Biological Activities and Quantitative Structure-Activity Relationships of Spiro[imidazolidine-4,4'(1'H)-quinazoline]-2,2',5(3'H)-triones as Aldose Reductase Inhibitors

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A series of spiro[imidazolidine-4,4'(1'H)-quinazoline]-2,2',5(3'H)-triones were prepared and tested for aldose reductase inhibitory activity. The 6'-halogenated derivatives were found to be highly potent in vitro inhibitors of male rabbit lens aldose reductase and in vivo inhibitors of polyol accumulation in the sciatic nerves of galactosemic rats. Of these, (4R)-6'-chloro-3'-methylspiro[imidazolidine-4,4'(1'H)-quinazoline]-2,2',5(3'H)-trione (**67**) showed the most potent in vitro and in vivo activities. An oral dose of 3 g/kg of compound **67** caused neither death nor behavioral abnormality in the preliminary acute toxicity study using mice and rats. Compound **67** was selected as a candidate for further evaluation. The quantitative structure-activity relationships in this series are also discussed.

Formation of sorbitol from glucose, catalyzed by aldose reductase (AR), is believed to cause diabetic complications such as retinopathy, neuropathy, nephropathy, and cataracts.[1] Aldose reductase inhibitors (ARIs) should be of value in preventing or treating these diabetic complications, and several compound are currently undergoing clinical trials. Orally active ARIs can be classified into two major categories; the five-membered cyclic imide analogues such as spirohydantoins and the carboxylic acid derivatives.[2]

In the course of our studies on quinazolines,[3] we have found new synthetic methods for spiro[imidazolidine-4,4'(1'H)-quinazoline]-2,2',5(3'H)-triones (**5**). These compounds have a common moiety in the structure for orally active ARIs (the spirohydantoin ring). Such a structural characteristic prompted us to carry out a systematic search for a new potent ARI agent. In this paper we describe the synthesis and pharmacology of spirohydantoin derivatives **5** and their quantitative structure-activity relationships (QSAR).

Chemistry

A reported approach to spiro[imidazolidine-4,4'(1'H)-quinazoline]-2,2',5(3'H)-triones involves the reaction of either 3-iminoisatin or 1-carbamoylisatins with isocyanates followed by base treatment.[4] However, this method is not applicable for the synthesis of the desired quinazolin-2-one derivatives having an unsubstituted spirohydantoin ring. Recently, we described a modification of this synthetic sequence using urea derivatives instead of isocyanates.[5-7] These two major synthetic routes reported by us, method A (the quinazolinone route) and method B (the arylhydantoin route) illustrated in Scheme I, were employed for many of our target compounds (Tables I, II, and VII). Method A is general and used for the preparation of **8**, **23-27**, **29-34**, **36**, **40-46**, **48-50**, **53**, and **59**. Thus, isatins (**3**) prepared from the corresponding anilines (**1**) via isonitrosoacetanilides (**2**),[8] were reacted with isocyanates to give intermediate 1-carbamoylisatins (**4**). These were then treated with 2-ethyl-2-isothiourea hydrobromide followed by heating with hydrochloric acid (HCl) to give the desired spirohydantoins. Method B is suitable for the preparation of the N-unsubstituted (**20**), *N*-amino (**21**), and *N*-hydroxy derivatives (**22**). Thus, a suitable 1-(ethoxycarbonyl)isatin (**6**) derived from **3** and ethyl chlorocarbonate was reacted with urea to give hydantoin **7**, which was converted into the desired spirohydantoins (**20-22**) by treatment with the corresponding amines.

The introduction of alkyl groups at the nitrogens of **8** was carried out as outlined in Schemes II and III. When

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(1) (a) Gabbay, K. H.; Merola, L. O.; Field, R. A. Sorbitol Pathway: Presence in Nerve and Cord with Substrate Accumulation in Diabetes. *Science* **1966**, *151*, 209-210. (b) Gabbay, K. H. Hyperglycemia, Polyol Metabolism, and Complications of Diabetes Mellitus. *Annu. Rev. Med.* **1975**, *26*, 521-536. (c) Kinoshita, J. H.; Kador, P. F.; Catiles, M. Aldose Reductase in Diabetic Cataracts. *JAMA, J. Am. Med. Assoc.* **1981**, *246*, 257-261. (d) Brownlee, M.; Cerami, A. The Biochemistry of the Complications of Diabetes Mellitus. *Annu. Rev. Biochem.* **1981**, *50*, 385-432. (e) Kador, P. F.; Robison, W. G.; Kinoshita, J. H. The Pharmacology of Aldose Reductase Inhibitors. *Annu. Rev. Pharmacol. Toxicol.* **1985**, *25*, 691-714. (f) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. Aldose Reductase Inhibitors: A Potential New Class of Agents for the Pharmacological Control of Certain Diabetic Complications. *J. Med. Chem.* **1985**, *28*, 841-849.

(2) (a) Lipinski, C. A.; Hutson, N. J. Aldose Reductase Inhibitors as a New Approach to the Treatment of Diabetic Complications. *Annu. Rep. Med. Chem.* **1984**, *19*, 169-177. (b) Benfield, P. Aldose Reductase Inhibitors and Late Complications of Diabetes. *Drugs* **1986**, *32* (Suppl. 2), 43-55. (c) Humber, L. G. The Medicinal Chemistry of Aldose Reductase Inhibitors. *Prog. Med. Chem.* **1987**, *24*, 299-343. (d) Larson, E. R.; Lipinski, C. A.; Sarges, R. Medicinal Chemistry of Aldose Reductase Inhibitors. *Med. Res. Rev.* **1988**, *8*, 159-186. (e) Masson, E. A.; Boulton, A. J. M. Aldose Reductase Inhibitors in the Treatment of Diabetic Neuropathy. A Review of the Rationale and Clinical Evidence. *Drugs* **1990**, *39*, 190-202.

(3) We synthesized 6-amino-2-fluoromethyl-3-(o-tolyl)-4(3H)-quinazolinone (afloqualone), which has been commercialized as a potent muscle relaxant; (a) Tani, J.; Yamada, Y.; Ochiai, T.; Ishida, R.; Inoue, I.; Oine, T. Studies on Biologically Active Halogenated Compounds. II. Chemical Modifications of 6-Amino-2-fluoromethyl-3-(o-tolyl)-4(3H)-quinazolinone and the CNS Depressant Activities of Related Compounds. *Chem. Pharm. Bull.* **1979**, *27*, 2675-2687. (b) Tani, J.; Yamada, Y.; Oine, T.; Ochiai, T.; Ishida, R.; Inoue, I. Studies on Biologically Active Halogenated Compounds. 1. Synthesis and Central Nervous System Depressant Activity of 2-(Fluoromethyl)-3-aryl-4(3H)-quinazolinone Derivatives. *J. Med. Chem.* **1979**, *22*, 95-99.

(4) (a) Capuano, L.; Welter, M.; Zander, R. Heterocyclizations, VII. New Hydantoins with Bridge-head Nitrogen or Spirane Structure. *Chem. Ber.* **1970**, *103*, 2394-2402. (b) Capuano, L.; Benz, K. Ring Transformations in the Isatin Series, IV. Spirocyclizations. *Chem. Ber.* **1977**, *110*, 3849-3861.

(5) Yamagishi, M.; Ozaki, K.; Ohmizu, H.; Yamada, Y.; Suzuki, M. Quinazolin-2-ones Having a Spirohydantoin Ring. I. Synthesis of Spiro[1,2,3,4-tetrahydroquinazoline-4,4'-imidazolidine]-2,2',5'-trione by Reaction of 1-Carbamoylisatin with Urea or Guanidine. *Chem. Pharm. Bull.* **1990**, *38*, 2926-2928.

(6) Yamagishi, M.; Yamada, Y.; Ozaki, K.; Tani, J.; Suzuki, M. Quinazolin-2-ones Having a Spirohydantoin Ring. II. Synthesis of Several Spiro[imidazolidine-4,4'(1'H)-quinazoline]-2,2',5(3'H)-triones via 5-Hydroxyhydantoin Derivatives. *Chem. Pharm. Bull.* **1991**, *39*, 626-629.

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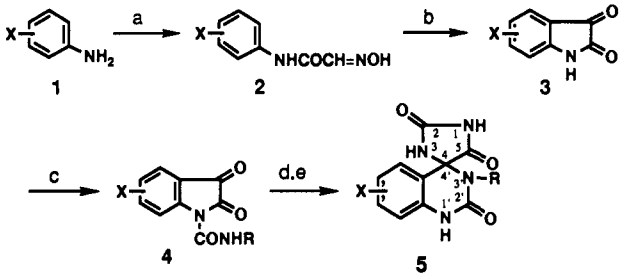
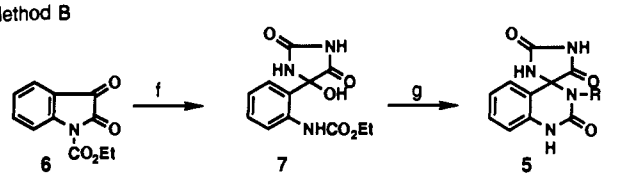
Scheme I[a]

Method A

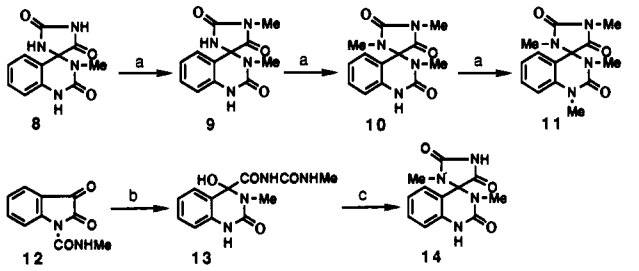


Method B



[a] (a) $CCl_3CHO \cdot H_2O$, NH_2OH; (b) concentrated H_2SO_4, Δ; (c) RNCO, Et_3N, THF; (d) $H_2NC(SEt)=NH \cdot HBr$, Et_3N, THF; (e) 10% HCl, Δ; (f) H_2NCONH_2, THF, Δ; (g) RNH_2, EtOH, toluene, Δ.

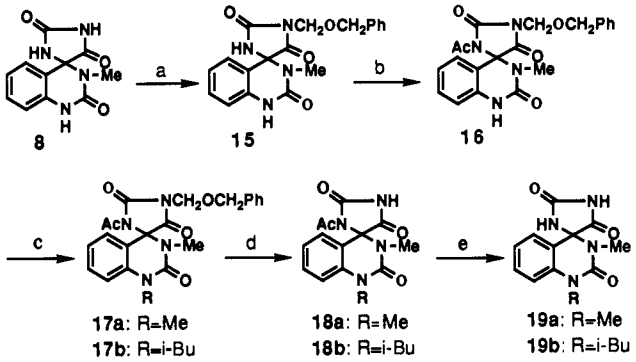
Scheme II[a]



[a] (a) MeI (1.0 equiv), NaH (1.0 equiv), DMF; (b) $H_2NCONHMe$, DBU, THF; (c) Δ.

