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Perspective

Aldose Reductase Inhibitors: A Potential New Class of Agents for the Pharmacological Control of Certain Diabetic Complications

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Although the administration of insulin can prolong the life of diabetics, its use to date has not prevented the debilitating, late-onset complications associated with this disease. Diabetic complications can occur in many tissues and affect various sensory organs, the nervous system, circulation, and renal excretion. Ocular diabetic complications include retinopathy, cataract, and corneal epitheliopathy while neuronal effects resulting in altered nerve motor function, sensory perception, and pain are categorized under diabetic neuropathy. Diabetic microangiopathy results from general vascular abnormalities associated with diabetes, including the formation of capillary microaneurysms, basement membrane thickening, and platelet aggregation. Glomerular thickening and proteinuria are two changes observed in diabetic nephropathy. The progression of these complications can result in loss of vision, sensory perception, limb function, and premature death. While the cause(s) of these complications remains unknown, they generally appear in tissues possessing insulin-independent glucose transport with their onset and severity appearing to be related to the management of blood glucose levels. Furthermore, mounting evidence suggests that the enzyme aldose reductase may provide a common biochemical link in the pathogenesis of many of these diabetic complications.[1-3]

The enzymes aldose reductase (alditol:$NADP^+$ oxidoreductase, EC 1.1.1.21) and sorbitol dehydrogenase (1-iditol dehydrogenase, EC 1.1.1.14) together form the sorbitol pathway. In this pathway aldose reductase initially catalyzes the stereospecific transfer of hydride from NADPH to the aldehyde form of glucose to form sorbitol. Sorbitol dehydrogenase in turn, utilizing NAD^+, oxidizes this intermediate polyol to fructose (Figure 1).[3] Aldose reductase and the sorbitol pathway were first described in 1956 by Hers in the seminal vesicles where it generates fructose for sperm.[4] Its discovery in the lens by van Heyningen (1958), however, quickly indicated that this pathway was not merely restricted to reproductive tissues.[5] Today, the sorbitol pathway has been found in a variety of tissues, including those that display diabetes-associated pathology. The physiological role of aldose reductase in most tissues, however, remains unknown with no physiologically significant amounts of accumulated sorbitol detected.

Adverse effects of the aldose reductase associated polyol pathway were first described by Kinoshita in the lens.[6-8] These studies, which formed the basis for the osmotic hypothesis of sugar cataract formation (polyol concept), indicate that the aldose reductase initiated intracellular accumulation of excess sorbitol results in biochemical changes that eventually lead to diabetic cataract formation. Through inhibition of this enzyme in diabetic animals this cataractogenic process can be delayed or even prevented.[9-11] Increased sorbitol levels have also been observed in other tissues displaying diabetes-associated pathology and several animal studies and preliminary clinical trials indicate that inhibition of aldose reductase may prevent the onset of other diabetic complications such as neuropathy,[12-18] corneal epitheliopathy,[19-22] retinopathy,[23-28] and

