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INHIBITION OF ALDEHYDE REDUCTASE BY ALDOSE REDUCTASE INHIBITORS

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Abstract—A broad group of structurally diverse aldose reductase inhibitors including flavonoids, carboxylic acids and hydantoins, have been examined for their ability to inhibit rat kidney aldehyde reductase (EC 1.1.1.19, EC 1.1.1.20) versus rat lens aldose reductase (EC 1.1.1.21). All aldose reductase inhibitors examined inhibited aldehyde reductase to some extent both in the reductive reaction as determined with glyceraldehyde as substrate and NADPH as coenzyme, and in the oxidative reaction where L-gulonic acid was oxidized to D-glucuronic acid in the presence of $NADP^+$. Of the inhibitors examined, 2,7-difluorospirofluorene-9,5′-imidazolidine-2′,4′-dione (Al1576) was the most potent inhibitor requiring only concentrations in the 10^{-8} M range to inhibit 50% of the *in vitro* activity of rat kidney aldehyde reductase (IC_{50} value), whereas 3-dioxo-1-*H*-benz[*de*]isoquinoline-2(3*H*)-acetic acid (alrestatin) was the least potent inhibitor requiring concentrations in the 10^{-5} M range. Both the reductive and oxidative steps appeared equally inhibited by these aldose reductases inhibitors. Moreover, all compounds appeared to inhibit either crude or highly purified rat kidney aldehyde reductase to essentially the same extent. Marked differences in the selectivity of these inhibitors, expressed as the ratio of IC_{50} values for rat kidney aldehyde reductase versus rat lens aldose reductase with glyceraldehyde as substrate, were observed with selectivity for aldose reductase ranging from *ca.* 2-fold for Al1576 to 119-fold for 3-(4-bromo-2-fluorobenzyl-4-oxo-3-phthalazine-1-ylacetic acid (Ponalrestat). Kinetic and competition studies suggest that these inhibitors interact with aldehyde reductase at a common site that is not identical to either the substrate or nucleotide binding site. These results suggest that the inhibitor binding sites of rat kidney aldehyde reductase and aldose reductase contain several common characteristics.

Mounting experimental evidence indicates that the NADPH-dependent reduction of glucose to the sugar alcohol sorbitol, catalyzed by the enzyme aldose reductase (EC 1.1.1.21), provides a common link in the onset of long-term diabetic complications that result in tissue and/or functional changes in the cornea, lens, retina, iris, peripheral nerves and kidney [1, 2]. In addition, pharmacological studies indicate that the onset and progression of these complications can be prevented or reduced significantly by the inhibition of sugar alcohol formation. These observations, which represent a novel pharmacological approach toward the control of diabetic complications that is independent of the insulin-dependent regulation of blood sugar levels, has spurred a worldwide-search for potent and selective aldose reductase inhibitors as potential therapeutic agents. Currently, a number of structurally diverse aldose reductase inhibitors have been developed as potential therapeutic agents.

Many of these aldose reductase inhibitors have also been observed to inhibit aldehyde reductase (EC 1.1.1.20), an enzyme also known as hexonate dehydrogenase (EC 1.1.1.19) [3–6]. This suggests that both aldose reductase and aldehyde reductase possess certain structural similarities necessary for the binding of aldose reductase inhibitors. Since the physiological role of neither reductase is presently known, defining the relationship between aldose reductase and aldehyde reductase and their potential roles in the onset of diabetic complications has become a topic of great current interest. Studies comparing the inhibitory susceptibility of aldose reductase and aldehyde reductase and their mechanisms of inhibition can give insight into the biochemical relationship between these two enzymes and their potential similarities and/or differences. Therefore, studies were initiated into the inhibition of rat kidney aldehyde reductase by a number of aldose reductase inhibitors, and the results compared to those obtained with rat lens aldose reductase.

MATERIALS AND METHODS

Materials

All chemicals employed were of reagent grade quality or as previously reported [7, 8]. NADPH and $NADP^+$ were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). Al1576 (1), was a gift from Alcon Laboratories (Fort Worth, TX); E-79175 (**3**), was a gift from Eisai Pharmaceutical Ltd. (Tokyo, Japan); tolrestat (**6**) and alrestatin (**10**), were gifts from Ayerst Research Inc., (Princeton, NJ); sorbinil (**8**) was a gift from Pfizer Central Research (Groton, CT); and Ponalrestat (**11**) was a gift from ICI Americas (Wilmington, DE). The source of all other inhibitors has been described

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previously [7]. Prior to each analysis, fresh 100 mM stock solutions of each inhibitor were prepared by dissolving each inhibitor in a minimal amount of dilute aqueous sodium hydroxide base.

