Synthesis and Aldose Reductase-Inhibitory Activity of Imidazopyrroloquinoline Esters

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Derivatives of imidazopyrroloquinoline (IPQ) and its esters were synthesized. Some of these compounds potently inhibited aldose reductases of rabbit lens and dog kidney, as well as the human recombinant enzyme, though the coenzyme pyrroloquinoline quinone (PQQ) was a relatively poor inhibitor. The IPQ esters with a methyl substituent at the C-3 carboxyl group were less potent inhibitors than the analogs without esterification at this position. An IPQ ester with the free carboxyl group at C-3 inhibited sorbitol accumulation in rat red blood cells.

Key words pyrroloquinoline quinone; imidazopyrroloquinoline; aldose reductase inhibitor; pyrroloquinoline quinone derivative; imidazopyrroloquinoline compound

In 1979, pyrroloquinoline quinone (PQQ, 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid, Fig. 1) was identified as a cofactor of methanol dehydrogenase.¹⁾ It was later found in several bacterial enzymes.^{2,3)} Recently, it was reported that PQQ is nutritionally important as a vitamin or growth factor in mammals,⁴⁾ and we detected trace levels of PQQ in human and rat tissues.⁵⁾ The following pharmacological activities of coenzyme PQQ have been reported: a protective effect against liver injury,⁶⁾ an inhibitory activity on the formation of hydrocortisone-induced cataract in embryos,⁷⁾ a radical scavenger-like activity,⁸⁾ an enhancing effect on the activity of DNA synthesis in human fibroblasts,⁹⁾ and a nerve growth factor-inducing activity.^{10,11)}

Aldose reductase (EC 1.1.1.21), which catalyzes the conversion of glucose to sorbitol, stimulates the accumulation of sorbitol in the tissues where diabetic complications, such as cataract, neuropathy, retinopathy and nephropathy, develop during hyperglycemia.¹²⁻¹⁸⁾ Therefore, aldose reductase inhibitors may be useful to prevent or treat chronic complications caused by diabetes.¹⁹⁾

In this study, we describe the aldose reductase-inhibitory activity of imidazopyrroloquinoline (7,10-dihydro-7-oxo-imidazo[4,5,1-*ij*]pyrrolo[2,3-*f*]quinoline-1,3,9-tricarboxylic acid (IPQ)) derivatives synthesized from coenzyme PQQ and various amino acids as well as IPQ esters, and we discuss the relationship between this activity and chemical structure.

Materials and Methods

Test Compounds Compounds used in this study were PQQ, derivatives of IPQ and IPQ esters, as shown in Fig. 1.

PQQ PQQ was prepared by a method previously described^{20,21)} which uses *Hyphomicrobium denitrificans* TK 0441.²²⁾

Trimethylester of PQQ The trimethylester of PQQ (4,5-dihydro-2,7,9-trimethoxycarbonyl-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline; PQQ-2,7,9-TME) was synthesized by treating PQQ with dimethyl sulfate.²³⁾ A solution of PQQ in *N,N*-dimethylformamide (DMF) with anhydrous potassium carbonate and dimethyl sulfate was stirred under N₂ at 25 °C. The reaction was stopped by lowering the pH to 1.0 with hydrochloric acid, and the products were extracted with chloroform-carbon tetrachloride (1:1, 4 times). The solution was washed with 1*N* HCl (2

times), aqueous saturated sodium bicarbonate (2 times) and water (2 times), dried over anhydrous sodium sulfate, and then evaporated under reduced pressure. The residue was dissolved in ethyl ether, and cooled at 5 °C until precipitates appeared. After filtration, the precipitates were dried, and PQQ-2,7,9-TME was obtained as orange powders.