(1) Dvornik, D. *Annu. Rep. Med. Chem.* **1978**, *13*, 159.
(2) Lipinski, C. A.; Hutson, N. J. *Annu. Rep. Med. Chem.* **1984**, *19*, 169.
(3) Kinoshita, J. H.; Kador, P. F.; Datiles, M. *J. Am. Med. Assoc.* **1983**, *246*, 257.
(4) Hers, H. G. *Biochim. Biophys. Acta* **1956**, *22*, 202.
(5) van Heyningen, R. *Nature (London)* **1959**, *184*, 194.
(6) Kinoshita, J. H. *Invest. Ophthalmol.* **1965**, *4*, 786.
(7) Kinoshita, J. H. *Invest. Ophthalmol.* **1974**, *13*, 713.
(8) Obazawa H.; Merola, L. O.; Kinoshita, J. H. *Invest. Ophthalmol.* **1974**, *13*, 204.
(9) Dvornik, D.; et al. *Science* **1973**, *182*, 1146.
(10) Fukushi, S.; Merola, L. O.; Kinoshita, J. H. *Invest. Ophthalmol. Visual Sci.* **1980**, *19*, 313.
(11) Peterson, M. J.; Sarges, R.; Aldinger, C. G.; MacDonald, D. P. *Metabolism* **1979**, *28*, 456.
(12) Gabbay, K. H.; Snider J. J. *Diabetes* **1972**, *21*, 295.
(13) Yue, D. K.; Hanwell, M. A.; Satchell, P. M.; Turtle, J. R. *Diabetes* **1982**, *31*, 789.
(14) Tomlinson, D. R.; Holmes, P. R.; Mayer, J. H. *Neurosci. Lett.* **1982**, *31*, 189.
(15) Robison, W. G., Jr. *Ann. Intern. Med.* **1984**, *101*, 85.
(16) Young, R. J.; Ewing, D. J.; Clarke, B. F. *Diabetes* **1983**, *32*, 938.
(17) Jaspan, J.; Herold, K.; Maselli, R.; Bartkus, C. *Lancet* **1983**, *1*, 758.
(18) Judzewitsch, R.; et al. *N. Engl. J. Med.* **1983**, *308*, 119.
(19) Kinoshita, J. H.; Fukushi, S.; Kador, P.; Merola, L. O. *Metabolism* **1979**, *28*, 462.
(20) Fukushi, S.; Merola, L. O.; Tanaka, M.; Datiles, M., Kinoshita, J. H. *Exp. Eye Res.* **1980**, *31*, 611.
(21) Datiles, M. B.; Kador, P. F.; Fukui, H. N.; Hu, T. S.; Kinoshita, J. H. *Invest. Ophthalmol. Visual Sci.* **1983**, *24*, 563.
(22) Cobo, L. M. *Ann. Intern. Med.* **1984**, *101*, 87.

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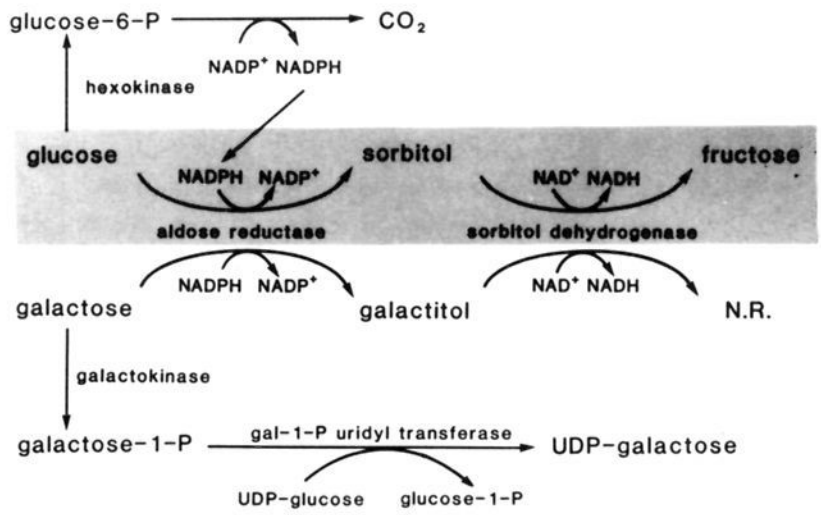


Figure 1. The polyol pathway (shaded) and alternate pathways of glucose and galactose metabolism.

microangiopathy.[29-31] Pharmacological agents that directly inhibit aldose reductase, therefore, appear to be useful for the prevention of certain diabetic complications and they may provide a new mode of treatment that is independent of the insulin-related control of blood glucose levels.