Enzyme assay

Reductase activity was assayed spectrophotometrically on a Guilford Response spectrophotometer by following the decrease in the absorption of NADPH at 340 nm over a 4-min period with DL-glyceraldehyde as substrate [8]. Dehydrogenase activity was assayed by following over a 4-min period the increase in the absorption of NADPH when L-gulonate was used as substrate [9]. This substrate was freshly prepared from L-gulonolactone by base hydrolysis [10]. Each 1.0-mL cuvette contained equal units of enzyme (*ca.* 30 milliunits, where one unit of activity is defined as the activity consuming 1 μM NADPH per min), 0.10 M $Na,^+K^+$ phosphate buffer, pH 6.2, 0.3 mM NADPH or $NADP^+$, with/without 10 mM substrate and inhibitor. Appropriate controls were employed to negate potential changes in the absorption of nucleotide and/or inhibitors at 340 nm in the absence of substrate.

Kinetic studies in which the concentrations of substrate or inhibitors were varied were conducted under the conditions as described above. Detailed kinetic studies with NADPH and $NADP^+$ cofactors in which their concentrations were varied from 2.5 to 50 μM and 10 to 400 μM, respectively, were conducted by monitoring the change at 340 nm on a Shimazu UV2100U spectrophotometer. The scan lengths for NADPH and $NADP^+$ kinetics were 10 and 30 sec respectively.

Calculations

Kinetic calculations were conducted with the PROPHET computer system (Division of Research Resources, National Institutes of Health, Bethesda, MD, and Bolt, Beranek & Newman, Inc., Cambridge, MA) using the public procedure BINKIN2 by fitting the means of 3–6 determinations to the enzyme kinetic equation $v = V_{max}[S]/[S] + K_m$ where v represents the initial velocity of the enzyme reaction, V_{max} represents the maximum velocity, $[S]$ represents the substrate concentrations, and K_m represents the Michaelis constant. The IC_{50} values were estimated from simple linear regressions of the log concentration–response curves calculated using the Marquardt–Levenberg iterative curve-fitting algorithm of the MLAB computer system developed by the Division of Computer Research and Technology, National Institutes of Health.

Enzyme preparations

Rat kidney aldehyde reductase. Aldehyde reductase was purified from rat kidneys (obtained frozen from Pel-Freez) and stored at −20° as previously described [9]. The thawed kidneys were dissected into cortical and medullary regions, and the cortices were homogenized in a polytron homogenizer with 100 mL of 0.25 M sucrose dissolved in 20 mM phosphate buffer, pH 7.5, containing 0.5 mM EDTA and 1 mM β-mercaptoethanol. After centrifugation at 15,000 *g* for 20 min, the supernatant fraction was fractionated with ammonium sulfate, and the 30–60% precipitate was collected, dissolved in a minimum volume of 10 mM Tris buffer, pH 7.4, containing 0.5 mM EDTA and 1 mM β-mercaptoethanol, and subjected to gel filtration through a Sephadex G-25 column (2.5 × 40 cm) equilibrated with the same Tris buffer to remove the ammonium sulfate. The enzyme solution was then separated into a major aldehyde reductase fraction and a minor aldose reductase fraction by anion exchange chromatography on a DEAE-cellulose column (2.5 × 50 cm), equilibrated with the same Tris buffer, and developed with a 0–0.2 M linear gradient of NaCl dissolved in the same Tris buffer. This partially purified aldehyde reductase solution, free of aldose reductase, represents crude aldehyde reductase utilized in subsequent inhibition studies.

The partially purified aldehyde reductase solution, concentrated over an Amicon YM-10 membrane, was purified further by gel filtration on a Sephadex G-75 column (2 × 70 cm) using 20 mM $Na,^+K^+$-phosphate buffer, pH 7.5, containing 0.5 mM EDTA and 1 mM β-mercaptoethanol. Fractions were collected in 120-drop aliquots, and those containing enzyme activity were subjected to affinity chromatography on a Matrex Gel Orange A column (1.25 × 20 cm) equilibrated with 20 mM $Na,^+K^+$-phosphate buffer, pH 7.5, containing 0.5 mM EDTA, 1 mM β-mercaptoethanol and 10% glycerol. The purified enzyme, obtained by elution with a 100-mL 0.1 mM linear gradient of NADPH dissolved in the same phosphate buffer, eluted as a single band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). This enzyme represents the highly purified enzyme preparation utilized for inhibition studies.

