a dark oil: $[\alpha]^{25}_{D} = -29^{\circ}$ (*c* = 1, CH₃OH); ¹H NMR (CDCl₃) δ 6.87 (m, 3 H), 4.35 (m, 1 H), 3.67 (d, 2 H), 2.13 (br s, 1 H), 1.24 (d, 3 H).

(*R*)-2-(3-Chloro-4-fluorophenoxy)propyl Bromide. To a mechanically stirred solution of 80.79 g (0.308 mol) of triphenylphosphine in 200 mL of toluene was added 15.7 mL of Br₂ over 30 min and the temperature was kept below 28 °C. After stirring at 23 °C for 1 h, a solution of 52.5 g (0.257 mol) of (*R*)-2-(3-chloro-4-fluorophenoxy)propanol in 57 mL of toluene was added, the temperature was kept below 27 °C, and the reaction was stirred at 23 °C for 20 h. Workup gave 60.2 g of a pale yellow oil containing a small amount of triphenylphosphine: ¹H NMR (CDCl₃) δ 7.47-6.47 (m, 3 H), 4.7-4.0 (m, 1 H), 3.4 (m, 2 H), 1.4 (d, 3 H).

2-(Benzoylamino)-2-[2-[(1R)-2-bromo-1-methylethoxy]-4chloro-5-fluorophenyl]acetic Acid. To 166 mL of CH₃SO₃H at 0 °C was added 58.8 g (0.22 mol) of 2-(3-chloro-4-fluorophenoxy)propyl bromide followed by 46.8 g (0.24 mol) of α -hydroxyhippuric acid. The reaction was stirred at 0 °C for 1 h and then stirred at 23 °C for 3 h. Workup gave 71.3 g of an off-white solid as a mixture of diastereoisomers.

4-(Benzoylamino)-7-chloro-6-fluoro-2(R)-methylchroman-4-carboxylic Acid. To a solution of 364.8 g (1.09 mol) of crude 2-(benzoylamino)-2-[2-[(1R)-2-bromo-1-methylethoxy]-4-chloro-5-fluorophenyl]acetic Acid in 794 mL of DMF was added 206 mL (2.18 mol) of Ac₂O followed by dropwise addition of 304 mL (2.18 mol) of TEA over 15 min. An exothermic reaction ensued and after 1 h the reaction was poured onto 2 L of H₂O. Workup gave 351 g of a mixture of diastereomers as a yellow foam.

N-(7-Chloro-6-fluoro-2(*R*)-methylchroman-4-yl)benzamide. To a mixture of 347 g (0.95 mol) of 4-(benzoylamino)-7-chloro-6-fluoro-2(*R*)-methylchroman-4-carboxylic acid in 1.6 L of CH₂Cl₂ was added 466 g (1.05 mol) of Pb(OAc)₄. The mixture was stirred at 23 °C for 15 min and then heated at reflux for 3 h. Workup gave 132 g of a brown solid. Material of this purity was used for hydrolysis to the chroman-4-one. A small sample was recrystallized from a 2:1 isopropyl alcohol/Et₂O to give a light tan solid: mp 191-192 °C; ¹H NMR (10:1 CDCl₃/DMSO-d₆) δ 8.43 (br s, 1 H), 7.76 (m, 2 H), 7.32 (m, 3 H), 6.93 (d, 1 H, J =9), 6.72 (d, 1 H, J = 7), 6.13 (d, 1 H, J = 4), 5.00 (m, 1 H), 1.42 (d, 3 H).

7-Chloro-6-fluoro-2(**R**)-methylchroman-4-one. To a slurry of 127 g (0.4 mol) of N-(7-chloro-6-fluoro-2(R)-methyl-2H-1benzopyran-4-yl)benzamide in 1.27 L of acetone was added 317 mL of 3 N HCl and the reaction heated at reflux for 1 h. Workup gave 25 g of a white solid. Recrystallization from 125 mL of hexane at reflux gave 12.9 g of product: mp 106.5–108 °C; $[\alpha]^{25}_{\rm D} = 70.7^{\circ}$ (c = 1, CH₃OH); ¹H NMR (CDCl₃) δ 7.65 (d, 1 H, J = 9), 7.12 (d, 1 H, J = 6), 4.68 (m, 1 H), 2.75 (m, 2 H), 1.60 (d, 3 H).