The Polyol Concept

Treatment of diabetic complications with inhibitors of aldose reductase is biochemically attractive because the aldose reductase initiated accumulation of sorbitol, and its resulting pathology, only appears to be significant under nonphysiological conditions of hyperglycemia. In many tissues, aldose reductase must directly compete with hexokinase for the utilization of glucose. The affinity of hexokinase for glucose, however, is greater than that of aldose reductase so that under normal physiological conditions available glucose is preferentially phosphorylated by hexokinase. In these tissues, significant increases of sorbitol are only produced under nonphysiological conditions, such as in diabetes, where hexokinase is saturated by elevated levels of glucose. Under these conditions, sorbitol is formed more rapidly than it is converted to fructose, resulting in a net accumulation of sorbitol. Sorbitol accumulation is also enhanced by the polarity of the polyol, which hinders facile penetration through membranes and subsequent removal from tissues through diffusion. The intracellular accumulation of polyols can thus produce a hyperosmotic effect that results in an influx of fluid. Increased fluid influx, in turn, is associated with membrane permeability changes and the subsequent onset of cellular pathology.[3,7] Recent NMR flux studies also suggest that polyol-induced pathology may be enhanced by changes in cellular redox potentials resulting from the rapid depletion of NADPH.[32]

The Pharmacology of Aldose Reductase

Diabetes-associated complications are difficult to study because of the long onset period and metabolic complexity of diabetes. Studies on the role of aldose reductase in various diabetic complications, however, are being aided by the broad substrate specificity of this enzyme and by the development of effective aldose reductase inhibitors (ARIs). Aldose reductase can reduce a variety of aromatic and aliphatic aldehydes, including the sugar galactose, which serves as a better substrate for the enzyme than glucose. Increased cellular levels of galactose can lead to more rapid and greater hyperosmotic effects than that of glucose because increased levels of glactose are more rapidly reduced to galactitol (dulcitol) than glucose to sorbitol. Moreover, the intracellular levels of galactitol remain high because galactitol is not further metabolized by sorbitol dehydrogenase (Figure 1). Use of the galactosemic animal model has, therefore, become instrumental in elucidating the relationship between aldose reductase and diabetic complications.

Certain guidelines have been proposed in order to establish the role of aldose reductase in the etiology of these complications.[33] To implicate aldose reductase in diabetes-associated pathology, the presence of the enzyme must be established in the tissue in question and similar diabetes-associated pathology must occur in the galactosemic state, with its onset being more rapid and severe than in the diabetic state. Administration of at least two structurally diverse aldose reductase inhibitors should also delay or prevent the onset of these complications. Accumulating evidence, recently reviewed in detail,[34] has implicated aldose reductase in the pathogenesis of the following diabetic complications.

Cataract. Depending on the strength of the inhibitor, the appearance of diabetic or galactosemic (sugar) cataracts can be essentially prevented through the use of ARIs administered either orally, by injection, or topically as eye drops.[35] Their efficacy, however, is dependent on administration at the early stages of cataract formation.[36]

Keratopathy. It has recently been observed that the diabetic corneal epithelium is less tolerant to stress encountered in ocular manipulations during photocoagulation, vitrectomy surgery, and even the wearing of contact lenses. Traumatized areas tend to heal more slowly and require supportive medical attention. A similar delay in reepithelialization can be observed in the denuded corneas of either severely diabetic (>600 mg %) or galactosemic rats and this delay can be prevented with either oral or topical ARI administration. Moreover, the treated corneas appear clear and transparent upon healing while the nontreated corneas appear hazy and edematous.[19,21,37]

Microangiopathy. Capillary basement membrane thickening is a morphological change commonly observed in diabetic tissues. This thickening can be similarly produced in galactose-fed animals and prevented upon concomitant administration of ARIs.[29-31]

Neuropathy. Decreases in motor nerve conduction velocity (MNCV), axonal transport, and sensory perception, commonly associated with diabetic neuropathy, can