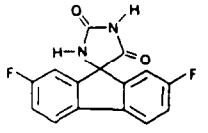
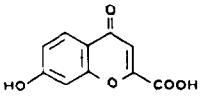
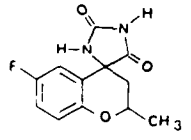
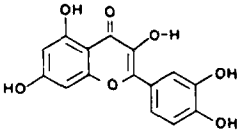
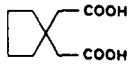
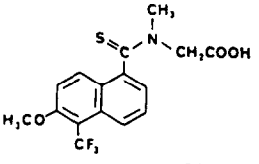
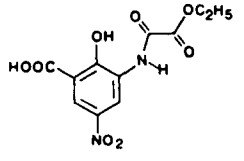
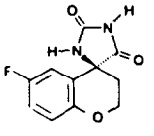
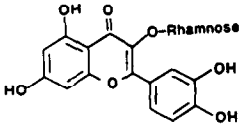
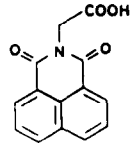
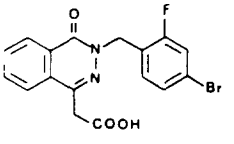
Rat lens aldose reductase. Aldose reductase was prepared from either fresh or frozen rat lenses as previously described [11].

RESULTS

A broad group of structurally diverse aldose reductase inhibitors, including flavonoids, carboxylic acids and hydantoins, were examined for their ability to inhibit rat kidney aldehyde reductase (Table 1). Of the compounds examined, 2,7-difluorospirofluorene-9,5′-imidazolidine-2′,4′-dione (Al1576); (**1**) was the most potent inhibitor requiring only concentrations in the 10^{-8} range to inhibit 50% of the *in vitro* activity of rat kidney aldehyde reductase (IC_{50} value), whereas 3-dioxo-1-*H*-benz[*de*]isoquinoline-2(3*H*)-acetic acid (alrestatin); (**10**) was the least potent inhibitor requiring concentrations in the 10^{-5} M range. All compounds examined appeared to inhibit either crude or highly purified rat kidney aldehyde reductase to essentially the same extent.

The apparent selectivity of these inhibitors for aldose reductase versus aldehyde reductase was examined by comparing the ratio of the IC_{50} values obtained for purified rat kidney aldehyde reductase versus rat lens aldose reductase. Based on these ratios, Al1576 (**1**), the most potent inhibitor examined, and 7-hydroxy-4-oxo-4*H*-chromen-2-carboxylic acid (**2**) were the least selective inhibitors with selectivity for aldose reductase inhibition only 2-fold greater than for aldehyde reductase. Selectivity for

Table 1. Inhibition of rat kidney aldehyde reductase (RKALR) and rat lens aldose reductase (RLAR) by aldose reductase inhibitors

	Inhibitor	IC_{50} (μM) RKALR Crude	RKALR Pure	RLAR	ALR/RL
1		0.018	0.046	0.024	1.9
2		6.1	4.9	2.2	2.2
3		0.11	0.16	0.028	5.7
4		6.2	7.1	1.1	6.5
5		41	31	2.2	14.1
6		0.11	0.24	0.011	21.8
7		3.6	2.7	0.1	27
8		1.9	2.0	0.07	28.5
9		28	23	0.61	37.7
10		70	58	1.5	38.7
11		2.4	1.9	0.016	118.8

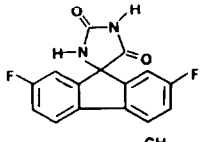
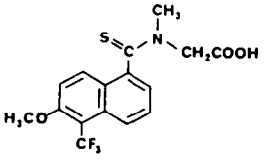
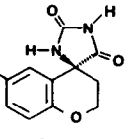
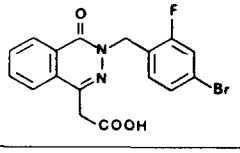
All studies were conducted using DL-glyceraldehyde as substrate as described in Materials and Methods. Values are the means of 3–6 determinations. Selectivity for aldose reductase inhibition was estimated by comparing the ratio of the IC_{50} values of purified RKALR/RLAR.