Ethyl 7-Chloro-6-fluoro-c-4-hydroxy-2(R)-methylchroman-4-acetate. A solution of 8.7 mL (6.2 mmol) of diisopropylamine in 150 mL of THF was cooled to 0 °C, 23.8 mL (6.2 mmol) of 2.6 M *n*-butyllithium in hexane was added, and the temperature was kept below 5 °C. The reaction was cooled to -78 °C, 6.0 mL (6.2 mmol) of EtOAc was added followed by a solution of 12.0 g (5.6 mmol) of 2(R)-7-chloro-6-fluoro-2methylchroman-4-one in 50 mL of THF, and the reaction temperature was kept below -65 °C. Workup gave the product (17.7 g) as an oil; ¹H NMR (CDCl₃) δ 7.10 (d, 1 H, J = 10), 6.68 (d, 1 H, J = 6), 4.30 (m, 1 H), 4.13 (q, 2 H), 2.73 (s, 2 H), 2.07 (m, 2 H), 1.33 (d, 3 H), 1.20 (t, 3 H).

7-Chloro-6-fluoro-c-4-hydroxy-2(R)-methylchroman-4acetic Acid (60). To a solution of 0.875 g (15.6 mmol) of KOH in 43 mL of ethanol was added 4.3 g (14.2 mmol) of ethyl 7chloro-6-fluoro-c-4-hydroxy-2(R)-methyl-chroman-4-acetate. The reaction was stirred at 23 °C for 4 h and then concentrated in vacuo. Workup gave 2.7 g of a solid foam which liquefied at 42-50 °C and effervesced at 60–95 °C: $[\alpha]^{25}_{D} = +104.9^{\circ}$ ($c = 1, CH_{3}OH$, corrected for ether content); ¹H NMR (CDCl₃) δ 7.20 (d, 1 H), 6.78 (d, 1 H), 4.20 (m, 1 H), 2.84 (s, 2 H), 2.26 (d, 1 H), 1.88 (t, 1 H), 1.37 (d, 3 H) (The NMR indicated about 20 mol % entrapped Et₂O in the foam.); HRMS calcd for $C_{12}H_{12}O_{4}^{35}ClF$ 274.0408, found 274.0378; chiral HPLC analysis of the derivatized methyl ester on a Pirkle 1A ionic column using 1.5% 2propanol/hexane eluant showed 1% of the earlier eluting 2S,45-enantiomer 61.

Sodium 7-Chloro-6-fluoro-*c*-4-hydroxy-2(*R*)-methylchroman-4-acetate. White crystalline material: mp 250–253 °C; $[\alpha]^{25}_{D} = 130.5^{\circ}$ (*c* = 1, CH₃OH); ¹H NMR (DMSO-*d*₆) δ 8.92 (br s, 1 H), 7.24 (d, 1 H), 6.75 (d, 1 H), 4.21 (m, 1 H), 2.27 (m, 2 H), 1.99 (d, 1 H), 1.62 (t, 1 H), 1.26 (d, 3 H). Anal. (C₁₂H₁₁-O₄ClFNa) C, H.

Sodium 7-Chloro-6-fluoro-c-4-hydroxy-2(S)-methylchroman-4-acetate. This material was prepared using the same procedure as for the 2*R*-enantiomer: mp 251–153 °C [α]²⁵_D = -129.2° (c = 1, CH₃OH); 96.2% one peak by chiral HPLC; 3.8% unidentified less polar material; S-enantiomer not detectable. Anal. (C₁₂H₁₁O₄CIFNa) C, H.