(23) Buzney, S. M.; Frank, R. N.; Varma, S. D.; Tanishima, T.; Gabbay, K. H. *Invest. Ophthalmol. Vis. Sci.* **1977**, *16*, 392.
(24) Poulsom, R.; Heath, H. *Biochem. Pharmacol.* **1983**, *32*, 1495.
(25) Engerman, R.; Bloodworth, J. M. B., Jr.; Nelson, S. *Diabetes* **1977**, *26*, 760.
(26) Engerman, R. L.; Kern, T. S. *Diabetes* **1984**, *33*, 97.
(27) Akagi, Y.; Kador, P. F.; Kuwabara, T.; Kinoshita, J. H. *Invest. Ophthalmol. Visual Sci.* **1983**, *24*, 1516.
(28) Kern, T. S.; Engerman, R. L. *Invest. Ophthalmol. Visual Sci.* **1984**, *25* (suppl), 159.
(29) Robison, W. G., Jr.; Kador, P. F.; Kinoshita, J. H. *Science* **1983**, *221*, 1177.
(30) Frank, R. N.; Keirn, R. J.; Kennedy, A.; Frank, K. W. *Invest. Ophthalmol. Visual Sci.* **1983**, *24*, 1519.
(31) Chandler, M. L.; Shannon, W. A.; DeSantis, L. *Invest. Ophthalmol. Visual Sci.* **1984**, *25* (suppl), 159.
(32) Gonzalez, R. G.; Barnett, P.; Aguayo, J.; Cheng, H. M.; Chylack, L. T., Jr. *Diabetes* **1984**, *33*, 196.
(33) Kinoshita, J. H. *Ann. Intern. Med.* **1984**, 101, 82.
(34) Kador, P. F.; Robison, W. G., Jr.; Kinoshita, J. H. *Annu. Rep. Pharmacol. Toxicol.*, in press.
(35) Kador, P. F.; Kinoshita, J. H. *Human Cataract Formation, Ciba Found. Symp.* **1984**, *106*, 110.
(36) Hu, T. S.; Datiles, M.; Kinoshita, J. H. *Invest. Ophthalmol. Visual Sci.* **1983**, *24*, 640.
(37) Datiles, M.; Hu, T.-S.; Kador, P.; Robison, W. G., Jr.; Kinoshita, J. *Invest. Ophthalmol. Visual Sci.* **1982**, *22* (suppl), 25.

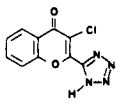
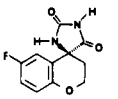
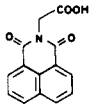
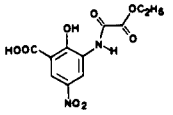
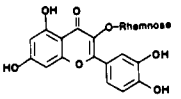
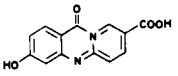
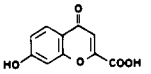
be reversed in animals or clinically through the oral administration of ARIs.[12-18]

Retinopathy. Aldose reductase has been histochemically localized in the pericytes (mural cells) of retinal capillaries and these cells have been observed to degenerate during human retinopathy.[27,38] A similar drop-out of retinal pericytes concomitant with microaneurysm formation and other clinical signs of retinopathy can be produced in dogs through galactose feeding.[26] Galactitol formation has also been detected in isolated retinal capillaries cultured in medium containing galactose and this formation can be prevented by the introduction of ARI.[28]

Nephropathy. The relationship between aldose reductase and diabetic kidney pathology has only recently been investigated and not fully elucidated. Preliminary studies indicate that kidney polyol accumulation and changes in proteinuria observed in diabetic rats can be diminished upon ARI administration.[39,40]

Biological Evaluation of Aldose Reductase Inhibitors Biochemical Studies. Aldose reductase is a member of the aldehyde reductase family of enzymes. In the literature this enzyme has been referred to by several names, including the low K_m aldehyde reductase, aldose reductase I(a)(b) and II(a)(b), AR A and AR B, and aldehyde reductase 2 (ALR 2).[41-43] Aldose reductase has been purified from several tissues, including the lens,[43-50] placenta,[51,52] brain,[53-56] kidney,[57] muscle,[58] and seminal vesicles[59] from such sources as cow, rat, rabbit, pig, dog, and human. These studies reveal that this sulfhydryl-containing enzyme generally appears to be a monomer with a molecular weight range between 28 and 45 K and that apparent isozymes of this enzyme may exist.[42,43,58,60] Aldose reductase is not a metalloprotein and there is no evidence of either bound phosphate or glycoprotein groups. Antibodies raised against the purified enzymes reveal significant differences in cross-reactivity between species and perhaps even tissues.