aldose reductase inhibition increased to a 6-fold difference with 2-methyl-6-fluorospirochroman-4,5′-imidazolidine-2′,4′-dione (M79175); (**3**) and 2-(3′,4′-dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4*H*-chromen (quercetin; **4**). A 14-fold difference in selectivity was observed with tetramethylene glutaric acid (TMG; **5**). This difference increased to 22-fold with *N*-([5-(trifluoro-methyl)-6-methoxy-1-naphthalenyl]-thioxomethyl)-*N*-methylglycine (tolrestat; **6**), and *ca.* 28-fold selectivity was observed with 2′-hydroxy-3′-carboxy-5′-nitro-oxanilic acid, ethyl ester, (**7**) and *S*-6-fluorospirochroman-4,5′-imidazolidine-2′,4′-dione (sorbinil; **8**). Substitution of a 3-rhamnosyl moiety to quercetin (**4**) to form quercitrin (**9**) increased selectivity for aldose reductase to 38-fold, and this selectivity was similar to that observed for alrestatin (**10**). The greatest selectivity (119-fold) for aldose reductase inhibition was observed with 3-(4-bromo-2-fluorobenzyl-4-oxo-3-phthalazine-1-ylacetic acid (Ponalrestat, Statil; **11**).

Since aldehyde reductase not only catalyzes the NADPH-dependent reduction of aldehydes but also catalyzes the $NADP^+$-dependent oxidation of L-gulonic acid to D-glucuronate, the ability of aldose reductase inhibitors to inhibit this oxidative step was also investigated with a series of inhibitors currently undergoing clinical evaluations. Examination of Al1576 (**1**), tolrestat (**6**), sorbinil (**8**), and Ponalrestat (**11**) revealed that both the reductive and oxidative steps were equally inhibited by these aldose reductase inhibitors (Table 2). Kinetic studies of the reduction of glyceraldehyde by rat kidney aldehyde reductase indicated that the inhibition by aldose reductase inhibitors was uncompetitive at low concentrations of inhibitor and mixed-type (intersecting noncompetitive) at higher concentrations (Fig. 1). Inhibition kinetics with alrestatin and sorbinil have been observed to be noncompetitive with glucuronate in human brain aldehyde reductase [3] and uncompetitive with glucuronate in sheep liver aldehyde reductase [6]. On the other hand, the inhibition by aldose reductase inhibitors of the oxidation of L-gulonate by rat kidney aldehyde reductase appeared competitive in nature. To determine if these inhibitors competed with the coenzyme NADPH and $NADP^+$ in the reductive and oxidative steps, respectively, kinetic studies were also conducted in which the concentration of nucleotide was varied. Uncompetitive inhibition was observed in both the reductive steps with DL-glyceraldehyde as substrate and the oxidative steps with L-gulonate as substrate. This is illustrated for sorbinil in Fig. 2.

Inhibition studies utilizing various combinations and concentrations of these inhibitors were also conducted to determine if aldose reductase inhibitors interact with rat kidney aldehyde reductase on a single site or multiple sites (Table 3). Inhibition studies utilizing Al1576 (**1**) (0.005 and 0.01 μM), tolrestat (**6**) (0.2 and 0.5 μM), sorbinil (**8**) (0.6 and 1.7 μM) and Ponalrestat (**11**) (0.4 and 1.0 μM) indicated that the inhibition produced by the various combinations and concentrations of these compounds was not additive, suggesting that they interact with the protein at a common site. This premise is supported by additional kinetic studies with sorbinil and Ponalrestat. Dixon plots of $1/v$ versus the concentration of one inhibitor at a fixed concentration of 10 mM glyceraldehyde while the concentration of the second inhibitor was varied yielded a family

Table 2. Effect of substrate on the inhibition of rat kidney aldehyde reductase by aldose reductase inhibitors

	Compound	IC_{50} (μM) DL-Glyceraldehyde	L-Gulonate
1		0.046	0.051
6		0.24	0.3
8		1.8	1.8
11		1.9	0.82

DL-Glyceraldehyde was reduced by glycerol, while L-gulonate was oxidized to D-glucuronate. Values are the means of 3–6 determinations.