6,7-Dichloro-c-4-hydroxy-2(R)-methylchroman-4-acetic Acid, Brucine Salt. To 2.3 L of CH₃CN was added 99.6 g (0.342 mol) of 6,7-dichloro-c-4-hydroxy-2-methylchroman-4-acetic acid and 147.0 g (0.342 mol) of brucine dihydrate. The mixture was brought to reflux to effect almost complete solution of the reagents, and slightly hazy particulate matter was removed by filtration of the hot solution. The filtrate was allowed to cool to 23 °C, and after 20 h, 96.5 g of white solid [mp 184–187 °C; $[\alpha]^{25}_{D} = +11^{\circ}$ $(c = 1, CH_3OH)$] was collected by filtration. The mother liquors were set aside for recovery of the 2S,4S-enantiomer and the entire batch of white solid was taken up in 1.5 L of CH₃CN at reflux to give a clear solution. The volume was reduced to 1.2 L by boiling off CH₃CN, the solution was allowed to cool to 23 °C, and after 20 h partially purified product was recovered by filtration as 65 g of white crystals: mp 191–195 °C; $[\alpha]^{25}_{D} = +30^{\circ}$ (c = 1, CH₃OH). This was dissolved in 1.4 L of CH₃CN at reflux, the solution was allowed to cool to 23 °C, and after 20 h there was isolated by filtration 52.7 g of product as white crystals: mp 193–197 °C; $[\alpha]^{25}_{D}$ = +36° (c = 1, CH₃OH). The absolute 2R,4R-configuration of this compound was established by X-ray crystallographic analysis.

6,7-Dichloro-c-4-hydroxy-2(R)-methylchroman-4-acetic Acid (58). The brucine salt (52.0 g) from the preceding example was partitioned between 500 mL of 0.5 N HCl and a mixture of 500 mL of Et₂O and 100 mL of EtOAc. The organic layer was washed with 4×300 mL of 0.5 N HCl followed by 300 mL of brine and then dried over $MgSO_4$. Concentration in vacuo gave 22.5 g of a glassy foam still containing some solvent; $[\alpha]^{25}_{D} = +102^{\circ}$ $(c = 1, CH_3OH)$. This material was treated with 200 mL of 1:1 CH_2Cl_2 /hexane at reflux and about 100 mg of a white gummy substance removed from the hot solution by filtration. On cooling to 30 °C, 100 mg of white gummy material formed and was removed by filtration. On standing at 23 °C for 20 h, large crystals formed and were collected by filtration to give 6.46 g of solids; mp 107–111 °C with gassing; $[\alpha]^{25}_{D} = +112.6^{\circ}$ ($c = 1, CH_{3}OH$). The mother liquors were concentrated in vacuo to a white solid $[12.0 \text{ g}, [\alpha]^{25}_{D} = +121^{\circ} (c = 1, CH_{3}OH)].$ To the latter solid was added 50 mL of hexane, the slurry was warmed, and Et₂O was added until solution occurred. Solvent was removed at reflux until the solution became slightly cloudy. On cooling to 23 °C two phases formed. After about 1 h crystals began growing in both phases. After standing 20 h, the resultant crystals were removed by filtration from the single solvent phase and on drying at 23 °C gave 10.4 g of product; mp 103–107 °C; $[\alpha]^{25}_{D} = +124^{\circ} (c = -1)^{25}$ 1, CH₃OH). Anal. $(C_{12}H_{12}O_4Cl_2)$ C, H, Cl.

NMR Studies. The conformation of the ethyl ester of 4 was studied in CDCl₃ solution. The ring exists as a chair rather than boat based on H3_{ax} to H2_{ax} and H3_{eq} to H2_{ax} couplings: 26% NOE H3_{ax}-H3_{eq}, 0% NOE H3_{ax} to CH₂CO₂C₂H₅, 7% NOE H2_{ax} to CH₂CO₂C₂H₅, 2% NOE H3_{ax} to H2_{ax}, and 7% NOE H5 to CH₂CO₂C₂H₅.

Studies on 41 are most consistent with an equatorial acetic acid side chain conformation, although there is some uncertainty as to the degree to which an internal hydrogen bond fixes the acetic acid moiety: 14% NOE H5 to CH_2CO_2H —contrast with 7% NOE in ester of 4 and 0% NOE for CH_3 to CH_2CO_2H .