IPQ Compounds Eleven IPQ compounds were synthesized from PQQ and various amino acids²⁴⁾; IPQ, methyl IPQ (IPQ-A), hydroxymethyl IPQ (IPQ-S), 1-methylethyl IPQ (IPQ-V), 2-methylpropyl IPQ (IPQ-L), 1-methylpropyl IPQ (IPQ-I), 2-carboxyethyl IPQ (IPQ-E), 2-carbamoyl ethyl IPQ (IPQ-Q), 2-methylthioethyl IPQ (IPQ-M), benzyl IPQ (IPQ-F), and 4-hydroxyphenyl methyl IPQ (IPQ-Y).

IPQ Esters Five carboxymethyl esters of IPQ were synthesized; 7,10-dihydro-1,3,9-trimethoxycarbonyl-7-oxo-imidazo[4,5,1-*ij*]pyrrolo[2,3-*f*]quinoline (IPQ-1,3,9-TME), 1,3-dicarboxy-7,10-dihydro-9-methoxycarbonyl-7-oxo-imidazo[4,5,1-*ij*]pyrrolo[2,3-*f*]quinoline (IPQ-1,3-DCA-9-ME = IPQ-9-ME), 1,9-dicarboxy-7,10-dihydro-3-methoxycarbonyl-7-oxo-imidazo[4,5,1-*ij*]pyrrolo[2,3-*f*]quinoline (IPQ-1,9-DCA-3-ME = IPQ-3-ME), 1-carboxy-7,10-dihydro-3,9-dimethoxycarbonyl-7-oxo-imidazo[4,5,1-*ij*]pyrrolo[2,3-*f*]quinoline (IPQ-1-CA-3,9-DME = IPQ-3,9-DME), and 3-carboxy-7,10-dihydro-1,9-dimethoxycarbonyl-7-oxo-imidazo[4,5,1-*ij*]pyrrolo[2,3-*f*]quinoline (IPQ-3-CA-1,9-DME = IPQ-1,9-DME).

Chemical Structures of IPQ Esters IPQ esters synthesized were subjected to analysis of melting point, elementary analysis, and visible and ultraviolet-, ¹H-NMR-, IR spectroscopy. All melting points were determined on a Yanagimoto micro melting point apparatus without

Fig. 1. Structures of PQQ and IPQ Derivatives

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correction. Absorption spectra were measured with a Hitachi 220-A spectrophotometer (Hitachi, Tokyo, Japan), and the samples were dissolved in acetonitrile. ^1H -NMR analysis was done with a JEOL JMN-PMX Si NMR spectrometer (JEOL, Tokyo) operating at 90 MHz, using tetramethylsilane (TMS) as an internal reference. The samples were dissolved in deuterated dimethylformamide ($\text{DMF-}d_7$). IR analysis was done with a Hitachi 260-50 infrared spectrometer (Hitachi, Tokyo) using the KBr method.

Assay of Aldose Reductase and Aldehyde Reductase Activities The activities of aldose reductase and aldehyde reductase were determined by the methods of Tanimoto *et al.*²⁵⁾ Assays were performed at 25 °C in 0.1 M sodium phosphate buffer (pH 6.2) containing 0.3 M ammonium sulfate, 10 mM DL-glyceraldehyde, 0.15 mM NADPH and enzyme in a total volume of 3.0 ml. The appropriate blanks to correct for nonspecific oxidation of NADPH and absorption of test samples were prepared. The reaction was initiated by the addition of the enzyme, and the rate of NADPH oxidation was followed by recording the decrease in absorbance at 340 nm on a Union High-Sen SM-401 spectrophotometer equipped with a temperature-controlled cuvette chamber and a National X-Y recorder ($n=3$).

Enzymes Rabbit lens aldose reductase was purified from rabbit lens by the methods of Tanimoto *et al.*^{25,26)} Aldose reductase and aldehyde reductase of dog kidney were purified according to Ohta *et al.*²⁷⁾ Recombinant human aldose reductase produced using *Spodoptera frugiperda* cells²⁸⁾ was purchased from Wako Pure Chemicals (Osaka, Japan).