8 was treated with 1 equiv of methyl iodide (MeI) in the presence of an equimolar amount of sodium hydride (NaH), methylation proceeded selectively at the 1-nitrogen of the hydantoin ring to give compound **9**, which was identical with an authentic sample.[4a] In this reaction, methylation did not occur at the 3-nitrogen of the hydantoin ring.[9] Further methylation of **9** afforded **10** and **11**. The structure of **10** was confirmed by a comparison with an authentic sample.[4b] Compound **14** was obtained by the reaction of 1-carbamoylisatin (**12**) with methylurea followed by heating of the resulting quinazolinone **13** in 1,2-dichlorobenzene. The 1′,3′-dialkylspirohydantoins **19a** and **19b** were prepared starting from **8** as shown in Scheme III. After protection of the reactive nitrogens on the hydantoin ring by benzyloxymethyl and acetyl groups, alkylation of the resulting **16** with MeI or isobutyl iodide provided **17a** and **17b**, respectively. Deprotection of **17a,b** by hydrogenolysis followed by alkaline hydrolysis furnished

Scheme III[a]



[a] (a) $ClCH_2OCH_2Ph$, NaH, DMF; (b) AcCl, NaH, DMF; (c) RI, NaH, DMF; (d) Pd black, H_2; (e) Na, EtOH.

the desired compounds **19a** and **19b**.

Some nitro compounds **47**, **51**, **55**, and **57** were prepared by regioselective mononitration of **29**, **42**, **32**, and **34** with sodium nitrate in sulfuric acid, respectively. Catalytic reduction (H_2/Pd black) or metal and acid reduction ($SnCl_2$/HCl) of the nitro group in **34** and **55** furnished anilines **35** and **56** in good yields. Phenol **37** was prepared from methyl ether **36** by reaction with 48% hydrobromic acid (HBr) at 130 °C. The phenol was acylated conventionally with benzoyl chloride and cinnamoyl chloride to give **38** and **39**.

Direct chlorination of **8** and **44** with sulfuryl chloride resulted in the formation of the dichloro compounds **54** and **58**, respectively. Compound **52** was prepared by nucleophilic substitution of **51** with morpholine.

For comparison of the biological activity, the related quinazolinones with the iminohydantoin (**60**),[5] aminothiazole (**61**),[7] and thiohydantoin rings (**62**)[7] were also prepared according to the previously reported methods (Table VII).

We have already reported that the absolute configuration of **63** [(+)-**8**] is *R*, as established by the X-ray crystallography.[10] In this work, the 6′-fluoro derivative **31** was also resolved using quinine to give the optically active compounds **65** and **66**.[11] The optically active 6′-chloro derivatives **67** and **68** were prepared by chlorination of the corresponding optically active compounds **63** and **64** with sulfuryl chloride.

Biological Results and Discussion

The in vitro inhibitory activities of these resulting compounds against the partially purified AR, which was obtained from male rabbit lens, were measured by the method of Hayman and Kinoshita.[12] Furthermore, the compounds showing the inhibitory activity at the concentration of less than 10^{-5} M were evaluated for their ability to inhibit polyol accumulation in the sciatic nerve of galactosemic rats (in vivo test).[13,14] These results are

(7) Yamagishi, M.; Ozaki, K.; Yamada, Y.; Da-te, T.; Okamura, K.; Suzuki, M. Quinazolin-2-ones Having a Spirohydantoin Ring. III. A General and Efficient Synthesis of 3′-Substituted Spiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-triones. *Chem. Pharm. Bull.* **1991**, *39*, 1694–1698.

(8) Popp, F. D. *Advances in Heterocyclic Chemistry*; Katritzky, A. R., Boulton, A. J., Eds.; Academic Press: New York, 1975; Vol. 18, pp 1–58.

(9) Orazi, O. O.; Corral, R. A.; Schuttenberg, H. Substitution in the Hydantoin Ring. Part VIII. Alkylation. *J. Chem. Soc., Perkin Trans. 1* **1974**, 219–221.

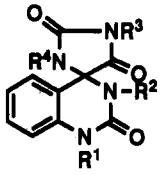
(10) Yamagishi, M.; Yamada, Y.; Ozaki, K.; Da-te, T.; Okamura, K.; Suzuki, M.; Matsumoto, K. Preparation of Optically Pure 3′-Methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione by Combination of Optical Resolution and Racemization. *J. Org. Chem.* **1992**, *57*, 1568–1571.

(11) The absolute configuration of the compounds (**65**, **66**) resolved optically was speculated as compound **65** showing a plus value on the polarimeter is *R*, while **66** showing minus one is *S* on the basis of the value of 6′-chloro compounds (**67**, **68**).

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

(13) Hayman, S.; Lou, M. F.; Merola, L. O.; Kinoshita, J. H. Aldose Reductase Activity in the Lens and Other Tissues. *Biochim. Biophys. Acta* **1966**, *128*, 474–482.

Table I. N-Substituted Spiroquinazolin-2-ones



compd	R^1	R^2	R^3	R^4	method[a]	yield, %	mp, °C	recrystn solvent	formula[b]	in vitro IC_{50},[c] M	in vivo, sciatic nerve[d] polyol contents[e] mean ± SE μmol/g	in vivo, sciatic nerve[d] % inhibn[f]
20[g]	H	H	H	H	B	59	>280	DMF-H_2O	$C_{10}H_8N_4O_3$	1.4×10^{-6}	10.7 ± 1.1	15.1
8[h,i]	H	Me	H	H	A	91	>280	DMSO	$C_{11}H_{10}N_4O_3$	2.8×10^{-7}	8.8 ± 0.4	32.5
21[g]	H	NH_2	H	H	B	57	>280	*l*	$C_{10}H_9N_5O_3 \cdot {}^1/_4H_2O$	4.0×10^{-6}	11.3 ± 0.5	12.2[m]
22[g]	H	OH	H	H	B	15	253 dec	*l*	$C_{10}H_8N_4O_4 \cdot {}^1/_2H_2O$	$>10^{-5}$		
23[i]	H	Et	H	H	A	75	>280	DMF-H_2O	$C_{12}H_{12}N_4O_3$	1.0×10^{-6}	9.6 ± 1.1	19.5[m]
24[i]	H	*i*-Pr	H	H	A	69	>280	DMF-H_2O	$C_{13}H_{14}N_4O_3$	$>10^{-5}$		
25[i]	H	*n*-Bu	H	H	A	53	263–266	*l*	$C_{14}H_{16}N_4O_3$	$>10^{-5}$		
26[i]	H	CH_2Ph	H	H	A	69	>280	DMF-H_2O	$C_{17}H_{14}N_4O_3$	$>10^{-5}$		
27[i]	H	Ph	H	H	A	72	>280	DMF-H_2O	$C_{16}H_{12}N_4O_3 \cdot {}^1/_2H_2O$	$>10^{-5}$		
19a[a]	Me	Me	H	H			223–225	DMF-H_2O	$C_{12}H_{12}N_4O_3 \cdot {}^2/_3H_2O$	8.0×10^{-7}	9.2 ± 0.4*	28.4
19b[a]	*i*-Bu	Me	H	H			270	EtOH-*i*-Pr_2O	$C_{15}H_{18}N_4O_3$	3.9×10^{-7}	8.1 ± 0.1	27.7
9[a,j]	H	Me	Me	H			269–270	DMF-H_2O	$C_{12}H_{12}N_4O_3$	$>10^{-5}$		
28[a]	H	Me	CH_2CO_2H	H			175	*l*	$C_{13}H_{12}N_4O_5 \cdot {}^1/_2H_2O$	$>10^{-5}$		
14[a]	H	Me	H	Me			>280	DMSO-H_2O	$C_{12}H_{12}N_4O_3$	$>10^{-5}$		
10[a,k]	H	Me	Me	Me			263–264	DMF-H_2O	$C_{13}H_{14}N_4O_3$	$>10^{-5}$		

[a] See Experimental Section. [b] All compounds were analyzed for C, H, and N. [c] Concentration that causes a 50% inhibition of enzymatic activity of partially purified AR from male rabbit lens. [d] Rats were fed a diet containing 20% galactose with or without the test compound at 30 mg/kg per day for 6 days. [e] Normal control, 3.4 ± 0.6 μmol/g (n = 5); galactosemic control, 9.9 ± 1.3 ~ 13.7 ± 0.8 μmol/g (n = 3). [f] Mean percent inhibition of polyol accumulation (n = 3). [g] Reference 6. [h] Reference 5. [i] Reference 7. [j] Reference 4a. [k] Reference 4b. [l] Reprecipitation. [m] Data obtained from rats fed a 20% galactose diet containing the test compound at 90 mg/kg per day. *$P < 0.05$: Significantly different from galactosemic control (Student's t-test).

summarized in Table I, II, VII, and VIII.

A. N-Substituted Derivatives. Unsubstituted spirohydantoin compound **20** exhibited strong AR inhibitory activity in the in vitro test, and the introduction of methyl group onto the 3′-position (compound **8**) increased remarkably the activity (Table I). Meanwhile, compounds having other alkyl, amino, hydroxy, and phenyl groups at the 3′-position showed almost the same activity as **20** or very weak activity. The introduction of alkyl substituents (**19a,b**) onto the 1′-nitrogen of compound **8** maintained the activity, while the compounds (**9, 10, 14, 28**) having alkyl substituents on either or both the 1- and the 3-nitrogen did not show the inhibitory activity.

B. Substituted Derivatives. To investigate substituent effects on the aromatic ring of **8** for the AR inhibitory activity, we prepared 32 compounds listed in Table II. The introduction of halogen increased the activity, while other substituents such as methyl, methoxy, amine, hydroxy, etc., gave no obvious effects or diminished the activity. Especially, halogen-substituted compounds at the 6′-position showed very strong inhibitory activities in both in vitro and in vivo assays.

QSAR. We analyzed their QSARs to find out about substituent effects on the aromatic ring in detail.[15] As indices of the hydrophobicity of substituents, we used the hydrophobicity parameters π derived from the logarithm of the partition coefficients (log P) of substituted benzenes in 1-octanol/water system.[16] The data necessary for the calculation were taken from the Pomona College database.[17] The electronic properties of the substituents were evaluated in terms of Hammett's electronic parameter (σ_m and σ_p).[18] To express the steric features of substituents, we used Verloop's STERIMOL parameters.[19] The steric parameters (L) were calculated on the basis of the CPK model with a computer program made by us. $I_{halo}(6')$ is the indicator variable which takes one for 6′-halogenated compounds and zero for others.