Biological Methods. Enzyme Preparation. Aldose reductase was partially purified from human placentae by a modification of Hayman and Kinoshita's¹⁸ purification of rat lens aldose reductase. Freshly obtained human placentae were homogenized in 3 volumes of 0.1 M K₃PO₄ buffer (pH 7.0) containing 5 mM 2-mercaptoethanol and centrifuged for 20 min at 33000g at 4 °C. The supernatants were subjected to a 50–75% NH₄SO₄ fractionation and the resulting pellets were pooled, resuspended in a minimum volume of buffer, and dialyzed overnight. The dialysate was chromatographed on a DEAE-cellulose column (2 cm × 25) and aldose reductase was eluted with a linear salt gradient (0–1 M NaCl). Peak fractions containing aldose reductase activity were pooled and aliquots stored frozen.

In Vitro Assay Procedure. Enzyme activity was assayed with an Abbott VP bichromatic clinical analyzer, which measured the rate of NADPH oxidation at 340 nm at 25 °C over 12 min in a reaction mixture of 0.25 mL of 50 mM K_3PO_4 buffer (pH 7.1) containing 0.4 M NH₄SO₄, 0.067 mM NADPH, and 1.0 mM

⁽¹⁸⁾ Hayman, S.; Kinoshita, J. H. Isolation and Properties of Lens Aldose Reductase. J. Biol. Chem. 1965, 240, 877-882.

Hydantoin Bioisosteres

dl-glyceraldehyde. Sufficient enzyme was added to produce a rate of NADPH oxidation equal to 4 milliunits (unit equal to 1 μ mol of NADPH oxidized at 25 °C/min). The coefficient of variation for this assay over a 4-year period was approximately 12%.

Acute in Vivo Model. According to the method of Peterson et al.,¹⁹ rats (male Sprague–Dawley, 180–220 g, 42–54 days old) were made diabetic by the injection of 85 mg/kg of streptozotocin at 0 h; test compounds were administered by oral gavage at 4, 7, and 24 h. Doses for this screen are given as mg/kg of compound at each of the three time points. The results are expressed as the mean (\pm SEM) percent inhibition of sorbitol accumulation versus untreated diabetic controls. Statistical significance was calculated on the basis of the absolute levels of sorbitol in the treated and untreated diabetic groups by using Student's t test.

Chronic in Vivo Model. Rats were made diabetic by iv injection of 85 mg/kg streptozotocin. After 7 days the test compounds were administered once-a-day for 5 days by oral gavage. Animals were sacrificed on day 12. Three hours after the last dose, rats were sacrificed, blood was collected, and 25-mg portions of each sciatic nerve were removed. The sorbitol content of neutralized perchloric acid extracts of tissues was determined enzymatically using sorbitol dehydrogenase. Reaction mixtures in a final volume of 1.5 mL contained 50 μ mol of glycine buffer (pH 9.4), 1.2 μ mol of NAD, 100 μ g of sheep liver sorbitol dehydrogenase (ca. 4 units), and up to 40 nmol of sorbitol.

Statistical Analysis. The IC_{50} for both the in vitro and acute in vivo assays was calculated by a previously described method.¹ This analysis assumes that the percent inhibition is a linear function of the logarithm of concentration within the dose-proportional range and that the slope of the line is the same for all compounds in the experiment. Observations are assumed to be mutually independent and of equal weight. Error in measuring inhibition is assumed to be normally distributed and approximately equal throughout the dose range for all compounds.

Measurement of ARIs in Nerve and Plasma. Nerve and plasma concentrations of ARIs were measured using an HPLC assay. An internal standard (another ARI of similar structure) was added to plasma samples containing aldose reductase inhibitors. Samples were acidified to pH 3 with HCl and were extracted into Et₂O and reconstituted in mobile phase. Aliquots were injected onto a μ Bondapak C-18 column and drug and internal standard were monitored by ultraviolet absorption at 294 nm. The mobile phase used for determinations of 18, 23, 32, 33, 35, and 36 was CH₃CN/THF/0.01 M NaH₂PO₄, pH 3.1, 45/10/45. The mobile phase used for determination of 4 was CH₃CN/H₂O (with 5 mM tetrabutylammonium phosphate), 65/35. Sciatic nerve samples (25 mg) were placed in 2 mL of H₂O containing internal standard and homogenized in a 5-mL glass/glass tissue homogenizer. Homogenates were acidified and drug and internal standards were extracted into Et₂O. The same HPLC systems used to quantify ARI in plasma was used for nerve samples.