Determination of IC_{50} Value PQQ and IPQ compounds were dissolved in water, and IPQ esters were dissolved in dimethyl sulfoxide (DMSO). The effects of the test compounds on the activities of aldose reductase and aldehyde reductase were determined by adding 4 μl of test solution to the reaction mixture. It was confirmed that 15% DMSO in the final assay solution did not inhibit the enzyme activity by more than 5%. The concentration of test compounds giving 50% inhibition of enzyme activity (IC_{50}) was estimated from the least-squares regression line of the log dose-response plot.

Inhibitory Activity of IPQ Esters on Sorbitol Accumulation in Red Blood Cells Inhibitory activity on sorbitol accumulation in red blood cells was determined by the method of Malone *et al.*²⁹⁾ and Aida *et al.*³⁰⁾ Red blood cells were collected in presence of heparin from five Wistar rats (male, 6 weeks, Charles River Japan Inc., Yokohama, Japan). To 1 ml of red blood cells, 3 ml of Krebs-Ringer bicarbonate buffer (pH 7.5) containing 100 mg/dl ("control" incubation medium) or 500 mg/dl of glucose plus various amounts of IPQ-1,3-DCA-9-ME ("test" incubation medium) was added ($n=5$). Red blood cell solutions containing glucose or glucose plus IPQ-1,3-DCA-9-ME were incubated in air/ CO_2 (95/5, (v/v)) at 37 °C for 3 h. IPQ-1,3-DCA-9-ME was dissolved in DMSO for addition to the red blood cells; the final concentration of DMSO was 1%.

Assay of Hemoglobin and Sorbitol Sorbitol concentration in the cells was determined by the method of Malone *et al.*²⁹⁾ and is expressed as the amount of sorbitol per amount of hemoglobin, which was determined using Hemoglobin test Wako (Wako Pure Chemicals, Osaka).

Results

Synthesis of IPQ Esters IPQ-1,3,9-TME was synthesized by treating IPQ with dimethyl sulfate. A solution of IPQ in DMF with dimethyl sulfate was stirred for 10 h. The reaction was stopped by reducing the pH to 1.0 with HCl, and the solution was cooled at 5 °C. The resultant precipitate was collected by filtration and dissolved in chloroform-acetonitrile. This solution was filtered, and the filtrate was evaporated under reduced pressure. The residue was washed with ethyl ether, and dried to afford IPQ-1,3,9-TME as orange powders.

IPQ-1,3-DCA-9-ME (IPQ-9-ME) was obtained through hydrolysis of IPQ-1,3,9-TME. An aqueous 0.1 M potassium carbonate solution was added to a suspension of IPQ-1,3,9-TME in acetonitrile with stirring at 25 °C. Stirring was continued for 28 min, then 1 N HCl was added to stop the reaction. The reaction mixture was concentrated and the precipitated orange solid was

collected by filtration and recrystallized from DMF-isopropyl ether to afford IPQ-9-ME.

IPQ-1,9-DCA-3-ME (IPQ-3-ME) was synthesized by treating 2,9-dicarboxy-4,5-dihydro-7-methoxycarbonyl-4,5-dioxo-1H-pyrrolo[2,3-*f*]quinone (PQQ-2,9-DCA-7-ME = PQQ-7-ME) which was synthesized from PQQ, with formaldehyde/ammonium chloride. A solution of PQQ· Na_2 in methanol was treated with concentrated hydrochloric acid and the mixture was stirred for 3 h at 35 °C. The reaction was stopped by addition of H_2O and adjusting the pH to 6.5 with NaOH. Then the pH was adjusted to 1.0 with concentrated hydrochloric acid, the mixture was extracted with ethyl acetate, and the organic solution was dried over anhydrous sodium sulfate. The solvent was removed by distillation to afford PQQ-7-ME as red powders. PQQ-7-ME was dissolved in methanol, then 37% formaldehyde solution and 20% ammonium chloride solution were added. The mixture was heated at 50 °C for 11 h, then cooled with ice. The resultant precipitate was collected by filtration, washed with acetonitrile and ethyl ether, then dried under reduced pressure to afford IPQ-3-ME as orange powders.