QSAR Analyses. The set of compounds **8** and **31–41** having various substituents at the 6′-position was analyzed first and gave the equation:

$$pI_{50} = \underset{(\pm 0.31)}{0.40\pi(6')} + \underset{(\pm 0.39)}{1.72\sigma_p(6')} - \underset{(\pm 0.10)}{0.25L(6')} + \underset{(\pm 0.37)}{0.58I_{halo}(6')} + \underset{(\pm 0.45)}{6.89} \quad (1)$$

$$n = 12,\ s = 0.19,\ r = 0.99,\ F_{4,7} = 67.6$$

In this and following equations, pI_{50} is log $(1/IC_{50})$, n is

(14) Dvornik, D.; Simard-Duquesne, N.; Krami, M.; Sestanj, K.; Gabbay, K. H.; Kinoshita, J. H.; Varma, S. D.; Merola, L. O. Polyol Accumulation in Galactosemic and Diabetic Rats: Control by an Aldose Reductase Inhibitor. *Science* **1973**, *182*, 1146–1148.

(15) Hansch, C.; Fujita, T. ρ-σ-π Analysis. A Method for the Correlation of Biological Activity and Chemical Structure. *J. Am. Chem. Soc.* **1964**, *86*, 1616–1626.

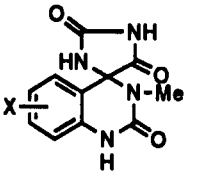
(16) Fujita, T.; Iwasa, J.; Hansch, C. A New Substituent Constant, π, Derived from Partition Coefficients. *J. Am. Chem. Soc.* **1964**, *86*, 5175–5180.

(17) Pomona College Medicinal Chemistry Database, Pomona College, Claremant, CA.

(18) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.

(19) Verloop, A.; Hoogenstraaten, W.; Tipker, J. In *Drug Design*; Ariëns, E. J., Ed.; Academic Press: New York, 1976; Vol. VI, pp 165–207.

Table II. Substituted Spiroquinazolin-2-ones



compd	X	yield, %[a]	mp, °C	recrystn solvent	formula[b]	in vitro IC_{50},[c] M	in vivo, sciatic nerve[d] polyol contents[e] mean ± SE, μmol/g	in vivo, sciatic nerve[d] % inhibn[f]
8	H					2.8×10^{-7}	8.8 ± 0.4	32.5
29	5′-Cl	54	>280	DMF–H_2O	$C_{11}H_9ClN_4O_3$	8.6×10^{-7}	11.9 ± 0.9	17.5
30	5′-Me	57	>280	*h*	$C_{12}H_{12}N_4O_3 \cdot {}^1/_2H_2O$	4.0×10^{-7}	9.2 ± 0.4	27.5
31	6′-F	66	>280	DMF–H_2O	$C_{11}H_9FN_4O_3 \cdot H_2O$	1.0×10^{-7}	6.7 ± 0.7*	58.7
32	6′-Cl	53	>280	DMF–H_2O	$C_{11}H_9ClN_4O_3$	5.6×10^{-8}	6.7 ± 0.4**	68.0
33	6′-Br	50	>280	*h*	$C_{11}H_9BrN_4O_3$	5.2×10^{-8}	5.6 ± 0.4**	74.7
34	6′-NO_2	26	>280	DMF–H_2O	$C_{11}H_9N_5O_5$	7.3×10^{-8}	12.1 ± 0.7	15.5
35	6′-NH_2	*g*	>280	*h*	$C_{11}H_{11}N_5O_3$	$>10^{-5}$		
36	6′-OMe	85	>280	DMF–H_2O	$C_{12}H_{12}N_4O_4$	4.5×10^{-6}	10.4 ± 0.6	13.6
37	6′-OH	*g*	>280	DMF–H_2O	$C_{11}H_{10}N_4O_4$	$>10^{-5}$		
38	6′-OCOPh	*g*	>280	*i*-PrOH	$C_{18}H_{14}N_4O_5$	2.0×10^{-6}	10.5 ± 0.6	12.3
39	6′-OCOCH=CHPh	*g*	>280	EtOH	$C_{20}H_{16}N_4O_5$	6.2×10^{-6}	10.4 ± 0.7	29.3
40	6′-CO_2Et	55	>280	EtOH–*i*-Pr_2O	$C_{14}H_{14}N_4O_5$	2.4×10^{-7}	11.1 ± 0.9	22.2
41	6′-Me	57	>280	*h*	$C_{12}H_{12}N_4O_3$	8.0×10^{-7}	9.0 ± 0.3*	30.9
42	7′-Cl	75	>280	DMF–H_2O	$C_{11}H_9ClN_4O_3$	9.4×10^{-8}	11.4 ± 1.2	22.3
43	7′-Me	65	>280	DMF–H_2O	$C_{12}H_{12}N_4O_3$	2.2×10^{-7}	8.4 ± 0.3	37.5
44	7′-OMe	73	270–272	*h*	$C_{12}H_{12}N_4O_4$	3.6×10^{-7}	9.8 ± 0.6	21.0
45	7′-NO_2	50	>280	*h*	$C_{11}H_9N_5O_5$	4.8×10^{-7}	10.3 ± 0.5	11.5
46	8′-F	50	>280	DMF–H_2O	$C_{11}H_9FN_4O_3$	4.2×10^{-8}	9.5 ± 0.5	29.9
47	5′-Cl,6′-NO_2	*g*	>280	DMF–H_2O	$C_{11}H_8ClN_5O_5$	4.7×10^{-7}	11.5 ± 0.6	0.0
48	6′,7′-Cl_2	68	>280	DMF–H_2O	$C_{11}H_8Cl_2N_4O_3$	7.4×10^{-8}	7.5 ± 0.2*	52.9
49	6′-Cl,7′-Me	50	>280	*h*	$C_{12}H_{11}ClN_4O_3$	3.0×10^{-8}	6.8 ± 0.4**	58.0
50	6′-Cl,7′-OMe	55	>280	*h*	$C_{12}H_{11}ClN_4O_4$	5.9×10^{-8}	8.3 ± 0.9	24.6
51	6′-NO_2,7′-Cl	*g*	>280	DMF–H_2O	$C_{11}H_8ClN_5O_5$	1.2×10^{-7}	10.6 ± 0.6	7.7
52	6′-NO_2,7′-morpholino	*g*	>280	DMF–H_2O	$C_{15}H_{16}N_6O_6$	4.3×10^{-7}	10.4 ± 0.5	10.3
53	6′,7′-OCH_2O–	51	>280	DMF–H_2O	$C_{12}H_{10}N_4O_5$	1.8×10^{-6}	9.2 ± 0.5	28.4
54	6′,8′-Cl_2	*g*	>280	*h*	$C_{11}H_8Cl_2N_4O_3$	3.7×10^{-8}	5.8 ± 0.2*	63.1
55	6′-Cl,8′-NO_2	*g*	>280	DMF–H_2O	$C_{11}H_8ClN_5O_5$	4.6×10^{-8}	8.0 ± 0.5	29.2
56	6′-Cl,8′-NH_2	*g*	>280	DMF–H_2O	$C_{11}H_{10}ClN_5O_3 \cdot {}^1/_2H_2O$	1.3×10^{-7}	7.0 ± 1.5	44.6
57	6′,8′-$(NO_2)_2$	*g*	>280	DMF–H_2O	$C_{11}H_8N_6O_7$	2.6×10^{-7}	10.1 ± 0.4	14.1
58	6′,8′-Cl_2,7′-OMe	*g*	>280	*h*	$C_{12}H_{10}Cl_2N_4O_4$	7.2×10^{-8}	8.4 ± 0.1	37.5
59	6′,7′,8′-F_3	55	>280	DMF–H_2O	$C_{11}H_7F_3N_4O_3$	1.4×10^{-7}	9.5 ± 1.0	29.9

[a] Yield from 4 by method A was shown. [b] All compounds were analyzed for C, H, and N. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I. [e] See footnote *e* in Table I. [f] See footnote *f* in Table I. [g] See Experimental Section. [h] Reprecipitation. *$P < 0.05$, **$P < 0.01$: Significantly different from galactosemic control (Student's *t*-test).

the number of compounds analyzed, s is the standard deviation, r is the multiple correlation coefficient and F is the value of F ratio between variances of calculated and observed activities. The figures in parentheses are the 95% confidence intervals. The π and σ terms having a positive sign in eq 1 show that hydrophobic and electron-withdrawing substituents are favorable for high activity. The steric parameter L was also significant in eq 1 and its negative coefficient indicated that the compounds with a smaller 6′-substituent are more active. The positive coefficient of $I_{halo}(6')$ suggests some additional effects of these substituents.

Next, we analyzed 7′-monosubstituted series of compounds (**8**, **42–45**) and found the equation:

$$pI_{50} = \underset{(0.47)}{0.59}\pi(7') + \underset{(0.20)}{6.49} \quad (2)$$

$$n = 5,\ s = 0.12,\ r = 0.92,\ F_{1,3} = 16.51$$

The hydrophobicity of substituents was the only significant parameter in the expression of inhibition. No electronic parameters were significant in eq 2.

We prepared 6′,7′-disubstituted compounds (**48–53**) to clarify the substituent effects of the 6′- and 7′-positions. QSAR analysis of these compounds together with mono-substituted compounds gave eq 3. The results were essentially the same as that of eqs 1 and 2. Introduction

$$pI_{50} = \underset{(\pm0.30)}{0.62}\pi(6') + \underset{(\pm0.33)}{1.31}\sigma_p(6') - \underset{(\pm0.10)}{0.29}L(6') + \underset{(\pm0.32)}{0.47}I_{halo}(6') + \underset{(\pm0.38)}{0.39}\pi(7') + \underset{(\pm0.37)}{7.03} \quad (3)$$

$$n = 22,\ s = 0.24,\ r = 0.97,\ F_{5,16} = 43.61$$

of $\Sigma[\pi(6') + \pi(7')]$ and $\Sigma[\sigma_p(6') + \sigma_m(7')]$ instead of each term made the correlation worse, its s, r, and F values being 0.34, 0.93, and 25.26, respectively. This result suggests two effects: (i) the hydrophobic effects of both the 6′- and the 7′-positions does not contribute to the process of permeation, but only to the interactions between the enzyme and inhibitors; (ii) the electronic effect of 6′-substituents is the site-specific one.