Table I. Effect of Purification on the Inhibitory Susceptibility of Human Placental Aldose Reductase[a]

compd	IC_{50}, μM: 30–70% $(NH_4)_2SO_4$	time, day	AH-Sepharose 4B column	time, day	orange A column
	41	1	38	2	280
		7	39	8	200
	0.7	1	4.7	2	2200
		7	7.2	8	1400
	6.5	1	22	2	140
		7	34	8	79
	7.3	1	4.5	2	3.2
		7	8.3	8	4.0
	7.1	1	14	2	16
		7	16	8	9.0
	4.5	1	5.0	2	19
		7	7.2	8	7.3
	1.8	1	4.7	2	5.9
		7	3.9	8	3.1

[a] Illustrates that ARIs display an increase in their IC_{50}s when evaluated against purified placental enzyme. Purification steps were carried out at 4 °C as previously described[52] and enzyme activity was photometrically assayed at 340 nm in a Guilford 2400-2 automated spectrophotometer using 10 mM DL-glyceraldehyde as substrate.[68] Inhibitor IC_{50}s were obtained from simple linear regressions of the Marquart-Levenberg iterative curve fitting algorithm.[83]

Table II. Pathological Changes Displayed by the Diabetic and Galactosemic Rat

rat models for aldose reductase evaluation		
complication	diabetes	galactose fed
cataract	12–16 weeks	30% diet 20 days, 50% diet 14 days
cornea	>600 mg % glucose necessary	effect after 7 days, 50% diet
neuropathy	MNCV 10 days, plateau at 30 weeks	MNCV <10 days
basement membrane	significant increase in 2 months	1.5× after 6 months

Inhibition studies with ARIs have been reported that use enzyme from several sources, including bovine, rat, rabbit and human lenses, human placenta, and the Engelbreth-Holm-Swarm (EHS) tumor cell line.[48,60-64] Depending on the structure of inhibitor employed, significant differences have been observed in the suscepti-

