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DEVELOPMENT OF POTENT ALDOSE REDUCTASE INHIBITORS HAVING A HYDANTOIN STRUCTURE

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Abstract—Seventeen hydantoin derivatives were tested as inhibitors of aldose reductase, an enzyme believed to participate in the initiation of diabetic complications. Nine compounds with high inhibitory activities (IC_{50} values against purified rat lens aldose reductase $\leq 1.06 \times 10^{-6}$ M) were tested further for their abilities to prevent sorbitol accumulation induced by exposure of excised rat lens and sciatic nerve to a high glucose concentration (50 mM). Seven active compounds among them inhibited sorbitol accumulation by about 50% or more at a concentration of 10^{-5} M. These seven compounds were given orally to streptozotocin-induced diabetic rats at a dose of 50 mg/kg/day and were assessed for their abilities to prevent both sorbitol accumulation in two tissues (lens and sciatic nerve) and *myo*-inositol depletion in the sciatic nerve. 1-[(2,4,5-Trichlorophenyl)sulfonyl]hydantoin, 1-[(2,5-dichlorophenyl)sulfonyl]hydantoin, and 1-[(β-naphthyl)sulfonyl]hydantoin were found to be the most effective: they inhibited sorbitol accumulation in the sciatic nerve completely and that in the lens by more than 92%. It is conceivable from this study that the three compounds are promising for further investigation targeted to the treatment of diabetic complications.

Intracellular accumulation of sorbitol, a reduction product formed from glucose by aldose reductase (alditol:$NADP^+$ 1-oxidoreductase, EC 1.1.1.21), has been implicated in the pathogenesis of diabetic complications such as peripheral neuropathy, cataract, nephropathy, microangiopathy, and retinopathy [1, 2]. Many aldose reductase inhibitors have been developed in an attempt to treat some of these complications [3].

We previously reported the inhibitory activities of fifty-four hydantoin derivatives against rat and bovine lens aldose reductases [4]. 1-(Phenylsulfonyl)hydantoin and its derivatives, 1-[(substituted phenyl)sulfonyl]hydantoins, were found to be potent aldose reductase inhibitors, with 1-[(4-bromophenyl)sulfonyl]hydantoin being the most potent among them. The last compound significantly delays the formation of nucleus cataract when given orally at a dose of 50 mg/kg/day to rats fed a 50% galactose diet [5] and also significantly decreases the blood glucose concentration of normal rabbits when administered by intubation at a dose of 50 mg/kg [6].

The purpose of this study was to search for more promising aldose reductase inhibitors having the basic structure of 1-(arylsulfonyl)hydantoin or 1-(alkylsulfonyl)hydantoin.

MATERIALS AND METHODS

Materials. Sorbitol dehydrogenase (from sheep liver) was obtained from Boehringer, Mannheim, West Germany. Streptozotocin was purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. 2,4,5-Trichlorobenzenesulfonyl chloride and 2,5-dichlorobenzenesulfonyl chloride were obtained from the Aldrich Chemical Co., Milwaukee, WI, U.S.A. α-Naphthalenesulfonyl chloride, β-naphthalenesulfonyl chloride, 4-iodobenzenesulfonyl chloride, 4-ethylbenzenesulfonyl chloride, 2-methylbenzenesulfonyl chloride, benzylsulfonyl chloride, 4-fluorobenzenesulfonyl chloride, and 2,5-dimethylbenzenesulfonyl chloride were purchased from the Tokyo Chemical Industry Co., Ltd., Tokyo, Japan.

Synthesis of hydantoins. Hydantoin derivatives, except for 1-[(3-nitro-4-hydroxyphenyl)sulfonyl]hydantoin, 1-[(4-hydroxyphenyl)sulfonyl]hydantoin, and 1-[(4-bromophenylsulfonyl)]-3-butylhydantoin, were synthesized essentially as described previously [3] by using arysulfonyl chloride and alkylsulfonyl chloride as starting materials. The synthesis of the above-mentioned three compounds will be reported elsewhere. All hydantoin derivatives were guaranteed to be pure by NMR spectrometry (using deuterated dimethyl sulfoxide as solvent) and by elemental analysis for carbon, hydrogen, and nitrogen atoms.

Assay of aldose reductase activity. Aldose reductase assays were conducted as described previously [7]. The effects of hydantoins on the enzyme activity were determined by including in the reaction mixture (1 ml) 10 μl of each hydantoin solution (in propylene glycol) at the desired concentrations.