Finally, we analyzed all compounds shown in Table II having various substituents at their 5′-, 6′-, 7′-, and 8′-positions and obtained the following:

$$pI_{50} = \underset{(\pm0.27)}{0.68}\pi(6') + \underset{(\pm0.27)}{1.18}\sigma_p(6') - \underset{(\pm0.08)}{0.29}L(6') + \underset{(\pm0.25)}{0.47}I_{halo}(6') + \underset{(\pm0.25)}{0.44}\pi(7') + \underset{(\pm0.31)}{6.98} \quad (4)$$

$$n = 32,\ s = 0.25,\ r = 0.95,\ F_{5,26} = 48.95$$

Table III. Activities and Physicochemical Parameters of Aromatic Substituted Compounds

	pI_{50}			logit			physicochemical parameters						
compd	obsd	calcd[a]	Δ	obsd	calcd[b]	Δ	$\pi(6')$	$\sigma_p(6')$	$L(6')$	I_{halo}	$\pi(7')$	$\Sigma\pi$	$\Sigma\sigma$
8	6.55	6.38	0.17	−0.32	−0.35	0.03	0.00	0.00	2.06	0	0.00	0.00	0.00
29	6.07	6.38	−0.31	−0.67	−0.62	−0.05	0.00	0.00	2.06	0	0.00	0.71	0.37
30	6.40	6.38	0.02	−0.42	−0.28	−0.14	0.00	0.00	2.06	0	0.00	0.56	−0.07
31	7.00	6.84	0.16	0.15	−0.15	0.30	0.14	0.06	2.65	1	0.00	0.14	0.06
32	7.25	7.17	0.08	0.33	−0.02	0.35	0.71	0.23	3.52	1	0.00	0.71	0.23
33	7.28	7.18	0.10	0.47	0.02	0.43	0.86	0.23	3.83	1	0.00	0.86	0.23
34	7.14	6.70	0.44	−0.74	−0.53	−0.21	−0.28	0.78	3.44	0	0.00	−0.28	0.78
35	4.77	4.56	0.21	NT[c]	−1.04	-	−1.23	−0.66	2.78	0	0.00	−1.23	−0.66
36	5.35	5.48	−0.13	−0.80	−0.75	−0.05	−0.02	−0.27	3.98	0	0.00	−0.02	−0.27
37	5.00	5.29	−0.29	NT[c]	−0.98	-	−0.67	−0.37	2.74	0	0.00	−0.67	−0.37
38	5.70	5.74	−0.04	−0.85	−0.52	−0.33	1.46	0.13	8.15	0	0.00	1.46	0.13
39	5.21	5.32	−0.11	−0.38	−0.68	0.30	1.80[e]	0.13[g]	10.39	0	0.00	1.80	0.13
40	6.62	6.12	0.50	−0.55	−0.45	−0.10	0.51	0.45	5.95	0	0.00	0.51	0.45
41	6.09	6.32	−0.23	−0.35	−0.36	0.01	0.56	−0.17	2.87	0	0.00	0.56	−0.17
42	7.03	6.69	0.34	−0.54	−0.19	−0.36	0.00	0.00	2.06	0	0.71	0.71	0.37
43	6.66	6.62	0.04	−0.22	−0.16	−0.06	0.00	0.00	2.06	0	0.56	0.56	−0.07
44	6.44	6.37	0.07	−0.58	−0.46	−0.12	0.00	0.00	2.06	0	−0.02	−0.02	0.12
45	6.32	6.25	0.07	−0.89	−0.86	−0.03	0.00	0.00	2.06	0	−0.28	−0.28	0.71
46	6.38	6.38	0.00	−0.37	−0.43	0.06	0.00	0.00	2.06	0	0.00	0.14	0.06
47	6.33	6.70	−0.37	NA[d]	−0.94	-	−0.28[f]	0.78[h]	3.44	0	0.00	0.43	1.15
48	7.13	7.48	−0.35	0.05	−0.13	0.18	0.71	0.23	3.52	1	0.71	1.42	0.60
49	7.52	7.41	0.11	0.14	0.24	−0.10	0.71	0.23	3.52	1	0.56	1.27	0.16
50	7.23	7.16	0.07	−0.49	−0.09	−0.40	0.71	0.23	3.52	1	−0.02	0.69	0.35
51	6.92	7.02	−0.10	−1.08	−0.68	−0.40	−0.28	0.78	3.44	0	0.71	0.43	1.15
52	6.37	6.57	−0.20	−0.94	−0.85	−0.09	−0.28	0.78	3.44	0	−0.31	−0.59	0.63
53	5.75	5.74	0.01	−0.40	−0.55	0.15	−0.02	−0.16	3.49	0	−0.02	−0.05	−0.32
54	7.43	7.17	0.26	0.23	0.08	0.15	0.71	0.23	3.52	1	0.00	1.42	0.46
55	7.34	7.17	0.17	−0.39	−0.42	0.03	0.71	0.23	3.52	1	0.00	0.43	1.01
56	6.89	7.17	−0.28	−0.09	−0.07	−0.02	0.71	0.23	3.52	1	0.00	−0.52	−0.43
57	6.59	6.70	−0.11	−0.79	−1.21	0.42	−0.28	0.78	3.44	0	0.00	−0.56	1.56
58	7.14	7.16	−0.02	−0.22	−0.12	−0.10	0.71	0.23	3.52	1	−0.02	1.40	0.58
59	6.58	6.90	−0.32	−0.37	−0.49	0.12	0.14	0.06	2.65	1	0.14	0.42	0.46

[a] Values were calculated by eq 4. [b] Values were calculated by eq 6. [c] NT = not tested. [d] NA = not active. [e] Estimated from the equation π(cinnamoyloxy) = log P ($CH_3OCOCH{=}CHPh$) + log P (CH_3COOPh) − log P (CH_3COOCH_3) − log P (benzene). [f] Estimated from the equation π(morpholino) = log P (1-Me-morpholine) + log P [C_6H_5-$N(Me)_2$] − log P [$N(Me)_3$] − log P (benzene). [g] Approximated by that of OCOPh. [h] Approximated by that of $N(Me)_2$.

Table IV. Squared Correlation Matrix

	$\pi(6')$	$\sigma_p(6')$	$L(6')$	$I_{halo}(6')$	$\pi(7')$	$\Sigma\pi$	$\Sigma\sigma$
$\sigma_p(6')$	0.016						
$L(6')$	0.450	0.030					
$I_{halo}(6')$	0.212	0.007	0.001				
$\pi(7')$	0.001	0.004	0.011	0.013			
$\Sigma\pi$	0.642	0.017	0.221	0.137	0.128		
$\Sigma\sigma$	0.001	0.666	0.001	0.004	0.011	0.025	
pI_{50}	0.093	0.290	0.067	0.431	0.098	0.130	0.230

Though 5′- and 8′-substituents varied in their hydrophobic and electronic properties, no parameters for these positions were significant in eq 4. The summational parameters of both π and σ didn't improve the correlation (s = 0.40, r = 0.86, F = 18.73) again. According to eq 4, hydrophobic, electron-withdrawing, and small substituents are favorable for high activity. Especially, chlorine (**32**) and bromine (**33**) at the 6′-position are the best 6′-substituents. This indicates that halogen atom at the 6′-position should have an important role for AR inhibitory activity in addition to the hydrophobic and the electronic properties. Introduction of a hydrophobic group at the 7′-position (**49**) improves somewhat the potency. Only 6′-substituents showed an electronic effect in the expression of activity. Kador and Sharpless have proposed the charge-transfer interaction between aldose reductase and its inhibitors based on the good correlation between the energy levels of lowest unoccupied molecular orbitals (LUMOs) of inhibitors and their potencies.[20] The significance of $\sigma_p(6')$ and $I_{halo}(6')$ may be due to the correlation with the energy levels of LUMOs. Further molecular modeling and quantum chemical studies on these compounds are now under way.

Table V. Development of Equation 4

const	$I_{halo}(6')$	$\sigma_p(6')$	$L(6')$	$\pi(6')$	$\pi(7')$	r	s	$F_{x,y}$[a]
6.18	0.99					0.66	0.56	$F_{1,30}$ = 22.69
6.03	0.93	1.04				0.82	0.43	$F_{1,29}$ = 20.71
6.49	0.90	1.16	−0.14			0.87	0.36	$F_{1,28}$ = 13.07
7.05	0.47	1.21	−0.31	0.70		0.94	0.27	$F_{1,27}$ = 23.84
6.98	0.47	1.18	−0.29	0.68	0.44	0.95	0.25	$F_{1,26}$ = 6.57

[a] F statistic for the significance of the addition of each variable.

Table VI. Development of Equation 6

const	pI_{50}	$\Sigma\sigma$	$\Sigma\pi$	r	s	$F_{x,y}$[a]
−2.59	0.33			0.51	0.35	$F_{1,27}$ = 9.71
−3.36	0.47	−0.54		0.76	0.27	$F_{1,26}$ = 19.61
−3.27	0.45	−0.50	0.18	0.82	0.24	$F_{1,25}$ = 6.68

[a] F statistic for the significance of the addition of each variable.

Relationship between in Vitro and in Vivo Activities. To analyze in vivo activity, we transformed the sciatic nerve inhibition potencies into their logit {logit = log [%-inhibition/(100 − %inhibition)]}.[21] We first calculated the linear correlation regression analysis and found eq 5. The

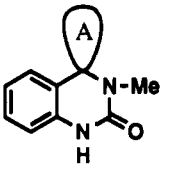
$$\underset{(\pm 0.22)}{\text{logit} = 0.33pI_{50}} \underset{(\pm 1.46)}{- 2.59} \quad (5)$$

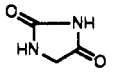
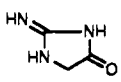
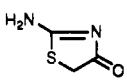
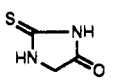
$$n = 29,\ s = 0.35,\ r = 0.51,\ F_{1,27} = 9.71$$

(20) Kador, P. F.; Sharpless, N. E. Pharmacophor Requirements of the Aldose Reductase Inhibitor Site. *Mol. Pharmacol.* **1983**, *24*, 521–531.

(21) Thomas, J.; Berkoff, C. E.; Flagg, W. B.; Gallo, J. J.; Haff, R. F.; Pinto, C. A. Antiviral Quinolinehydrazones. A Modified Free-Wilson Analysis. *J. Med. Chem.* **1975**, *18*, 245–250.