IPQ-1-CA-3,9-DME (IPQ-3,9-DME) was synthesized by treating 9-carboxy-4,5-dihydro-2,7-dimethoxycarbonyl-4,5-dioxo-1H-pyrrolo[2,3-*f*]quinone (PQQ-9-CA-2,7-DME = PQQ-2,7-DME) with formaldehyde/ammonium chloride. PQQ-2,7-DME was synthesized by treating 7,9-dicarboxy-4,5-dihydro-2-methoxycarbonyl-4,5-dioxo-1H-pyrrolo[2,3-*f*]quinoline (PQQ-7,9-DCA-2-ME = PQQ-2-ME) with methanol and sulfuric acid. PQQ-2-ME was obtained by hydrolyzing PQQ-2,7,9-TME. Aqueous 0.1 M potassium carbonate was added to a suspension of PQQ-2,7,9-TME in acetonitrile under stirring at 25 °C. The reaction was stopped by lowering the pH to 1.0 with HCl, and the suspension was cooled at 5 °C. The resultant precipitate was collected by filtration, washed with water, and dried under reduced pressure to afford PQQ-2-ME as red powders. A suspension of PQQ-2-ME in methanol was treated with sulfuric acid under stirring at 60 °C. The reaction was stopped by adjusting the pH to 5.0 with aqueous 0.1 M potassium carbonate solution, and the reaction products were extracted with ethyl acetate. The organic solution was dried over anhydrous sodium sulfate, then evaporated under reduced pressure. The residue was purified on a silica gel column with a mixture of 1 : 2 ethyl acetate and acetate. The product was dissolved in ethyl acetate, and the solution was cooled at 5 °C. The resultant precipitate was collected by filtration and dried to afford PQQ-2,7-DME as orange powders. A solution of PQQ-2,7-DME in DMF-methanol (9 : 20) was mixed with 37% formaldehyde solution and aqueous 20% ammonium chloride solution. The mixture was heated at 60 °C for 19 h, then cooled with ice. The resultant precipitate was collected by filtration, washed with 0.1 N HCl, dried under reduced pressure, and recrystallized from DMF to afford IPQ-3,9-DME as orange powders.

IPQ-3-CA-1,9-DME (IPQ-1,9-DME) was synthesized by treating 7-carboxy-4,5-dihydro-2,9-dimethoxycarbonyl-4,5-dioxo-1H-pyrrolo[2,3-*f*]quinone (PQQ-7-CA-2,9-DME = PQQ-2,9-DME) which was synthesized from PQQ-2,7,9-TME, with formaldehyde/ammonium chlor-

ide. A solution of PQQ-2,7,9-TME in a mixed solvent of trifluoroacetic acid and water (3/1) was heated at 60 °C for 12 h. The reaction was stopped by addition of cold water to the reaction solution. PQQ-2,9-DME was extracted with chloroform, then the solution was dried over anhydrous sodium sulfate, and the solvent was removed by distillation. PQQ-2,9-DME was dissolved in methanol, then 37% formaldehyde solution and 20% ammonium chloride solution were added. The mixture was stirred at room temperature for 5 h at pH 5–6 (controlled with 4 N sodium hydroxide). The reaction was stopped by reducing the pH to 1.5 with 2 N HCl. The reaction solution was concentrated in an evaporator to half of the original volume. The precipitated solid was collected by filtration, washed with 0.1 N HCl, ethanol and ethyl ether, and dried under reduced pressure to afford IPQ-1,9-DME as orange powders.