Preparation of rat lens aldose reductase. Aldose reductase was purified from rat lenses according to our method [8]. Briefly, a 40–70% ammonium sulfate fraction was chromatographed on DEAE-Sephacel, followed by two column chromatographic steps, i.e.

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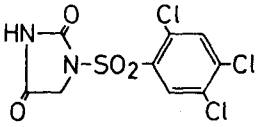
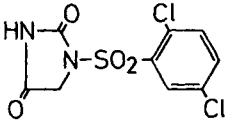
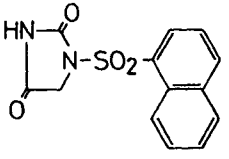
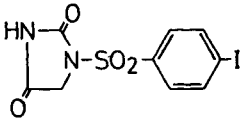
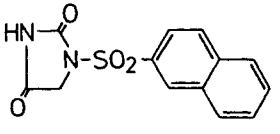
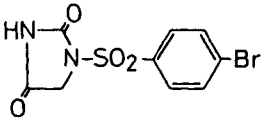
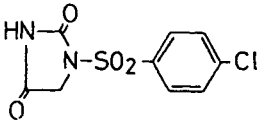
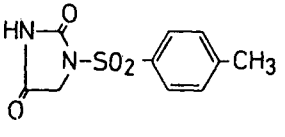
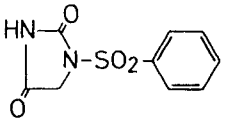
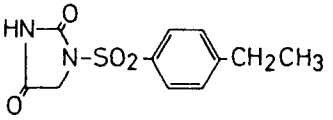
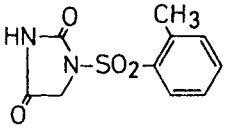
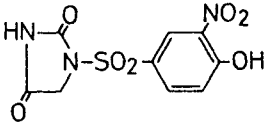
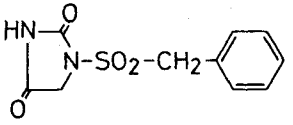
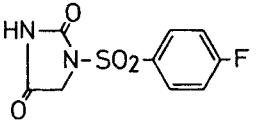
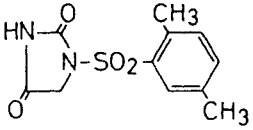
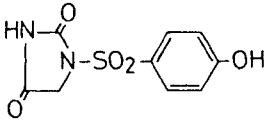
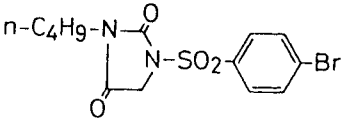
Table 1. Chemical structures, melting points, elemental analysis data, and IC_{50} values of hydantoin derivatives

Compound No.	Structure	Name (abbreviation)	m.p. (°C)	Analysis (%) Calcd (Found)			IC_{50}* (M) × 10^6
				C	H	N	
1		1-[(2,4,5-Trichlorophenyl) sulfonyl]hydantoin (Tri-Cl-PSH)	232–235	30.16 (29.97	1.56 1.85	7.76 7.51)	0.28
2		1-[(2,5-Dichlorophenyl)sulfonyl]-hydantoin(Di-Cl-PSH)	261–264	34.97 (34.92	1.96 1.86	9.06 8.99)	0.36
3		1-[(α-Naphthyl)sulfonyl]hydantoin (α-NSH)	210–214	53.78 (53.75	3.47 3.32	9.65 9.65)	0.58
4		1-[(4-Iodophenyl)sulfonyl]hydantoin (4-I-PSH)	190–192	29.52 (29.44	1.93 1.86	7.65 7.61)	0.62
5		1-[(β-Naphthyl)sulfonyl]hydantoin (β-NSH)	221–224	53.78 (53.79	3.47 3.33	9.65 9.58)	0.66
6†		1-[(4-Bromophenyl)sulfonyl]hydantoin (4-Br-PSH)					0.70
7†		1-[(4-Chlorophenyl)sulfonyl]hydantoin (4-Cl-PSH)					0.92
8†		1-[(4-Methylphenyl)sulfonyl]hydantoin (4-Me-PSH)					1.00