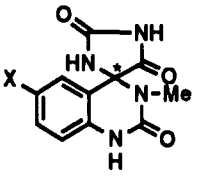
Table VII. Spiroquinazolin-2-ones with Modified Hydantoin Rings



compd	A	mp, °C	recrystn solvent	formula[a]	in vitro IC_{50},[b] M	in vivo, sciatic nerve[c] polyol contents[d] mean ± SE, μmol/g	in vivo, sciatic nerve[c] % inhibn[e]
8[f,g]		>280	DMSO	$C_{11}H_{10}N_4O_3$	2.8×10^{-7}	8.8 ± 0.4	32.5
60[f]		258–259	DMSO–H_2O	$C_{11}H_{11}N_5O_2 \cdot {}^1/_2H_2O$	$>10^{-5}$		
61[g]		>280	MeOH–H_2O	$C_{11}H_{10}N_4O_2S \cdot {}^3/_5H_2O$	$>10^{-5}$		
62[g]		>280	DMF–H_2O	$C_{11}H_{10}N_4O_2S \cdot {}^1/_2H_2O$	$>10^{-5}$		

[a] All compounds were analyzed for C, H, and N. [b] See footnote *c* in Table I. [c] See footnote *d* in Table I. [d] See footnote *e* in Table I. [e] See footnote *f* in Table I. [f] Reference 5. [g] Reference 7.

Table VIII. Enantiomers of Spiroquinazolin-2-ones



compd	X	configuration	mp, °C	recrystn solvent	$[\alpha]^{25}_D$ (c 1, EtOH)	formula[a]	in vitro IC_{50},[b] M	in vivo, sciatic nerve[c] polyol contents[d] mean ± SE, μmol/g	in vivo, sciatic nerve[c] % inhibn[e]
63[f]	H	*R*	174–176	MeOH–H_2O	+34.7	$C_{11}H_{10}N_4O_3 \cdot {}^1/_2H_2O$	3.1×10^{-7}	8.4 ± 0.6*	39.0
64[f]	H	*S*	174–176	MeOH–H_2O	−34.7	$C_{11}H_{10}N_4O_3 \cdot {}^1/_2H_2O$	3.4×10^{-6}		NT[h]
65[g]	F	*R*	267	MeOH–H_2O	+37.6	$C_{11}H_9FN_4O_3 \cdot H_2O$	6.4×10^{-8}	6.6 ± 0.3**	61.0
66[g]	F	*S*	267	MeOH–H_2O	−39.6	$C_{11}H_9FN_4O_3 \cdot H_2O$	4.5×10^{-6}	12.1 ± 0.8	15.5
67[g]	Cl	*R*	172–173	EtOH–H_2O	+36.4	$C_{11}H_9ClN_4O_3 \cdot H_2O$	2.2×10^{-8}	3.4 ± 0.3**	100.0
68	Cl	*S*	169–173	EtOH–H_2O	−33.7	$C_{11}H_9ClN_4O_3 \cdot H_2O$	7.7×10^{-7}	10.7 ± 0.5	26.3[i]

[a] All compounds were analyzed for C, H, and N. [b] See footnote *c* in Table I. [c] See footnote *d* in Table I. [d] See footnote *e* in Table I. [e] See footnote *f* in Table I. [f] Reference 10. [g] See Experimental Section. [h] NT = not tested. [i] See footnote *m* in Table I. *$P < 0.05$, **$P < 0.01$: Significantly different from galactosemic control (Student's *t*-test).

linear correlation between in vitro and in vivo activities is rather poor. This result suggests that not only high in vitro activity but also the physicochemical properties suitable for the appropriate pharmacokinetic behavior are important to express high in vivo activity. Next, we used the summational parameters which well described the overall hydrophobic and electronic properties of whole molecules. The $\Sigma\pi$ parameter is the summation of $\pi(5')$, $\pi(6')$, $\pi(7')$, and $\pi(8')$, and $\Sigma\sigma$ is the summation of $\sigma_m(5')$, $\sigma_p(6')$, $\sigma_m(7')$, and $\sigma_p(8')$.

$$\text{logit} = \underset{(\pm 0.17)}{0.45pI_{50}} + \underset{(\pm 0.15)}{0.18\Sigma\pi} - \underset{(\pm 0.23)}{0.50\Sigma\sigma} - \underset{(\pm 1.07)}{3.27} \quad (6)$$

$$n = 29,\ s = 0.24,\ r = 0.82,\ F_{3,25} = 16.86$$

Eq 6 could even predict the weak potency of compound 47 which was not included in the analysis. Overall hydrophobicity of the whole molecule contributes to high in vivo activity, which may be due to the increased bioavailability. Electron-donating substituents are favorable. This suggests that the higher electron density of the 1′-nitrogen of the quinazoline may be favorable for the transport and/or the resistance against the metabolic degradation of the compound.[22]

C. Modified Spirohydantoin Derivatives. In order to investigate the role of the hydantoin ring for the AR inhibitory activity, we synthesized and assayed the modified hydantoins (**60–62**). These compounds were less potent than the original hydantoin (**8**) as shown in Table VII. These results suggested that the hydantoin ring should play an important role for the AR inhibitory activity in this series.

D. Optically Active Derivatives. The results of the AR inhibitory activities in both in vitro and in vivo assays for optically pure compounds showed that *R*-configuration compounds were clearly superior to *S*-configuration compounds as summarized in Table VIII. This suggests that the orientation of the hydantoin ring would be critically important for the AR inhibitory activity, and a highly stereospecific binding site for spirohydantoins might exist at the enzyme. From our SAR and QSAR explorations in

(22) Dearden, J. C. Molecular Structure and Drug Transport. In *Comprehensive Medicinal Chemistry*, 1st ed.; Hansch, C., Sammes, P. G, Taylor, J. B., Eds.; Pergamon Press: Oxford, 1990; Vol. 4, pp 375–411.

Table IX

compd	in vitro IC_{50},[a] M	in vivo[b] dose, mg/kg per day	sciatic nerve polyol contents[c] mean ± SE, μmol/g	sciatic nerve % inhibn[d]	lens polyol contents[e] mean ± SE, μmol/g	lens % inhibn[d]
67	2.2×10^{-8}	3	7.5 ± 0.6*	23.4	58.9 ± 1.5	0.0
		15	4.8 ± 0.2*	85.9	59.2 ± 0.6	6.0
		90	2.8 ± 0.2**	100.0	42.4 ± 1.1***	33.3
sorbinil (69)[f]	2.5×10^{-7}	15	4.2 ± 0.2***	90.2	23.2 ± 0.8***	69.3

[a] See footnote c in Table I. [b] See footnote d in Table I. [c] See footnote e in Table I. [d] See footnote f in Table I. [e] Normal control, 1.4 ± 0.1 μmol/g (n = 5); galactosemic control, 62.2 ± 1.1 ~ 62.9 ± 0.1 μmol/g (n = 3). [f] Reference 23. *P < 0.05, **P < 0.01, ***P < 0.001: Significantly different from galactosemic control (Student's t-test).

the series of quinazolin-2-ones having a spirohydantoin ring, it was found that the 6'-halogen atom and the spirohydantoin ring were very important for AR inhibitory activities. It is interesting to note that these results are similar to those of the chroman hydantoin series reported by Pfizer workers.[23]

E. Further Evaluation of Compound 67. In the series of quinazolin-2-ones having a spirohydantoin ring, we have found a number of efficient ARIs. Of these, (4*R*)-6'-chloro-3'-methylspiro[imidazolidine-4,4'(1'*H*)-quinazoline]-2,2',5(3'*H*)-trione (**67**) was the most potent in vitro and in vivo. The compound **67** was further evaluated for the ability to inhibit polyol accumulation in the sciatic nerve and lens of galactosemic rats. These biological data are summarized in Table IX. Compound **67** at a dose of 30 mg/kg per day inhibited polyol accumulation in the sciatic nerve by 86% in galactosemic rats. This inhibitory activity of **67** was almost the same as that of sorbinil (**69**),[23] (4*S*)-6-fluoro-2,3-dihydrospiro[4*H*-1-benzopyran-4,4'-imidazolidine]-2',5'-dione. In the lens, although the in vitro AR inhibitory activity of **67** was 10 times stronger than that of sorbinil (**69**), the in vivo polyol accumulation inhibitory activity of **67** was rather weaker than that of sorbinil (**69**). This suggests that the permeability of **67** into the lens would be very low as compared with that of sorbinil (**69**). The in vivo AR inhibitory activities of **67** in the sciatic nerve and lens seem to be rather similar to those of tolrestat, *N*-[[5-(trifluoromethyl)-6-methoxy-1-naphthalenyl]thioxomethyl]-*N*-methylglycine, which is a typical carboxylic acid type ARI.[24]

On the other hand, ARIs should need extremely low toxicity and no side effects as well as high AR inhibitory activity for a long term administration.[2b,c,e] In the preliminary acute toxicity study using mice and rats, compound **67** at an oral dose of 3 g/kg caused neither death nor behavioral abnormality.

Conclusions

From our SAR and QSAR studies, it was found that the 6'-halogen atom and the stereochemical relationship between the spirohydantoin ring and the quinazoline ring play an important role for potent AR inhibitory activities in the spirohydantoin series. These explorations in our series have yielded a number of ARIs with potent in vitro and in vivo activities. Of these, compound **67** inhibited most potently polyol accumulation in the sciatic nerve of galactosemic rats and had low toxicity in mice and rats. Thus, compound **67** was selected for further evaluation as a therapeutic agent of diabetic complications. Further biological evaluation and studies on the mechanism of AR inhibitory activity are now in progress.

Experimental Section

Melting points (mp) were measured by the use of a Yamato MP-21 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-420 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Hitachi R-40 (90-MHz) spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were taken on a Hitachi M-60 spectrometer at an ionizing potential of 30eV. Specific rotations were measured with a Perkin-Elmer 243 digital readout polarimeter using a 10-cm cell. Thin-layer chromatography (TLC) was carried out with Merck silica gel 60F-254 plates.

Materials. The following materials were prepared from the corresponding aniline or indole according to reported procedures: 4-chloroisatin,[25] 4-methylisatin,[26] 5-chloroisatin,[27] 5-bromoisatin,[28] 5-methylisatin,[27] 5-methoxyisatin,[29] 6-chloroisatin,[25] 6-methylisatin,[26] 6-methoxyisatin,[29] 6-nitroisatin,[30] 7-chloroisatin,[31] 5,6-dichloroisatin,[32] 5-chloro-6-methylisatin,[33] 5-chloro-6-methoxyisatin,[34] and 5,6-methylenedioxyisatin.[35]

Preparation of 1-Carbamoylisatins (4). To a solution of 0.1 mol of triethylamine and 0.1 mol of isatin, prepared from corresponding aniline according to the Sandmeyer procedure,[8]

(23) (a) 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. (b) Sarges, R.; Schnur, R. C.; Belletire, J. L.; Peterson, M. J. Spiro Hydantoin Aldose Reductase Inhibitors. *J. Med. Chem.* **1988**, *31*, 230–243.