(38) Kuwabara, T.; Cogan, D. G. *Arch. Ophthalmol.* **1963**, *69*, 492.
(39) Varagiannis, E.; Beyer-Mears, A.; Cruz, E. *Diabetes* **1984**, *33* (suppl), 43a.
(40) Beyer-mears, A.; Ku, L.; Cohen, M. P. *Diabetes* **1984**, *33*, 604.
(41) Turner, A. J.; Flynn, T. G. *Prog. Clin. Biol. Res.* **1982**, *114*, 401.
(42) Gabbay, K. H.; Cathcart, E. S. *Diabetes* **1974**, *23*, 460.
(43) Tanimoto, T.; Fukuda, H.; Kawamura, J. *Chem. Pharm. Bull.* **1983**, *31*, 2395.
(44) Hayman, S.; Kinoshita, J. H. *J. Biol. Chem.* **1965**, *240*, 877.
(45) Herrmann, R. K.; Kador, P. F.; Kinoshita, J. H. *Exp. Eye. Res.* **1983**, *37*, 467.
(46) Conrad, S. M.; Doughty, C. C. *Biochim. Biophys. Acta* **1982**, *708*, 348.
(47) Sheaff, C. M.; Doughty, C. C. *J. Biol. Chem.* **1976**, *251*, 2696.
(48) Inagaki, K.; Miwa, I.; Okuda, J. *Arch. Biochem. Biophys.* **1982**, *216*, 337.
(49) Branlant, G. *Eur. J. Biochem.* **1982**, *129*, 99.
(50) Kador, P. F.; Millen, J.; Akagi, Y.; Kinoshita, J. H. *Invest. Ophthalmol. Vis. Sci.* **1984**, *25* (suppl), 47.
(51) Clements, R. S.; Winegrad, A. I. *Biochem. Biophys. Res. Commun.* **1972**, *47*, 1473.
(52) Kador, P. F.; Carper, D.; Kinoshita, J. H. *Anal. Biochem.* **1981**, *114*, 53.
(53) Wermuth, B.; Burgisser, H.; Bohren, K.; von Wartburg, J. P. *Eur. J. Biochem.* **1982**, *127*, 279.
(54) O'Brien, M. M.; Schofield, P. J. *Biochem. J.* **1980**, *187*, 21.
(55) Boghosian, R. A.; McGuinness, E. T. *Biochim. Biophys. Acta* **1979**, *567*, 278.
(56) Dons, R. F.; Doughty, C. C. *Biochim. Biophys. Acta* **1976**, *452*, 1.
(57) Kern, T. S.; Engerman, R. L. *Histochem. J.* **1982**, *14*, 507.
(58) Cromlish, J. A.; Flynn, T. G. *J. Biol. Chem.* **1983**, *258*, 3416.
(59) Ludvigson, M. A.; Sorenson, R. L. *Diabetes* **1980**, *29*, 438.
(60) Tanimoto, T.; Fukuda, H.; Kawamura, J. *Chem. Pharm. Bull.* **1984**, *32*, 1025.
(61) Kador, P. F.; Merola, L. O.; Kinoshita, J. H. *Doc. Ophthalmol. Proc. Ser.* **1979**, *18*, 117.
(62) Kador, P. F.; Kinoshita, J. H.; Tung, W. H.; Chylack, L. T. *Invest. Ophthalmol. Visual Sci.* **1980**, *19*, 980.
(63) Millen, J.; Kador, P. F.; Kinoshita, J. H.; Vogeli, G. *Invest. Ophthalmol. Visual Sci.* **1984**, *25* (suppl), 154.
(64) Okuda, J.; Miwa, I.; Inakagi, K.; Horie, T.; Nakayama, M. *Biochem. Pharmacol.* **1982**, *31*, 3807.

Table III. Retinal Changes Displayed by the Diabetic and Galactosemic Dog

	dog retinopathy model	
retinal change	alloxan diabetic[a]	30% galactose fed
capillary aneurysms		
by fluorescein	3	32 months
by histology	36–51 months	32 months
mural cell ghosts	60 months	32 months
hemorrhages	60 months	32 months
cotton wool spots and soft exudates	60 months	32 months
capillary basement membrane thickening		60 months

[a] 320–450 mg/dL.

bility to inhibition of aldose reductases from different tissues and species.[61,62] While no specific trends have been detected, the human placental enzyme is generally less susceptible to inhibition, suggesting that inhibitors destined for man should eventually be screened against human enzyme.[62] Apparent decreased susceptibility to inhibition has also been observed to occur with increased enzyme purification or storage times, suggesting that only fresh crude or stable, partially purified enzyme preparations be employed for the screening of ARIs. This is especially true for human placental aldose reductase as illustrated in Table I.

Kinetic studies on aldose reductases indicate that all enzymes, independent of source or tissue, display similar, broad substrate specificity in reducing the aldehyde group of several substrates, including certain steroids, aromatic aldehydes, aliphatic aldehydes, and aldose sugars.[41,44,52,65] This activity is enhanced by the presence of sulfate ion. Kinetic studies further indicate that currently known ARIs are not competitive with either the substrate or NADPH cofactor.[64,66–70] On the basis of kinetics and inhibitor competition studies, three distinct enzyme regions have been identified—a substrate site, nucleotide fold, and inhibitor site.[71] Through protein modification studies the presence of arginine has been detected at the nucleotide fold and the presence of a nucleophilic amino acid, believed to be tyrosine, has been observed at the inhibitor site.[71,72] Evidence for the presence of a tyrosine moiety at the substrate site has also been reported.[72] Observed similarities in substrate specificity combined with immunological and inhibitory susceptibility differences suggest the existence of structural regions outside the substrate site or nucleotide fold that are different.