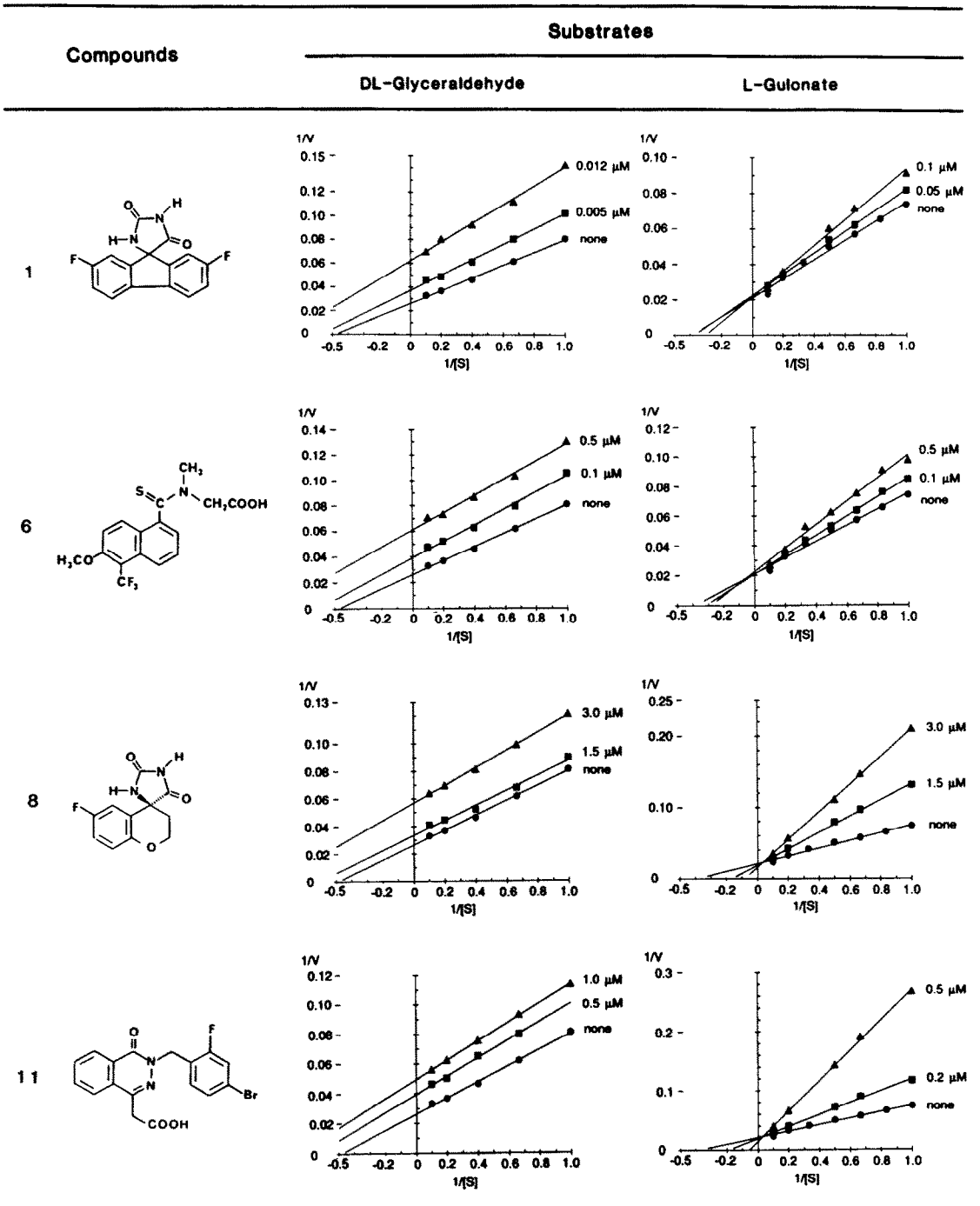


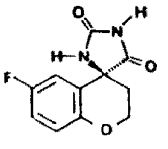
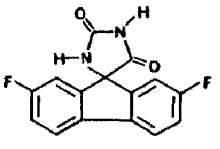
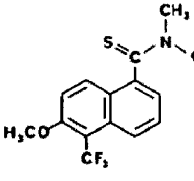
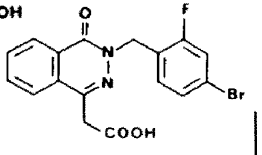
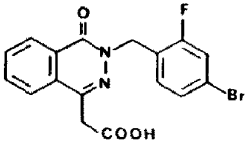
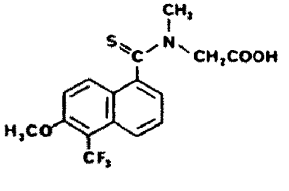
Fig. 1. Kinetic studies of purified rat kidney aldehyde reductase in the presence of the aldose reductase inhibitors Al1576 (**1**), tolrestat (**6**), sorbinil (**8**) and Ponalrestat (**11**) with DL-glyceraldehyde or L-gulonate as substrates. Graphs represent Lineweaver–Burk plots from these kinetic studies conducted by varying the concentration of either substrate in the presence of inhibitor as described in Materials and Methods. Each point is the mean of 3–6 determinations.