(24) Malamas, M. S.; Sestanj, K.; Millen, J. Synthesis and Biological Evaluation of Tolrestat Metabolites. *Eur. J. Med. Chem.* **1991**, *26*, 197–200.

(25) Senear, A. E.; Sargent, H.; Mead, J. F.; Koepfli, J. B. The Synthesis of Potential Antimalarials. 7-Chloro-α-(2-piperidyl)-4-quinolinemethanol. *J. Am. Chem. Soc.* **1946**, *68*, 2695–2697.

(26) Mayer, F.; Schulze, R. About 4- and 6-Methylisatins. *Chem. Ber.* **1925**, *58*, 1465–1469.

(27) Gassman, P. G.; Cue, B. W.; Luh, T-Y. A General Method for the Synthesis of Isatins. *J. Org. Chem.* **1977**, *42*, 1344–1348.

(28) Heller, G. New Isomerisms in the Isatin Series (IV). *Chem. Ber.* **1920**, *53*, 1545–1551.

(29) Ferber, E.; Schmolke, G. Synthesis of 5-Methoxyisatins. *J. Prakt. Chem.* **1940**, *155*, 234–240.

(30) Noland, W. E.; Rieke, R. D. New Synthetic Route to 6-Nitroisatin via Nitration of 3-Indolealdehyde. *J. Org. Chem.* **1962**, *27*, 2250–2252.

(31) Singh, P.; Dhami, K. S.; Sharma, G. M.; Narang, K. S. Thiopegan Derivatives. XV. Synthesis of Some 8-Chloro- and 8-Methyl-10,11-thiopegans and Their Derivatives. *Chem. Abstr.* **1958**, *52*, 18378f.

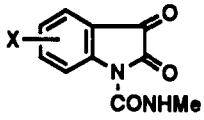
(32) Baker, B. R.; Schaub, R. E.; Joseph, J. P.; McEvoy, F. J.; Williams, J. H. An Antimalarial Alkaloid from Hydrangea. XV. Synthesis of 5-, 6-, 7-, and 8-Derivatives with Two Identical Substituents. *J. Org. Chem.* **1952**, *17*, 149–156.

(33) Baker, B. R.; Joseph, J. P.; Schaub, R. E.; McEvoy, F. J.; Williams, J. H. An Antimalarial Alkaloid from Hydrangea. XVI. Synthesis of 5-, 6-, 7-, and 8-Derivatives with Two Different Substituents. *J. Org. Chem.* **1952**, *17*, 157–163.

(34) Gillespie, J. S.; Rowlett, R. J.; Davis, R. E. Antimalarials. Substituted 2-Phenyl-4-quinolinemethanols. *J. Med. Chem.* **1968**, *11*, 425–429.

(35) Gulland, J. M.; Robinson, R.; Scott, J.; Thornley, S. 6:7-Dimethoxyisatin, 5:6-Methylenedioxyisatin, and the Nuclear Degradation of 3:4-Methylenedioxyquindoline. *J. Chem. Soc.* **1929**, 2924–2941.

Table X. Physical Data for Substituted 1-(Methylcarbamoyl)isatins (4)



compd	X	yield, %	mp, °C	recrystn solvent	formula	anal.
4b	4-Cl	74	158–160	DMF–*i*-Pr_2O	$C_{10}H_7ClN_2O_3$	C, H, N, Cl
4c	4-Me	85	170–172	AcOEt	$C_{11}H_{10}N_2O_3$	C, H, N
4d	5-F	65	230–232	AcOEt	$C_{10}H_7FN_2O_3$	C, H, N, F
4e	5-Cl	75	234–236	AcOEt	$C_{10}H_7ClN_2O_3$	C, H, N, Cl
4f	5-Br	71	228–230	DMF	$C_{10}H_7BrN_2O_3$	C, H, N, Br
4g	5-OMe	74	205–208	AcOEt	$C_{11}H_{10}N_2O_4$	C, H, N
4h	5-CO_2Et	56	138–141	AcOEt	$C_{13}H_{12}N_2O_5$	C, H, N
4i	5-Me	87	228–230	AcOEt	$C_{11}H_{10}N_2O_3$	C, H, N
4j	5-NO_2	66	168 dec	DMF–*i*-Pr_2O	$C_{10}H_7N_3O_5$	C, H, N
4k	6-Cl	53	220–222	DMF–*i*-Pr_2O	$C_{10}H_7ClN_2O_3$	C, H, N, Cl
4l	6-OMe	54	217–219	DMF	$C_{11}H_{10}N_2O_4$	C, H, N
4m	7-F	53	145–146	DMF–*i*-Pr_2O	$C_{10}H_7FN_2O_3$	C, H, N, F
4n	5,6-Cl_2	61	220–221	DMF–*i*-Pr_2O	$C_{10}H_6Cl_2N_2O_3$	C, H, N, Cl
4o	5-Cl,6-Me	52	217–219	DMF	$C_{11}H_9ClN_2O_3$	C, H, N, Cl
4p	5-Cl,6-OMe	60	202–205	AcOEt	$C_{11}H_9ClN_2O_4$	C, H, N, Cl
4q	5,6-OCH_2O-	84	238–240	AcOEt	$C_{11}H_8N_2O_5$	C, H, N
4r	5,6,7-F_3	56	169–171	AcOEt	$C_{10}H_5F_3N_2O_3$	C, H, N, F

in 80 mL of *N*,*N*-dimethylformamide (DMF) was added dropwise 0.1 mol of isocyanate (e.g., methyl isocyanate) at 0 °C over 10 min with stirring. After being stirred for 1 h at the same temperature, the resulting precipitates were collected, washed with diisopropyl ether, dried, and recrystallized (e.g., from DMF–H_2O) to give the 1-carbamoylisatin [e.g., 1-(methylcarbamoyl)isatin (**4a**)]. Yields and physical data for substituted 1-carbamoylisatins are summarized in Table X.

Preparation of Spiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-triones. General Method A.[7] A mixture of 0.1 mol of 1-carbamoylisatin (**4**), 0.11 mol of triethylamine, and 0.1 mol of 2-ethyl-2-isothiourea hydrobromide in 250 mL of tetrahydrofuran (THF) was stirred at room temperature for 3 h. The reaction mixture was then concentrated in vacuo. To the residue was added 75 mL of 10% HCl, and the mixture was heated at 80 °C for 3 h. After being cooled, the resulting precipitates were collected, washed with H_2O, dried, and recrystallized [e.g., dimethyl sulfoxide (DMSO)] to give the target compound [e.g., 3′-methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (**8**)].

General Method B.[6] A solution of 0.1 mol of 5-[2-[(ethoxycarbonyl)amino]phenyl]-5-hydroxyimidazolidine-2,4-dione (**7**),[5] which was obtained by the reaction of 1-(ethoxycarbonyl)isatin (**6**) and urea, and 0.4 mol of amine (e.g., 10% ethanolic ammonia) in 750 mL of toluene and 75 mL of ethanol was heated at 120 °C for 4 h in a sealed tube. After being cooled, the precipitates were collected, washed with ethanol, and dissolved in H_2O. This solution was acidified with 10% HCl to pH 2. The resulting crystals were then collected, washed with H_2O, dried, and recrystallized (e.g., DMF–H_2O) to give the target compound [e.g., spiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (**20**)].

1,3′-Dimethylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (9). A solution of 1.23 g (5 mmol) of **8** in 10 mL of DMF was treated in portions with 0.20 g (5 mmol) of 60% NaH. The mixture was stirred at room temperature for 30 min, 0.71 g (5 mmol) of MeI was added, and stirring was continued for 1 h. After being poured into ice–water, the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated in vacuo. The crude product was recrystallized from DMF–H_2O to give 1.00 g (77%) of **9**: mp 269–270 °C (lit.[4a] mp 288 °C); IR (Nujol) 1788, 1720, 1655 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.72 (3 H, s), 3.00 (3 H, s), 6.70–7.50 (4 H, m), 9.26 (1 H, s), 9.90 (1 H, s); MS, *m*/*e* 260 (M^+). Anal. ($C_{12}H_{12}N_4O_3$) C, H, N.

1,3,3′-Trimethylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (10). This compound was prepared by the following procedure described for the preparation of the compound **9**. From 1.30 g (5 mmol) of **9**, 0.20 g (5 mmol) of 60% NaH, and 0.71 g (5 mmol) of MeI was obtained 1.10 g (80%) of **10** (from DMF–H_2O): mp 263–264 °C (lit.[4b] mp 272 °C); IR (Nujol) 1780, 1720, 1675 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.60 (3 H, s), 2.70 (3 H, s), 3.04 (3 H, s), 6.76–7.45 (4 H, m), 10.00 (1 H, s); MS, *m*/*e* 274 (M^+). Anal. ($C_{13}H_{14}N_4O_3$) C, H, N.

1,1′,3,3′-Tetramethylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (11). This compound was prepared by the following procedure described for the preparation of the compound **9**. From 1.37 g (5 mmol) of **10**, 0.20 g (5 mmol) of 60% NaH, and 0.71 g (5 mmol) of MeI was obtained 1.10 g (76%) of **11** (from DMF–H_2O): mp 175–176 °C; IR (Nujol) 1780, 1720, 1660 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.60 (3 H, s), 2.76 (3 H, s), 3.06 (3 H, s), 3.34 (3 H, s), 6.95–7.60 (4 H, m). Anal. ($C_{14}H_{16}N_4O_3$) C, H, N.

3,3′-Dimethylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (14). A mixture of 5.10 g (18.6 mmol) of compound **12**, 3.70 g (50 mmol) of methylurea, and 0.20 g (1.3 mmol) of 1,8-diazabicyclo[5.4.0]undec-7-ene in 100 mL of THF was refluxed for 5 h. After being cooled, the precipitates were collected, dried, and recrystallized from DMSO–H_2O to give 2.00 g (29%) of **13**: mp 203–205 °C; ^1H NMR (DMSO-d_6) δ 2.69 (3 H, d, J = 5 Hz), 2.75 (3 H, s), 6.67–7.50 (4 H, m), 7.55–8.21 (2 H, m), 9.30–9.90 (2 H, m). Anal. ($C_{12}H_{14}N_4O_4$) C, H, N.