Chemical Characterization of IPQ Esters The IPQ esters synthesized as described above were analyzed by melting point determination, elementary analysis, and visible and ultraviolet-, ¹H-NMR-, IR spectroscopy. The

Table 1. Melting Point, and Visible and Ultraviolet Spectral Data of IPQ and IPQ Esters

Compound	Melting point ^{a)}	$\lambda_{\max}$ (nm)		
IPQ	>280 °C	251	276	423
IPQ-1,3-DCA-9-ME	>300 °C	257	278	417
IPQ-1,9-DCA-3-ME	>280 °C	255	277	421
IPQ-1-CA-3,9-DME	>250 °C	254	278	416
IPQ-3-CA-1,9-DME	>300 °C	255	276	417
IPQ-1,3,9-TME	>250 °C	258	275	427

a) Decomposition occurred during the assay for all compounds.

Table 2. IR Spectral Data for IPQ and IPQ Esters

Compound	Wave number (cm ⁻¹)
IPQ	2950 ^{br} , 2400 ^{br} , 1585 ^s , 1200 ^s , 1170 ^{vs} , 875 ^m , 735 ^m
IPQ-1,3-DCA-9-ME	3382 ^{br,m} , 3172 ^w , 2985 ^m , 1505 ^s , 1639 ^{vs} , 1497 ^m , 1255 ^{vs} , 998 ^m
IPQ-1,9-DCA-3-ME	3380 ^{br,m} , 3196 ^w , 2960 ^w , 1722 ^s , 1693 ^s , 1626 ^{vs} , 1490 ^m , 1232 ^{vs} , 1012 ^m
IPQ-1-CA-3,9-DME	3234 ^w , 1722 ^s , 1662 ^{vs} , 1531 ^m , 1257 ^{vs} , 1022 ^m
IPQ-3-CA-1,9-DME	3430 ^{br,m} , 3271 ^w , 2980 ^w , 1714 ^s , 1645 ^{vs} , 1251 ^{vs} , 995 ^w
IPQ-1,3,9-TME	3450 ^{br,m} , 3178 ^w , 2960 ^w , 1731 ^s , 1662 ^{vs} , 1512 ^m , 1259 ^{vs} , 1234 ^s , 991 ^m

Table 4. Characteristics of IPQ and IPQ Esters

Compound	Substituents			Molecular formula	Molecular mass relative	Analysis					
	R ₁	R ₂	R ₃			Calcd			Found		
						C	H	N	C	H	N
IPQ	H	H	H	C ₁₅ H ₇ N ₃ O ₇	341.2	52.80	2.07	12.31	52.65	2.17	12.22
IPQ-1,3-DCA-9-ME	H	H	CH ₃	C ₁₆ H ₉ N ₃ O ₇	355.3	54.09	2.55	11.83	53.91	2.70	11.55
IPQ-1,9-DCA-3-ME	H	CH ₃	H	C ₁₆ H ₉ N ₃ O ₇	355.3	54.09	2.55	11.83	53.95	2.65	11.66
IPQ-1-CA-3,9-DME	H	CH ₃	CH ₃	C ₁₇ H ₁₁ N ₃ O ₇	369.3	55.29	3.00	11.38	55.00	3.16	11.15
IPQ-3-CA-1,9-DME	CH ₃	H	CH ₃	C ₁₇ H ₁₁ N ₃ O ₇	369.3	55.29	3.00	11.38	55.10	3.16	11.15
IPQ-1,3,9-TME	CH ₃	CH ₃	CH ₃	C ₁₈ H ₁₃ N ₃ O ₇	383.3	56.40	3.42	10.96	56.00	3.49	10.71

results are shown in Tables 1 to 4. The structures of IPQ esters, which were supported by these results, are shown in Table 4.