X-ray Crystal Structures. A representative crystal was surveyed and a 1-Å data set (maximum sin $\vartheta/\lambda = 0.5$) was collected on a Nicolet $R3m/\mu$ diffractometer for 59 and a Syntex P1 diffractometer for all other X-rays. Atomic scattering factors were taken from the International Tables for X-ray Crystallography.²⁰ All crystallographic calculations were facilitated by the SHELXTL2 system and all diffractometer data were collected at room temperature. The brucine salt of 58 was crystallized in space group $P2_12_12_1$ as a 1:1 adduct bishydrate from acetonitrile with 4 molecules/unit cell. The chirality of 58 is assigned from the known absolute configuration of brucine. The final R index was 0.065. 59 was crystallized from CH_2Cl_2 /hexane in space group $P2_12_12_1$ with four pairs of dimeric nonequivalent molecules/unit cell. The final R index was 0.038. The chirality follows from that of 58. Sorbinil crystals were grown by slow evaporation of an acetone/CH₃OH solution in space group $P2_12_12_1$ with 1.55 molecules/unit cell. The final R index was 0.040. The absolute configuration has previously been reported as S.²¹ 2-Methyltetralone hydantoin (4,1'(S)-3'(R)-methyl-7'-fluorospiro[imidazolidine-4',1'(2'H)-naphthalene]-4'(3'H),2,5-trione) was crystallized $below 60 °C from acetone in space group <math>P2_12_12_1$ with four molecules/unit cell. The final R index was 0.047. The absolute configuration is the same as in sorbinil at the spiro carbon center.