A suspension of 1.10 g (4.0 mmol) of **13** in 20 mL of 1,2-dichlorobenzene was refluxed for 5 h. After being cooled, the precipitates were collected, dried, and recrystallized from DMSO–H_2O to give 1.00 g (97%) of **14**: mp > 280 °C; ^1H NMR (DMSO-d_6) δ 3.59 (3 H, s), 3.78 (3 H, s), 6.70–7.58 (4 H, m), 10.07 (1 H, s), 11.50 (1 H, br). Anal. ($C_{12}H_{12}N_4O_3$) C, H, N.

1-[(Benzyloxy)methyl]-3′-methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (15).[10] A solution of 7.38 g (30 mmol) of **8** in 100 mL of DMF was treated in portions with 1.20 g (30 mmol) of 60% NaH. The mixture was stirred at room temperature for 30 min, 4.71 g (30 mmol) of (benzyloxy)methyl chloride was added, and stirring was continued for 1 h. After being poured into ice–water, the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated in vacuo. The crude product was recrystallized from DMF–H_2O to give 10.40 g (95%) of **15**: mp 226–227 °C; IR (Nujol) 1800, 1735, 1660 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.77 (3 H, s), 4.60 (2 H, s), 5.01 (2 H, s), 6.60–7.50 (9 H, m), 9.50 (1 H, s), 10.00 (1 H, s). Anal. ($C_{19}H_{18}N_4O_4$) C, H, N.

3-Acetyl-1-[(benzyloxy)methyl]-3′-methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (16). A solution of 10.98 g (30 mmol) of **15** in 50 mL of DMF was treated in portions with 1.20 g (30 mmol) of 60% NaH. The mixture was stirred at room temperature for 30 min, 2.40 g (30 mmol) of acetyl chloride was added at 0 °C, and stirring was continued at room temperature for 1 h. After being poured into ice–water, the mixture was extracted with AcOEt. The organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated in vacuo. The crude product was recrystallized from 2-propanol–diisopropyl ether to

give 8.5 g (69%) of **16**: mp 209–211 °C; ^{1}H NMR (DMSO-d_6) δ 2.50 (3 H, s), 2.70 (3 H, s), 4.70 (2 H, s), 5.18 (2 H, s), 6.65–7.60 (9 H, m), 10.13 (1 H, s). Anal. ($C_{21}H_{20}N_4O_5$) C, H, N.

3-Acetyl-1′,3′-dimethylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (18a). 3-Acetyl-1-[(benzyloxy)methyl]-1′,3′-dimethylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (**17a**) was prepared by the following procedure described for the preparation of the compound **9**. From 3.06 g (7.5 mmol) of **16**, 0.30 g (7.5 mmol) of 60% NaH, and 1.06 g (7.5 mmol) of MeI was obtained 3.00 g (95%) of **17a** as a syrup.

Pd black (0.20 g) was added to a solution of 3.00 g (7.1 mmol) of **17a** in 20 mL of EtOH, and the mixture was subjected to hydrogenolysis on a Parr apparatus for 5 h at a H_2 pressure of 45 psi. The catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in saturated aqueous $NaHCO_3$ and then acidified with 10% HCl. The resulting precipitates were collected. The white solids were washed with H_2O, dried, and recrystallized from DMF–H_2O to give 0.85 g (42%) of **18a**: mp 261–263 °C; IR (Nujol) 1800, 1760, 1715, 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.40 (3 H, s), 2.80 (3 H, s), 3.36 (3 H, s), 3.00–4.00 (1 H, br), 6.90–7.60 (4 H, m); MS, *m/e* 302 (M^+). Anal. ($C_{14}H_{14}N_4O_4$) C, H, N.

3-Acetyl-1′-isobutyl-3′-methylspiro[imidazolidine-4,4′-(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (18b). This compound was prepared from **16** (4.08 g, 10 mmol) as described for the preparation of the compound **18a**. Recrystallization from DMF–H_2O gave 0.55 g (16%) of **18b**: mp 195 °C; ^{1}H NMR (DMSO-d_6) δ 0.8–1.3 (6 H, m), 1.8–2.3 (1 H, m), 2.36 (3 H, s), 2.72 (3 H, s), 3.5–4.1 (2 H, m), 6.8–7.5 (4 H, m); MS, *m/e* 344 (M^+). Anal. ($C_{17}H_{20}N_4O_4$) C, H, N.

1′,3′-Dimethylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (19a). To a solution of 161 mg (7 mmol) of sodium in 20 mL of EtOH was added 1.00 g (3.3 mmol) of **18a** at room temperature, and the mixture was stirred for 3 h at the same temperature. After evaporation of EtOH in vacuo, the residue was acidified with 10% HCl. The resulting precipitates were collected, washed with H_2O, dried, and recrystallized from DMF–H_2O to give 0.67 g (74%) of **19a**: mp 223–225 °C; IR (Nujol) 1782, 1730, 1630 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.86 (3 H, s), 3.33 (3 H, s), 7.00–7.63 (4 H, m), 9.10 (1 H, s), 11.40 (1 H, br). Anal. ($C_{12}H_{12}N_4O_3 \cdot {}^2/_3H_2O$) C, H, N.

1′-Isobutyl-3′-methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (19b). This compound was prepared by the following procedure described for the preparation of the compound **19a**. From 0.50 g (1.45 mmol) of **18b** and 69 mg (3.0 mmol) of sodium in 10 mL of EtOH was obtained 0.23 g (52%) of **19b** (from EtOH–2-propanol): mp 270 °C; IR (Nujol) 1790, 1723, 1645 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.90 (6 H, d, *J* = 7 Hz), 1.80–2.30 (1 H, m), 2.79 (3 H, s), 3.60–4.00 (2 H, m), 6.90–7.60 (4 H, m), 9.02 (1 H, s), 11.19 (1 H, s); MS, *m/e* 302 (M^+). Anal. ($C_{15}H_{18}N_4O_3$) C, H, N.

1-(Carboxymethyl)-3′-methylspiro[imidazolidine-4,4′-(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (28). A solution of 12.31 g (50 mmol) of **8** in 100 mL of DMF was treated in portions with 2.0 g (50 mmol) of 60% NaH. The mixture was stirred at room temperature for 30 min, 8.35 g (50 mmol) of ethyl bromoacetate was added, and stirring was continued for 1 h. The reaction mixture was concentrated in vacuo, and the residue was crystallized from H_2O. The precipitates were collected, washed with H_2O, dried, and recrystallized from DMF–H_2O to give 11.00 g (66%) of 1-[(ethoxycarbonyl)methyl]-3′-methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione: mp 220–222 °C; IR (Nujol) 1790, 1750, 1725, 1670 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.22 (3 H, t, *J* = 7 Hz), 2.80 (3 H, s), 4.20 (2 H, q, *J* = 7 Hz), 4.33 (2 H, s), 6.81–7.50 (4 H, m), 9.50 (1 H, s), 10.00 (1 H, s); MS, *m/e* 332 (M^+). Anal. ($C_{15}H_{16}N_4O_5$) C, H, N.

A mixture of 3.32 g (10 mmol) of the 1-[(ethoxycarbonyl)methyl] derivative obtained above and 1.12 g (20 mmol) of potassium hydroxide in 30 mL of H_2O and 20 mL of MeOH was stirred at 50 °C for 1.5 h. After being cooled, the resulting solids were filtered off. The filtrate was acidified with 10% HCl, and the resulting precipitates were collected to give 2.00 g (64%) of **28**: mp 175 °C; IR (Nujol) 3550, 3400, 3200, 1790, 1720, 1660, 1610 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.80 (3 H, s), 4.23 (2 H, s), 6.80–7.43 (4 H, m), 9.45 (1 H, s), 9.97 (1 H, s); MS, *m/e* 304 (M^+). Anal. ($C_{13}H_{12}N_4O_5 \cdot {}^1/_2H_2O$) C, H, N.

6′-Amino-3′-methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (35). Pd black (0.10 g) was added to a solution of 2.91 g (10 mmol) of **34** and 1.38 g (10 mmol) of potassium carbonate (K_2CO_3) in 50 mL of H_2O, and the mixture was subjected to catalytic hydrogenation on a Parr apparatus for 5 h at a H_2 pressure of 45 psi. The catalyst was filtered off, and the filtrate was acidified with 10% HCl. The resulting precipitates were collected and washed with H_2O. The product was purified by dissolution in alkali and reprecipitation with acid to give 1.00 g (38%) of **35**: mp > 280 °C; IR (Nujol) 3400, 3350, 3300, 1780, 1725 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.76 (3 H, s), 3.10–6.00 (2 H, br), 6.33 (1 H, s), 7.60 (2 H, s), 8.93 (1 H, s), 9.43 (1 H, s), 11.20 (1 H, br); MS, *m/e* 261 (M^+). Anal. ($C_{11}H_{11}N_5O_3$) C, H, N.

6′-Hydroxy-3′-methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (37). A mixture of 4.70 g (17 mmol) of **36** and 100 mL of 48% HBr was stirred at 130 °C for 7 h. The reaction mixture was concentrated in vacuo. The resulting precipitates were collected, washed with H_2O, dried, and recrystallized from DMF–H_2O to give 3.00 g (67%) of **37**: mp > 280 °C; IR (Nujol) 3400, 3320, 3150, 1780, 1732, 1662 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.77 (3 H, s), 6.50 (1 H, s), 6.77 (2 H, s), 9.05 (1 H, s), 9.65 (1 H, s), 11.35 (1 H, s); MS, *m/e* 262 (M^+). Anal. ($C_{11}H_{10}N_4O_4$) C, H, N.

3′-Methyl-6′-(benzoyloxy)spiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (38). To a solution of 1.31 g (5 mmol) of **37** and 0.83 (6 mmol) of K_2CO_3 in 15 mL of H_2O was added dropwise 0.7 mL (6 mmol) of benzoyl chloride at 0–5 °C over 15 min, and the mixture was stirred for 2.5 h at room temperature. The precipitates were collected, washed with H_2O, dried, and recrystallized from 2-propanol to give 1.25 g (68%) of **38**: mp > 280 °C; IR (Nujol) 1785, 1730, 1680 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.83 (3 H, s), 6.90–8.00 (6 H, m), 8.10–8.50 (2 H, m), 9.30 (1 H, s), 10.14 (1 H, s), 11.10–11.80 (1 H, br); MS, *m/e* 366 (M^+). Anal. ($C_{18}H_{14}N_4O_5$) C, H, N.