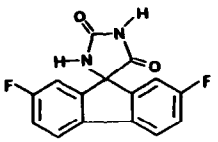
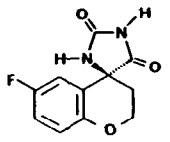
of parallel curves which indicate that sorbinil and Ponalrestat are mutually exclusive (Fig. 3) [12]. Computer analyses of K_i data for these inhibitors indicated optimal results with only a single binding site model.

DISCUSSION

A number of similarities and differences have been observed through detailed studies of aldose reductase and aldehyde reductase (hexonate

Table 3. Competition studies with the carboxylic acid containing aldose reductase inhibitors Ponalrestat and tolrestat and the hydantoins sorbinil and Al1576 against purified rat kidney aldehyde reductase

		 (20.7)	(43.5)	 (24.5)	(43.6)	 (23.7)	(39.9)	 (21.7)	(42.4)
	(21.7)	*(42.4)* 35.0±1.6	*(65.2)* 51.4±1.7	*(46.2)* 41.3+0.9	*(65.3)* 55.2±1.0	*(45.4)* 38.5±2.3	*(61.6)* 52.1±2.2	*(43.4)* 34.2±0.8	*(64.1)* 49.0±2.2
	(42.4)	*(63.1)* 48.2±1.1	*(85.9)* 59.7±0.6	*(66.9)* 54.9±1.6	*(86.0)* 61.9±0.3	*(66.1)* 53.5±1.9	*(82.3)* 59.7±0.8		*(84.8)* 66.1±1.1
	(23.7)	*(44.4)* 37.8±0.5	*(67.2)* 53.9±0.5	*(48.2)* 41.9±0.6	*(67.3)* 5.5±0.9	*(47.4)* 39.7±1.1	*(63.6)* 49.7±1.6		
	(39.9)	*(60.6)* 50.2±2.0	*(83.4)* 59.7±0.3	*(64.4)* 54.1±0.8	*(83.5)* 64.0±0.3		*(79.8)* 56.4±0.3		

	(24.5)	*(45.2)* 40.2±0.8	*(68.0)* 52.8±1.9	*(49.0)* 41.8±1.1	*(69.1)* 58.2±0.7			
	(43.6)	*(64.3)* 53.3±2.0	*(87.1)* 62.9±2.32		*(87.2)* 66.7±1.2			
	(20.7)	*(41.4)* 35.1±2.0	*64.2)* 49.8±2.4					
	(43.5)		*(87.0)* 61.4±0.3					

Concentrations of inhibitor used and percent inhibition produced were: Ponalrestat, 0.4 μM 21.7%, 1.0 μM 42.45%; tolrestat, 0.2 μM 23.7%, 0.5 μM 39.9%; sorbinil, 0.6 μM 20.7%, 1.7 μM 43.5%; and Al1576, 0.005 μM 24.5%, 0.01 μM 43.6%. Inhibitors were added consecutively to the cuvettes containing purified enzyme and activity was assayed spectrophotometrically as described in Materials and Methods using DL-glyceraldehyde as substrate. The numbers in parentheses represent percent inhibition of each inhibitor and theoretical additive inhibition of the two inhibitors.

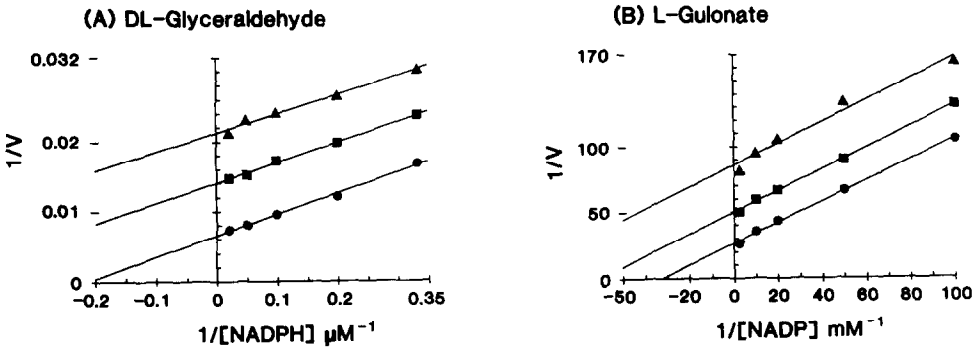


Fig. 2. Kinetic studies of purified rat kidney aldehyde reductase in which the concentration of nucleotide was varied in the presence of 1.5 and 3.0 μM concentrations of the aldose reductase inhibitor sorbinil (**8**). (A) Lineweaver–Burk plot of the reductive reaction with 10 mM DL-glyceraldehyde as substrate as the concentration of NADPH is varied. (B) Lineweaver–Burk plot of the oxidative reaction with 10 mM L-gulonate as substrate as the concentration of $NADP^+$ is varied. The closed circles represent the reaction in the absence of inhibitor, while the squares and triangles indicate the presence of 1.5 and 3.0 μM sorbinil respectively. Each point is the mean of 3–6 determinations.