9†		1-(Phenylsulfonyl)hydantoin (PSH)					1.06
10		l-[(4-Ethylphenyl)sulfonyl]hydantoin (4-Et-PSH)	190–192	49.24 (48.69	4.51 4.42	10.44 10.30)	1.09
11		1-[(2-Methylphenyl)sulfonyl]-hydantoin (2-Me-PSH)	190–193	47.25 (47.12	3.97 3.94	11.02 11.05)	1.16
12		1-[(3-Nitro-4-hydroxyphenyl)-sulfonyl]hydantoin (3-NO_2-4-OH-PSH)	271–272	35.88 (35.90	2.34 2.26	13.95 13.83)	1.23
13		1-(Benzylsulfonyl)hydantoin (BSH)	227–229	47.25 (47.00	3.97 3.91	11.02 10.82)	1.29
14		1-[(4-Fluorophenyl)sulfonyl]-hydantoin(4-F-PSH)	245–246	41.86 (41.78	2.73 2.77	10.85 10.73)	2.80
15		1-[(2,5-Dimethylphenyl)sulfonyl]-hydantoin(Di-Me-PSH)	215–217	49.06 (49.27	4.87 4.46	10.40 10.32)	2.80
16		1-[(4-Hydroxyphenyl)sulfonyl]-hydantoin(4-OH-PSH)	239–240	42.18 (41.92	3.14 3.07	10.93 10.79)	2.80
17		1-[(4-Bromophenyl)sulfonyl]-3-butylhydantoin(4-Br-PSBH)	175–176	47.20 (47.16	4.57 4.60	8.47 8.49)	>100

* Values are for purified rat lens aldose reductase.

† Melting points, elemental analysis data, and IC_{50} values of these compounds were reported previously [3].

affinity chromatography using Mātrex gel red A and gel filtration on sephadex G-75. By this procedure, aldose reductase was purified 380-fold.

Measurement of sorbitol accumulation in vitro. Lenses and sciatic nerves were excised from male Wistar strain rats (180–200 g) after anesthesia was induced with pentobarbital sodium. A lens or a pair of sciatic nerves was put into a test tube and incubated at 37° in 5 ml of high-glucose medium consisting of 4.95 ml of buffer A (see below) and 0.05 ml of 10^{-2}–10^{-4} hydantoin solution (in propylene glycol) or 0.05 ml of vehicle only. Buffer A (pH 7.4) contained 110 mM NaCl, 3.8 mM KCl, 0.54 mM $MgSO_4$, 0.9 mM $KHCO_3$, 0.27 mM NaH_2PO_4, 0.23 mM KH_2PO_4, 1.25 mM $CaCl_2$, 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES), and 50 mM glucose. Incubation was conducted for 4 hr for lenses and 8 hr for sciatic nerves, after which the tissues were washed with buffer A and kept frozen at −70° until required for sorbitol assay. For deproteinization by the method of Somogyi [9], tissues were minced with scissors, homogenized in 0.5 ml of 2.0% $ZnSO_4 \cdot 7H_2O$, mixed with 0.5 ml of 1.8% $Ba(OH)_2 \cdot 8H_2O$, and then centrifuged at 13,000 *g* for 5 min. Sorbitol in supernatant fractions was assayed enzymatically. The reaction mixture contained 8 mM NAD^+, 1.6 units/ml sorbitol dehydrogenase, 60 mM Glycine-NaOH buffer (pH 9.5), and an appropriate amount of sample solution. Incubation was performed for 20 min at 30°, and NADH formation was determined from the increase in absorbance at 340 nm.

Measurement of sorbitol and myo*-inositol accumulation* in vivo. Male Wistar strain rats (220–240 g) were raised on a normal laboratory chow. Diabetes was induced by a single intraperitoneal injection of 12.5 mg/ml streptozotocin (in 0.01 M citrate buffer, pH 5.1) at a dose of 50 mg/kg. On day 4 after injection, blood samples were taken by tail-prick for blood glucose assay. Protein-free solutions were prepared from the blood samples by the procedure of Somogyi [9]. Blood glucose was determined with a commercial kit, Blood Sugar-GOD-Perid-Test (Boehringer, Mannheim, West Germany). Streptozotocin-treated rats with nonfasting blood glucose concentrations from 300 to 600 mg/dl were used for further experiments. Beginning with day 4 after administration of streptozotocin, inhibitor suspensions or a placebo were given each morning by gastric intubation to diabetic rats. The inhibitor suspensions and the placebo were prepared as described previously [5] and given at a dose of 50 mg/kg/day for 12 days. The placebo was also given to a group of normal rats. On the day following the final administration, lenses and sciatic nerves were removed and processed for gas–liquid chromatographic assay of sorbitol and *myo*-inositol according to our previous method [5] for assaying polyols.