Inhibition of Aldose Reductase and Aldehyde Reductase by PQQ and IPQ Derivatives The inhibitory activities of PQQ and IPQ derivatives towards aldose reductase and aldehyde reductase are shown in Table 5. The aldose reductase (AR)-inhibitory activity was examined using aldose reductases of human, rabbit and dog. The inhibitory patterns of IPQ derivatives towards the three kinds of aldose reductases showed a similar trend. PQQ inhibited the aldose reductase very weakly, whereas IPQ derivatives strongly inhibited aldose reductases. Among the IPQ derivatives, the IC₅₀ values varied somewhat, ranging from 0.1 to 4 μM. The IC₅₀ value of IPQ was comparable to those of Epalrestat and Sorbinil, developed for preventing and treating chronic complications of diabetes.^{27,30–32} The aldehyde reductase (ALR)-inhibitory activities were examined using dog kidney aldehyde reductase activity. The IC₅₀ values of IPQ derivatives were relatively high, and ranged from 1 to 35 μM. The ratios of IC₅₀ values for aldehyde reductase to aldose reductase from dog kidney were 13.3 for IPQ, 21.2 for IPQ-A, and 34.6 for IPQ-I.

Kinetics of Inhibition of the Aldose Reductase by IPQ Effect of IPQ on the Lineweaver–Burke plot of aldose reductase activity with DL-glyceraldehyde as the substrate is shown in Fig. 2. The plot for IPQ at a concentration of 0.1 μM exhibited apparently parallel lines characteristic of uncompetitive inhibition.

Inhibition of Human Aldose Reductase by IPQ Esters

Table 3. ¹H-NMR Spectral Data for IPQ and IPQ Esters

Compound	δ value, ppm (DMF-d ₇ , internal standard: TMS)
IPQ	7.22 (d, $J=2$ Hz, 1H), 8.27 (s, 1H), 9.21 (s, 1H), 13.16 (br, 1H)
IPQ-1,3-DCA-9-ME	3.91 (s, 3H), 7.37 (d, $J=2$ Hz, 1H), 8.62 (s, 1H), 9.68 (s, 1H), 13.89 (br, 1H)
IPQ-1,9-DCA-3-ME	4.06 (s, 3H), 7.28 (d, $J=2$ Hz, 1H), 8.33 (s, 1H), 9.23 (s, 1H), 13.19 (br, 1H)
IPQ-1-CA-3,9-DME	3.95 (s, 3H), 4.14 (s, 3H), 7.41 (d, $J=2$ Hz, 1H), 8.62 (s, 1H), 9.29 (s, 1H), 12.75 (br, 1H)
IPQ-3-CA-1,9-DME	3.99 (s, 3H), 4.20 (s, 3H), 7.43 (d, $J=2$ Hz, 1H), 8.41 (s, 1H), 9.57 (s, 1H), 13.53 (br, 1H)
IPQ-1,3,9-TME	3.99 (s, 3H), 4.14 (s, 3H), 4.17 (s, 1H), 7.64 (d, $J=2.2$ Hz, 1H), 8.49 (s, 1H), 9.37 (s, 1H), 12.50 (br, 1H)

Table 5. Inhibition of Aldose Reductase (AR) and Aldehyde Reductase (ALR) by PQQ and IPQ Derivatives

Compound	IC ₅₀ (μM)				Ratio ALR/AR (Dog)
	AR Human	AR Rabbit	AR Dog	ALR Dog	
PQQ	61.0	47.0	6.0	7.0	1.1
IPQ	0.19	0.17	0.092	1.2	13.3
IPQ-A	0.31	0.43	0.52	11.0	21.2
IPQ-I	0.45	0.43	0.26	9.0	34.6
IPQ-Q	0.54	0.77	0.74	11.0	14.9
IPQ-L	0.96	0.37	0.58	7.0	12.1
IPQ-S	1.3	1.1	0.78	7.4	9.5
IPQ-M	1.7	0.9	1.0	6.5	6.5
IPQ-V	1.9	1.6	2.3	12.0	5.2
IPQ-Y	2.2	2.8	1.3	3.3	2.5
IPQ-E	2.3	1.5	0.69	8.6	12.5
IPQ-F	2.4	4.1	1.4	6.1	4.4

Fig. 2. Effect of IPQ on the Lineweaver-Burke Plot of Aldose Reductase Activity with DL-Glyceraldehyde as the Substrate

Enzyme: dog kidney aldose reductase. ●, without inhibitor; ○, with 0.1 μM IPQ.