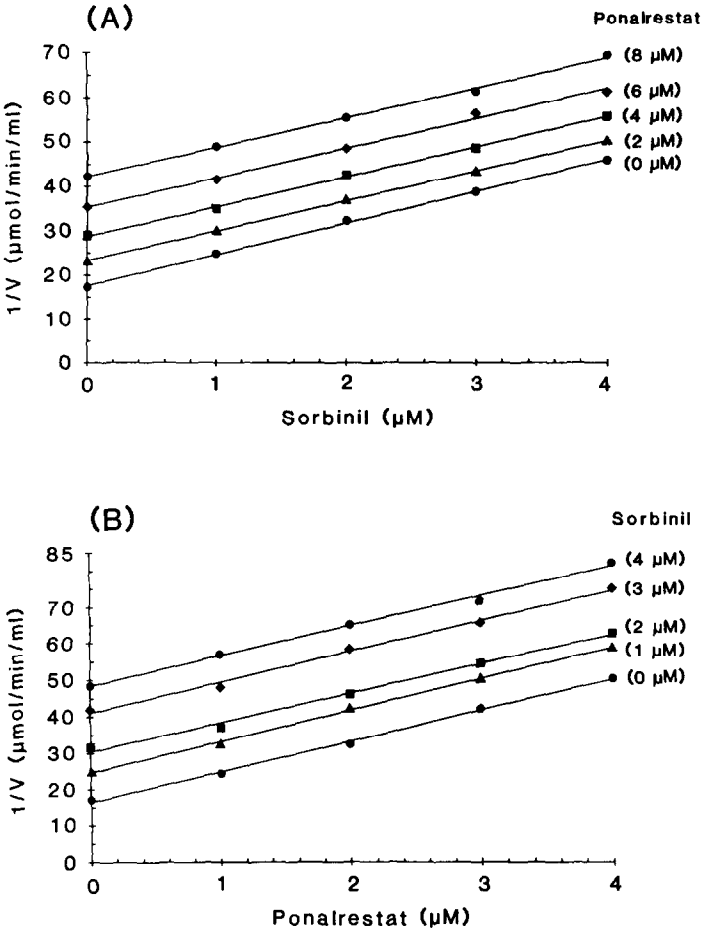


Fig. 3. Dixon plots of (A) $1/v$ versus the concentration (μM) of sorbinal at fixed concentrations (0, 2, 4, 6 and 8 μM) of Ponalrestat, and (B) $1/v$ versus the concentration of Ponalrestat at fixed concentrations (0, 1, 2, 3 and 4 μM) of sorbinil. DL-Glyceraldehyde (10 mM) was used as substrate. Each point is the mean of 3–4 determinations.

dehydrogenase), and these have raised questions concerning their physiological role(s). Similarities include the monomeric nature of these enzymes, their partially overlapping substrate specificities, and their similar utilization of the coenzyme NADPH; however, they differ in their immunological cross-reactivities, and distinct physical properties, which include molecular weights and isoelectric points. Previously, the aldose reductase inhibitors alrestatin (**10**) and sorbinil (**8**) have been reported to inhibit aldehyde reductase from human brain [3] and sheep liver [6], while sorbinil (**8**) M79175 (**3**), and Ponalrestat (**11**) have been observed to inhibit crude aldehyde reductase from bovine retina [5]. In the present study, the inhibitory susceptibility of rat lens aldose reductase versus rat kidney aldehyde reductase for aldose reductase inhibitors has been directly compared for a series of structurally diverse inhibitors. This study utilized DL-glyceraldehyde, a common substrate for both enzymes, in the absence of organic solvent that can potentially affect the conformation of either enzyme and subsequently the inhibitory results (Table 1). The results revealed that all aldose reductase inhibitors examined also inhibited aldehyde reductase to some extent and that this inhibition occurred both in the reductive reaction as determined with glyceraldehyde as substrate and NADPH as coenzyme, and in the oxidative reaction where L-gulonic acid is oxidized to D-glucuronic acid in the presence of $NADP^+$ (Table 2). Unlike aldose reductase where the susceptibility to inhibition has been observed to decrease for select aldose reductase inhibitors such as sorbinil (**8**) with increased purification and length of storage time [13], all of these compounds inhibited the reductive reaction of either crude or highly purified aldehyde reductase to essentially the same extent. This indicates that either partially purified or purified aldehyde reductase can be utilized equally for reproducible screenings of inhibitor activities. Furthermore, this suggests that the secondary or tertiary structure of rat kidney aldehyde reductase remains structurally more stable upon purification than that of aldose reductase since the site of interaction between these aldose reductase inhibitors and rat kidney aldehyde reductase does not appear to be altered significantly by either purification or prolonged storage.