6′-(Cinnamoyloxy)-3′-methylspiro[imidazolidine-4,4′-(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (39). To a suspension of 2.10 g (8 mmol) of **37** in 30 mL of pyridine was added dropwise 1.34 g (8 mmol) of cinnamoyl chloride at 0–5 °C over 15 min, and the mixture was stirred at the same temperature for 4.5 h. The mixture was concentrated in vacuo, and the residue was acidified with 10% HCl. The precipitates were collected and washed with H_2O. A suspension of this compound obtained above, aqueous $NaHCO_3$, and diisopropyl ether was stirred for 30 min, and the mixture was filtered off. The filtrate was concentrated in vacuo. To the residue was added diisopropyl ether and the precipitates were collected and recrystallized from EtOH to give 0.43 g (14%) of **39**: mp > 280 °C; IR (Nujol) 3220, 1790, 1730, 1675, 1635 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.76 (3 H, s), 6.6–8.0 (10 H, m), 9.07 (1 H, s), 9.91 (1 H, s), 11.23 (1 H, s); MS, *m/e* 392 (M^+). Anal. ($C_{20}H_{16}N_4O_5$) C, H, N.

Nitration of Spiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-triones (47, 51, 55, 57). To a solution of 3.6 mmol of the corresponding spirohydantoin **5** in 5 mL of concentrated sulfuric acid was added 3.6 mmol of sodium nitrate at 0–5 °C, and the mixture was stirred at room temperature for 1 h. After being poured into ice–water, the resulting crystals were collected, washed with H_2O, dried, and recrystallized from DMF–H_2O to give the target compound [e.g., 5′-chloro-3′-methyl-6′-nitrospiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5-(3′*H*)-trione (**47**)].

3′-Methyl-7′-morpholino-6′-nitrospiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (52). A mixture of 0.20 g (0.6 mmol) of **51** and 2 mL (23 mmol) of morpholine was heated at 90–100 °C for 1 h. The reaction mixture was concentrated in vacuo, and the residue was acidified with 10% HCl. The resulting precipitates were collected, washed with H_2O, dried, and recrystallized from DMF–H_2O to give 0.19 g (82%) of **52**: mp > 280 °C; ^{1}H NMR (DMSO-d_6) δ 2.78 (3 H, s), 3.00 (4 H, br), 3.70 (4 H, br), 6.57 (1 H, s), 7.60 (1 H, s), 9.05 (1 H, s), 10.28 (1 H, s), 11.00 (1 H, br). Anal. ($C_{15}H_{16}N_6O_6$) C, H, N.

6′,8′-Dichloro-3′-methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (54). A mixture of 1.23 g (5 mmol) of **8**, 6.75 g (50 mmol) of sulfuryl chloride, and a catalytic amount of iodine in 50 mL of acetic acid was stirred at 60 °C for 9 h. After being cooled to room temperature, the mixture was diluted with 100 mL of H_2O. The resulting precipitates were

collected and purified by dissolution in alkali and reprecipitation with acid to give 1.35 g (86%) of **54**: mp > 280 °C; IR (Nujol) 1799, 1722, 1640 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.77 (3 H, s), 7.06 (1 H, d, J = 3 Hz), 7.59 (1 H, d, J = 3 Hz), 9.10 (1 H, s), 9.53 (1 H, s), 11.10–11.70 (1 H, br). Anal. ($C_{11}H_8Cl_2N_4O_3$) C, H, Cl, N.

8′-Amino-6′-chloro-3′-methylspiro[imidazolidine-4,4′-(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (56). To a mixture of 6.10 g (32 mmol) of stannous chloride and 20 mL of concentrated HCl was added 2.60 g (8 mmol) of **55** at room temperature, and the mixture was stirred for 2 h at the same temperature. The reaction mixture was adjusted to pH 3 with 10% NaOH. The resulting precipitates were collected, washed with H_2O, dried, and recrystallized from DMF–H_2O to give 2.00 g (82%) of **56**: mp > 280 °C; IR (Nujol) 3410, 3350, 3220, 3050, 1790, 1720 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.75 (3 H, s), 5.55 (2 H, s), 6.16 (1 H, d, J = 2 Hz), 6.62 (1 H, d, J = 2 Hz), 8.96 (1 H, s), 9.05 (1 H, s), 11.17 (1 H, s); MS, m/e 295 (M^+). Anal. ($C_{11}H_{10}ClN_5O_3 \cdot {}^1/_2H_2O$) C, H, Cl, N.

6′,8′-Dichloro-7′-methoxy-3′-methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (58). A mixture of 276 mg (1 mmol) of **44**, 270 mg (2 mmol) of sulfuryl chloride, and a catalytic amount of iodine in 5 mL of acetic acid was stirred at 50 °C for 30 min. After being poured into ice–water, the resulting precipitates were collected to give 170 mg (49%) of **58**: mp > 280 °C; IR (Nujol) 1800, 1715, 1645 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.79 (3 H, s), 3.82 (3 H, s), 7.15 (1 H, s), 9.10 (1 H, s), 9.55 (1 H, s), 11.38 (1 H, s). Anal. ($C_{12}H_{10}Cl_2N_4O_4$) C, H, Cl, N.

Optical Resolution of 6′-Fluorospiro[imidazolidine-4,4′-(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (31) with Quinine. A mixture of 8.10 g (28.7 mmol) of **31** and 13.00 g (40 mmol) of quinine in 450 mL of MeOH–H_2O (2:1) was heated until it became a clear solution, and the solution was then allowed to cool slowly. The precipitated solids were collected to give 4.00 g (24%) of **65** as the quinine adduct, $[\alpha]^{25}_D$ −20.8° (*c* 1, DMF), and the filtrate (A) was saved. This complex was treated with 2% HCl. The precipitates were collected and recrystallized from MeOH–H_2O to give 1.10 g (14%) of **65**: mp 267 °C; $[\alpha]^{25}_D$ +37.6° (*c* 1, EtOH); IR (Nujol) 3610, 3500, 3320, 3270, 1720, 1700 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.82 (3 H, s), 6.80–7.35 (3 H, m), 9.07 (1 H, s), 9.95 (1 H, s), 11.35 (1 H, s); MS, m/e 264 (M^+). Anal. ($C_{11}H_9FN_4O_3 \cdot H_2O$) C, H, F, N.

The original filtrate A was concentrated in vacuo, and the residue was acidified with 2% HCl to pH 2. The resulting solid (compound **31**) was filtered off, and the filtrate was concentrated in vacuo to 20–30 mL. The resulting crystals were collected and recrystallized from MeOH–H_2O (2:1) to give 0.7 g (9%) of **66**: mp 267 °C; $[\alpha]^{25}_D$ −39.6° (*c* 1, EtOH); IR (Nujol) 3610, 3500, 3320, 3270, 1720, 1700 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.82 (3 H, s), 6.80–7.35 (3 H, m), 9.07 (1 H, s), 9.92 (1 H, s), 11.37 (1 H, s); MS, m/e 264 (M^+). Anal. ($C_{11}H_9FN_4O_3 \cdot H_2O$) C, H, F, N.

(4*R*)-6′-Chloro-3′-methylspiro[imidazolidine-4,4′(1′*H*)-quinazoline]-2,2′,5(3′*H*)-trione (67). To a suspension of 17.24 g (70 mmol) of **63** in 170 mL of acetic acid was added dropwise 14.17 g (105 mmol) of sulfuryl chloride at 0 °C over 10 min with stirring. After being stirred for 1 h at room temperature, the mixture was poured into ice–water and stirred at 0–5 °C for 30 min. The precipitates were collected, washed with H_2O, and dried. This material was dissolved in 1 L of EtOH, the resulting precipitates were filtered off, and the filtrate was concentrated in vacuo to about 100 mL. To this solution was added 300 mL of H_2O, and the mixture was kept at 0 °C for 15 h. The precipitates were collected, washed with H_2O, dried, and recrystallized from EtOH–H_2O to give 14.0 g (67%) of **67**: mp 172–173 °C; $[\alpha]^{25}_D$ +36.4° (*c* 1, EtOH); IR (Nujol) 1765, 1730, 1660 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.80 (3 H, s), 6.92 (1 H, d, J = 9 Hz), 7.06 (1 H, d, J = 2 Hz), 7.40 (1 H, dd, J = 9 and 2 Hz), 9.11 (1 H, s), 10.07 (1 H, s), 11.40 (1 H, s); MS, m/e 280 (M^+). Anal. ($C_{11}H_9ClN_4O_3 \cdot H_2O$) C, H, Cl, N.

Biological Methods. Enzyme Assay. Aldose reductase was partially purified from rabbit lenses according to the method of Hayman.[12] The in vitro inhibitory activity of test compounds was determined using glyceraldehyde as the substrate. The test compound was dissolved in DMSO and added to the reaction medium containing 67 mM dipotassium phosphate buffer (pH 7.4), 400 mM lithium sulfate, 0.1 mM NADPH, and 1 mM glyceraldehyde in a total volume of 1.5 mL at a final concentration of 10^{-8} to 10^{-5} M. The assay was carried out at 25 °C and initiated by addition of the enzyme preparation. The decrease in optical density at wave length of 340 nm was monitored for about 3 min using a Hitachi 557 spectrophotometer. All determinations were carried out in duplicate. The concentration associated with 50% inhibition of enzyme activity (IC_{50}) was graphically determined from log concentration–percent inhibition plots.

In Vivo Assay. Groups of three male rats (SIc: Wistar, 4-week-old) were used. Rats were allowed free access to water and a diet containing 20% galactose with or without the test compound at a final concentration of 30 mg/kg per day or 90 mg/kg per day for 6 days. The galactose control group was fed on the 20% galactose diet alone during the same period. The normal control group was fed on a standard diet.

Animals were bled to death under ether anesthesia on day 7, and the sciatic nerves were isolated for the measurements of their polyol contents. For compound **67**, the lens polyol level was also determined at various doses.

The polyol content was measured by the modified polyol assay described by Dvornik.[14] Mean percent inhibition of polyol accumulation in the sciatic nerve or lens was calculated from the following equation:

$$\% \text{ inhibition} = \frac{(\text{Pol})_{GC} - (\text{Pol})_{GT}}{(\text{Pol})_{GC} - (\text{Pol})_{NC}} \times 100 \qquad (7)$$

where $(Pol)_{GC}$, $(Pol)_{GT}$, and $(Pol)_{NC}$ mean the polyol content in the sciatic nerve or lens of the galactose control, galactose + test compound, and normal control group, respectively. Statistical significance was calculated on the basis of the absolute levels of polyol in the treated and untreated diabetic groups by using Student's t-test. A probability level of 0.05 or less was considered significant.

Acute Toxicity. Acute toxicity of the compound **67** was studied using mice and rats. Groups of three male ddY mice weighing 22–25 g and groups of three male Wistar rats aged 4 weeks were used. General signs were observed, and the number of deaths was counted for 2 weeks after the single oral administration of the compound at 3 g/kg.

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