For the enhancement of ARI activity and the improvement of absorbability into the cells, several IPQ esters were synthesized. Inhibitory potency (IC₅₀) against recombinant human aldose reductase is shown in Table 6. IPQ-1,3-DCA-9-ME and IPQ-3-CA-1,9-DME inhibited aldose reductase as strongly as did IPQ, but IPQ-1,9-DCA-3-ME and IPQ-1-CA-3,9-DME were weaker inhibitors. IPQ-1,3,9-TME were not inhibitory.

Inhibitory Effect on Sorbitol Accumulation in Red Blood Cells The inhibitory effect of IPQ-1,3-DCA-9-ME on sorbitol accumulation in red blood cells was studied, because this compound showed inhibitory potency against aldose reductase *in vitro*, and was relatively stable among the IPQ esters.¹¹⁾ The sorbitol was accumulated in rat red blood cells by incubation in the presence of 500 mg/dl glucose for 3 h. The increase of sorbitol accumulation was 72% suppressed by the addition of 10 μM IPQ-1,3-DCA-9-ME, and was completely suppressed by the addition of 30 μM, as shown in Fig. 3.

Discussion

PQQ weakly inhibited aldose reductase with IC₅₀ values from 6 to 61 μM. On the other hand, IPQ derivatives were

Table 6. Inhibition of Human Aldose Reductase by IPQ and IPQ Esters

Compound	Substituents			IC ₅₀ (μM)
	R ₁	R ₂	R ₃	
IPQ	H	H	H	0.19
IPQ-1,3-DCA-9-ME	H	H	CH ₃	0.26
IPQ-3-CA-1,9-DME	CH ₃	H	CH ₃	0.58
IPQ-1,9-DCA-3-ME	H	CH ₃	H	2.9
IPQ-1-CA-3,9-DME	H	CH ₃	CH ₃	1.3
IPQ-1,3,9-TME	CH ₃	CH ₃	CH ₃	100

Fig. 3. Inhibitory Effect of IPQ-1,3-DCA-9-ME on Sorbitol Accumulation in Red Blood Cells

Values are mean ± S.E., n=5. (): % inhibition.

strong inhibitors (IC₅₀ values from 0.1 to 4.1 μM). IPQ was the strongest inhibitor, with an IC₅₀ value of 0.1 to 0.2 μM, which is comparable to those of Epalrestat and Sorbinil.^{27,30-32)} For the determination of the relation between aldose reductase-inhibitory activity and chemical structure of IPQ, the aldose reductase inhibitory activity of IPQ esters was examined. IPQ-1,3,9-TME, IPQ-1-CA-3,9-DME and IPQ-1,9-DCA-3-ME with a methyl group substituent at the C-3 carboxyl group were less potent than IPQ, IPQ-1,3-DCA-9-ME and IPQ-3-CA-1,9-DME, which lack esterification at this position. Therefore, the C-3 carboxyl group of the IPQ analogs seems to be needed for aldose reductase-inhibitory activity. Furthermore, IPQ-1,3-DCA-9-ME, which is the most stable compound among the carboxy methyl esters of IPQ,¹¹⁾ showed strong inhibitory activity on sorbitol accumulation in rat red blood cells. These results, together with the nerve growth factor-inducing activity of IPQ derivatives,¹¹⁾ suggest that IPQ-1,3-DCA-9-esters are candidate drugs for preventing and treating chronic complications of diabetes.

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