Marked differences in the selectivity of these inhibitors, expressed as the ratio of IC_{50} values for rat kidney aldehyde reductase versus rat lens aldose reductase with glyceraldehyde as substrate, were observed with selectivity for aldose reductase ranging from *ca.* 2-fold for Al1576 (**1**) to 119-fold for Ponalrestat (**11**). None of the aldose reductase inhibitors appeared more selective for inhibiting aldehyde reductase. These results differ from those previously reported with *p*-nitrobenzaldehyde dissolved in 1.3% (v/v) methanol as substrate, in which the selectivity of aldose reductase inhibitors for crude bovine retina aldose reductase versus aldehyde reductase, also expressed as IC_{50} ratios, ranged from 1.95 for sorbinil (**8**) to < 0.18 for M79175 (**3**) [5].

Although aldose reductase inhibitors appear structurally diverse, they contain certain structural and electronic similarities that have been deduced with the aid of computer modelling and molecular orbital calculations. From these studies it has been deduced that aldose reductase inhibitors possess a primary and possibly secondary hydrophobic (aromatic) binding region and a common region that is susceptible to nucleophilic attack [13–16]. Moreover, kinetic and competition studies with purified rat lens aldose reductase indicate that aldose reductase inhibitors interact with aldose reductase at a common site that is not identical to either the substrate or nucleotide binding site. Similarly, the present studies suggest that in the reductive reaction aldose reductase inhibitors interact with rat kidney aldehyde reductase at a common region that is not identical to either the aldehyde substrate binding region or the nucleotide binding site. Moreover, based on estimated common pharmacophor requirements for aldose reductase inhibitors [14], examination of the structures of the inhibitors in Table 1 suggests that increased steric bulk in the inhibitor binding region near the secondary lipophilic site and the "sterically constrained charge-transfer pocket" favors selectivity of the inhibitors for aldose reductase inhibition. This is illustrated by the increased selectivity of quercitrin (**9**) versus quercetin (**4**). On the other hand, the absence of stearic bulk in this region along with increased lipophilic binding resulted in increased selectivity for aldehyde reductase as illustrated by Al1576 (**1**) > M79175 (**3**) > sorbinil (**8**). Some evidence for the occurrence of a nucleophilic interaction between aldehyde reductase and the aldose reductase inhibitors similar to that observed with aldose reductase inhibitors has also been observed through limited investigations with affinity labeled aldose reductase inhibitors [15, 16]. Preliminary studies with isothiocyanato and *N*-haloacetamido analogs substituted in the 5-position of alrestatin (**10**) indicate that these compounds can also irreversibly inhibit aldehyde reductase as a result of nucleophilic attack [17].

Interestingly, kinetic studies of the inhibition of the oxidation of L-gulonate by aldehyde reductase indicated that aldose reductase inhibitors were competitive with the substrate. Similar competitive kinetics have also been observed in the ability of Al1576 (**1**) to inhibit the rat lens [18] and human placental [19] aldose reductase mediated oxidation of benzyl alcohol using the 3-acetylpyridine analog of $NADP^+$ as cofactor. Although it has not been established whether substrates being "oxidized or reduced" interact at the same substrate binding site on either enzyme, the kinetic results which indicate competitive inhibition in the oxidative step and uncompetitive inhibition in the reductive step suggest that the inhibitors are in closer proximity to substrates being oxidized than are inhibitors and substrates being reduced.

The present studies demonstrate clear similarities between the inhibition of rat kidney aldehyde reductase and rat lens aldose reductase by aldose reductase inhibitors, suggesting that inhibition occurs by similar mechanisms. Nevertheless, selectivity in the inhibition of aldose reductase versus aldehyde reductase can be introduced through the introduction of selective steric substitutions and altered lipophilicity, indicating that the inhibitor binding site on each enzyme is different. More

detailed studies are required in order to differentiate these differences.

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