Statistics. The statistical analyses were performed by Student's *t*-test with the level of significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the chemical structure, melting point, elemental analysis data, and IC_{50} value (inhibitor concentration necessary for 50% inhibition) of each of the seventeen hydantoin compounds used in this study. These compounds, consisting of sixteen 1-(arylsulfonyl)hydantoins and a single 1-(alkylsulfonyl)hydantoin, are arranged in the table in ascending order of IC_{50} value. The melting point, elemental analysis data, and IC_{50} value of 4-Br-PSH (No. 6), 4-Cl-PSH (No. 7), 4-Me-PSH (No. 8), and PSH (No. 9) were reported previously [3]. Five compounds, i.e. Tri-Cl-PSH (No. 1), Di-Cl-PSH (No. 2), α-NSH (No. 3), 4-I-PSH (No. 4), and β-NSH (No. 5), were more potent in inhibitory activity against rat lens aldose reductase than 4-Br-PSH (No. 6), which had been the most potent among the 1-(arylsulfonyl)hydantoins so far examined. The fact that 1-(benzylsulfonyl)hydantoin (No. 13), a kind of 1-(alkylsulfonyl)hydantoin, had almost the same potency as PSH (No. 9) reveals that the arylsulfonyl-type structure is not essential for the inhibitory activity against aldose reductase. It is obvious from the comparison between 4-Br-PSH (No. 6) and 4-Br-PSBH (No. 17) that substitution at the N-3 position of the hydantoin ring is severely detrimental.

For selection of the compounds to be assessed *in vivo*, nine compounds (No. 1–9) with effective inhibitory activity against purified rat lens aldose reductase were tested for their abilities to protect against sorbitol accumulation in rat lenses and sciatic nerves *in vitro*. Tissues were incubated in high-glucose medium at 37° for definite periods (4 hr for lenses and 8 hr for sciatic nerves) in the presence or absence of 10^{-5} M hydantoin compound. Tri-Cl-PSH (No. 1) and Di-Cl-PSH (No. 2) were the most effective compounds (Table 2), as was also observed in the inhibition against purified aldose reductase. At first glance, the order of potency of the nine compounds in the *in vitro* system using excised tissues parallels well that in the cell-free system using purified enzyme. Since β-NSH (No. 5) was found to be one of the most promising inhibitors (as stated later), we determined the ED_{50} values of this compound in the *in vitro* system. The values for lenses and sciatic nerves were 5.6×10^{-6} M and 7.0×10^{-6} M respectively.

We chose seven compounds (No. 1–7) out of nine hydantoin derivatives, which wer used in the *in vitro* experiment, for further study *in vivo*. Hydantoin compounds were given orally to diabetic rats at a dose of 50 mg/kg/day for 12 days, and their abilities to prevent both sorbitol accumulation in two tissues (lens and sciatic nerve) and *myo*-inositol depletion in the sciatic nerve were investigated. A decrease in nerve *myo*-inositol content in experimental diabetes has been suggested to be related to nerve dysfunctions [10, 11], although the mechanism underlying this putative involvement has not yet been clarified. Some aldose reductase inhibitors have been shown to prevent the fall in nerve *myo*-inositol [12–14]. These facts led us to measure nerve *myo*-inositol content in this study. Tri-Cl-PSH (No. 1), Di-Cl-PSH (No. 2), and β-NSH (No. 5) were the most effective compounds for prevention of sorbitol accumulation in both tissues (Table 3). These compounds inhibited sorbitol accumulation in the sciatic nerve completely, even lowering it below the normal level, and that in the lens by more than 92%. β-NSH

Table 2. Effects of hydantoin derivatives on sorbitol accumulation in rat lenses and sciatic nerves *in vitro*

Compound No. (abbreviation)	Lens sorbitol		Sciatic nerve sorbitol	
	μmol/g wet wt	% Inhibition	nmol/g wet wt	% Inhibition
Incubation time of control:				
0 hr	0.37 ± 0.03		57 ± 4	
4 or 8 hr*	4.27 ± 0.07		1258 ± 49	
1 (Tri-Cl-PSH)	0.64 ± 0.05	92.9	321 ± 20	78.0
2 (Di-Cl-PSH)	1.13 ± 0.09	80.2	348 ± 30	75.8
3 (α-NSH)	1.63 ± 0.17	70.1	478 ± 24	64.9
4 (4-I-PSH)	1.38 ± 0.06	73.8	558 ± 16	58.3
5 (β-NSH)	2.04 ± 0.09	56.6	593 ± 40	55.4
6 (4-Br-PSH)	2.70 ± 0.12	39.4	661 ± 55	49.7
7 (4-Cl-PSH)	2.46 ± 0.16	45.5	712 ± 56	45.5
8 (4-Me-PSH)	2.94 ± 0.08	33.2	802 ± 18	38.0
9 (PSH)	2.64 ± 0.05	41.0	752 ± 38	42.1

Sorbitol contents are expressed as the mean ± SD of three experiments.
* Lenses were incubated for 4 hr; and nerve tissue, for 8 hr.