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Registry No. 1, 111502-99-9; 2, 111503-00-5; 3, 140226-36-4; 4, 111477-59-9; 5, 111477-55-5; 5 ethyl ester, 111502-76-2; 6, 111503-01-6; 7, 111477-52-2; 8, 111503-02-7; 9, 140226-37-5; 10, 111503-04-9; 11, 111503-05-0; 12, 111503-06-1; 13, 111532-19-5; 14, 140226-38-6; 15, 140226-39-7; 16, 111503-07-2; 17, 111532-19-5; 18, 111477-50-0; 19, 111478-56-9; 20, 111503-08-3; 21, 111503-09-4; 22, 111477-57-7; 23, 112711-08-7; 24, 111503-10-7; 25, 111503-43-6; **26**, 111503-11-8; **27**, 111503-12-9; **28**, 111503-13-0; **29**, 111503-14-1; 30, 111503-19-6; 31, 111503-15-2; 32, 111477-54-4; 33, 111478-59-2; 34, 111503-20-9; 35, 111478-57-0; 36, 111478-58-1; 37, 111503-21-0; 38, 111503-22-1; 39, 111503-53-8; 40, 140226-40-0; 41, 111532-17-3; 42, 140226-41-1; 43, 111532-18-4; 44, 111502-88-6; 45, 111502-89-7; 46, 111502-90-0; 47, 111502-91-1; 48, 140226-42-2; 49, 140226-43-3; **50**, 111502-92-2; **51**, 140226-44-4; **52**, 111502-93-3; **53**, 111502-95-5; 54, 111502-97-7; 55, 111503-16-3; 56, 111503-17-4; 57, 111503-18-5; 58, 111555-76-1; 58 brucine salt, 111686-81-8; 59, 140387-61-7; 60, 111477-47-5; 60 ethyl ester, 111477-46-4; 60 sodium salt, 111477-48-6; 61, 140387-62-8; 61 sodium salt, 140387-63-9; AR, 9028-31-3; EtOAc, 141-78-6; CH₃CHO, 75-07-0; 3,4-Cl₂C₆H₃CH₂Cl, 102-47-6; (±)-6-chloro-2-methylchroman-4-one, 82320-21-6; 4chlorophenol, 106-48-9; (\pm) - β -butyrolactone, 36536-46-6; (\pm) -3-(4-chlorophenoxy)butyric acid, 140226-45-5; (±)-3-(3-chloro-4fluorophenoxy)butyric acid, 140226-46-6; (±)-3-(4-chloro-3methoxyphenoxy)butyric acid, 140226-47-7; (±)-7-chloro-6fluoro-2-methylchroman-4-one, 140387-64-0; (±)-6-chloro-7methoxy-2-methylchroman-4-one, 140360-39-0; 2,4-dichlorophenol, 120-83-2; 4-chloro-1-naphthol, 604-44-4; 4-chloro-3-methoxyphenol, 18113-07-0; (E0-crotonic acid, 107-93-7; (±)-6,8-dichloro-2methylchroman-4-one, 140226-48-8; (±)-6-chloro-3,4-dihydro-2methyl-2H-benzo[h]chroman-4-one, 140226-49-9; 1-(2-hydroxy-4,5-dichlorophenyl)-1,3-butanedione, 111477-92-0; (±)-6,7-dichloro-2-methylchroman-4-one, 140387-65-1; 5-fluoro-2hydroxyacetophenone, 394-32-1; (±)-6-fluoro-2-methylchroman-4-one, 82320-16-9; (±)-6-fluoro-2-phenylchroman-4-one, 93602-26-7; 6-fluoro-4H-1-benzopyran-4-one, 105300-38-7; (±)-6fluoro-2-(3,4-dichlorobenzyl)chroman-4-one, 140360-40-3; 3chloro-4-fluorophenol, 2613-23-2; ethyl L-(+)-(S)-lactate, 687-47-8; ethyl (R)-2-(3-chloro-4-fluorophenoxy)propionate, 111503-55-0; (R)-2-(3-chloro-4-fluorophenoxy)propanol, 140226-50-2; (R)-2-(3-chloro-4-fluorophenoxy) propyl bromide, 111503-57-2; (\pm) - α hydroxyhippuric acid, 109125-34-0; 2(R)-(benzoylamino)-2-[2-[(1R)-2-bromo-1-methylethoxy]-4-chloro-5-fluorophenyl]acetic acid, 111503-58-3; 2(S)-(benzoylamino)-2-[2-[(1R)-2-bromo-1methylethoxy]-4-chloro-5-fluorophenyl]acetic acid, 111503-24-3; 4(R)-(benzoylamino)-7-chloro-6-fluoro-2(R)-methylchroman-4carboxylic acid, 111532-20-8; 4(S)-(benzoylamino)-7-chloro-6fluoro-2(R)-methylchroman-4-carboxylic acid, 111532-22-0; N-[7-chloro-6-fluoro-2(R)-methylchroman-4-yl]benzamide, 140226-51-3; 7-chloro-6-fluoro-2(R)-methylchroman-4-one, 111503-60-7; sorbinil, 68367-52-2.

Supplementary Material Available: Tables of analytical data for compounds of Table I and their precursors and compounds from Table II, and crystal X-ray data for 58, 59, sorbinil, and the 2-methyltetralone hydantoin derivative (9 pages). Ordering information is given on any current masthead page.

⁽¹⁹⁾ Peterson, M. J.; Sarges, R.; Aldinger, C. E.; MacDonald, D. P. CP-45,634: A Novel Aldose Reductase Inhibitor That Inhibits Polyol Pathway Activity in Diabetic and Galactosemic Rats. *Metab. Clin. Exp.* 1979, 28 (Suppl. 1), 456-461.

⁽²⁰⁾ International Tables for X-ray Crystallography; Kynoch Press: Birmingham, UK, 1974; Vol. IV, pp 55, 99, 149.

⁽²¹⁾ Sarges, R.; Bordner, J.; Dominy, B. W.; Peterson, M. J.; Whipple, E. B. Synthesis, Absolute Configuration, and Conformation of the Aldose Reductase Inhibitor Sorbinil. J. Med. Chem. 1985, 28, 1716-1720.