(No. 5) led to a significant increase in nerve *myo*-inositol content as compared with the control level, whereas the increment of the content brought about by Tri-Cl-PSH (No. 1) and Di-Cl-PSH (No. 2) was not significant statistically. α-NSH (No. 3) increased nerve *myo*-inositol content over the normal level, but was not sufficiently effective in preventing sorbitol accumulation in either the lens or sciatic nerve. The data from the seven compounds in Table 3 suggest no strict, reverse correlation between *myo*-inositol content and sorbitol content in the sciatic nerve. This is not compatible with the view [15] that increased polyol pathway activity may be a likely factor in the fall in nerve *myo*-inositol content. It should be emphasized that α-NSH (No. 3) was more potent than β-NSH (No. 5) both in inhibition of purified aldose reductase and in prevention of sorbitol accumulation *in vitro*, whereas the potencies of the two compounds in prevention of sorbitol accumulation *in vivo* were unequivocally reversed. This may be explained by differences between the two compounds in intestinal absorption rate and/or plasma half-life of disappearance, although there is no evidence in hand. Since the hydantoin derivatives tested in this study are reminiscent of hypoglycemic sulfonylureas, it is highly expected that the com-

Table 3. Effects of hydantoin derivatives on polyol accumulation in lenses and sciatic nerves of diabetic rats *in vivo*.

Compound No. (abbreviation)	Lens	Sciatic nerve	
	Sorbitol (μmol/g wet wt)	Sorbitol (nmol/g wet wt)	*Myo*-inositol (nmol/g wet wt)
Normal rats	0.28 ± 0.10 (9)	107 ± 21 (8)	2854 ± 447* (11)
Control (diabetic rats)	37.04 ± 2.98 (14)	1731 ± 379 (9)	2127 ± 283 (13)
1 (Tri-Cl-PSH)	3.06 ± 0.77 (5)	ND† (5)	2612 ± 275‡§ (5)
2 (Di-Cl-PSH)	1.27 ± 0.07 (4)	61 ± 15‖ (4)	2427 ± 18¶§ (3)
3 (α-NSH)	17.16 ± 4.21 (7)	488 ± 87** (7)	3165 ± 444*§ (6)
4 (4-I-PSH)	3.09 ± 1.15 (4)	282 ± 82** (8)	2632 ± 220‡§ (4)
5 (β-NSH)	2.24 ± 0.40 (11)	106 ± 19§ (11)	2913 ± 306*§ (12)
6 (4-Br-PSH)	17.52 ± 1.76 (4)	378 ± 45** (4)	2168 ± 30¶†† (3)
7 (4-Cl-PSH)	10.68 ± 2.71 (7)	401 ± 87** (6)	2634 ± 162*§ (5)

Data represent the mean ± SD with the number of experiments given in parentheses.
* Significantly different from control ($P < 0.001$).
† Not detectable.
‡ Significantly different from control ($P < 0.01$).
§ Not significantly different from normal.
‖ Significantly different from normal ($P < 0.01$).
¶ Not significantly different from control.
** Significantly different from normal ($P < 0.001$).
†† Significantly different from normal ($P < 0.05$).

pounds would have hypoglycemic activity, as was already proved with 4-Br-PSH (No. 6) [6]. Indeed, β-NSH (No. 5), one of the three most potent compounds in the *in vivo* experiment, markedly reduced the blood glucose concentration when given orally to normal rabbits at a dose of 50 mg/kg (unpublished data). However, the blood glucose levels of diabetic rats after oral administration of hydantoin compounds (No. 1–7) at 50 mg/kg/day for 12 days were not significantly different from that (450 ± 87 mg/dl) of control (untreated diabetic) rats.

In conclusion, this study has shown that among the seventeen hydantoin derivatives tested as aldose reductase inhibitors, 1-[(2,4,5-trichlorophenyl)sulfonyl]hydantoin (No. 1), 1-[(2,5-dichlorophenyl)-sulfonyl]hydantoin (No. 2), and 1-[(β-naphthyl)-sulfonyl]hydantoin (No. 5) are the most promising compounds for further investigation focused on the treatment of diabetic complications.

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