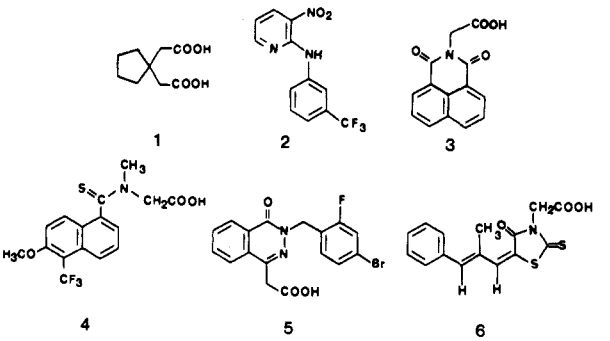
Animal Studies. Several animal models have been employed for the in vivo evaluation of ARIs. The most convenient and widely employed has been the diabetic or galactosemic rat (Table II). This animal, in either the diabetic or galactosemic state, can display cataract formation,[7,35] corneal epitheliopathy,[19,21] capillary basement membrane changes,[29–31] MNCV and sensory perception changes,[13,73] and apparent alterations in kidney function.[39,40] Moreover, the frank appearance of visible dense nuclear opacities in the eyes of these animals provides a convenient end point for the initial in vivo evaluation of ARIs. The rat, however, cannot satisfactorily mirror retinal changes observed with human retinopathy, including the selective degeneration of retinal pericytes, the formation of microaneurysms, and the appearance of cotton wool spots. These changes can be observed in the diabetic or galactosemic dog (Table III), indicating that this model should be employed for the evaluation of ARIs in retinopathy.[25,26]

Aldose Reductase Inhibitors

Development of ARIs. Historically, the development of ARIs focused on the prevention of diabetic cataract formation. These studies, in turn, have led to the development of a variety of structurally diverse inhibitors. Through their use, evidence for the involvement of aldose reductase in other serious diabetic complications has evolved.

The first compound capable of modifying the cataractous process through inhibition of aldose reductase was tetramethyleneglutaric acid (1, TMG).[7] Its development in the late 1960s evolved from observations that long-chain fatty acids could inhibit aldose reductase in lens homogenates.[44,66] The inability of TMG to penetrate membranes led to *N*-(3-nitro-2-pyridyl)-3-(trifluoromethyl)aniline (2, AY-20,263).[7] However, significant solubility problems with this compound in turn resulted in the development of the water-soluble inhibitor alrestatin (1,3-dioxo-1*H*-benz[*d*,*e*]isoquinoline-2(3*H*)-acetic acid (3, AY-22,284).[9] Since the development of alrestatin, a number of more potent organic acids have evolved as ARIs. These include *N*-[[5-(trifluoromethyl)-6-methoxy-1-naphthalenyl]thioxomethyl]-*N*-methylglycine (4, tolrestat, AY 27,773), [3-(4-bromo-2-fluorobenzyl)-4-oxo-3*H*-phthalazin-1-yl]acetic acid (5, ICI 128,436), and (*E*)-5-[(*E*)-2-methyl-3-phenylpropenylidene]rhodanine-3-acetic acid (6, ONO 2235).[73–75]

Carboxylic Acids

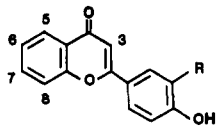


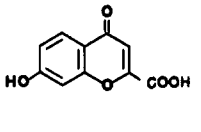
Since the mid-1970s, a number of flavonoids have been reported to have aldose reductase inhibitory activity.[64,67,70,76,77] These included quercetin (7, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4*H*-chromene), its

(65) Wermuth, B.; Monder, C. *Eur. J. Biochem.* **1983**, *131*, 423.
(66) Jedziniak, J. A.; Kinoshita, J. H. *Invest. Ophthalmol.* **1971**, *10*, 357.
(67) Varma, S. D.; Kinoshita, J. H. *Biochem. Pharmacol.* **1976**, *25*, 2505.
(68) Kador, P. F.; Sharpless, N. E. *Biophys. Chem.* **1978**, *8*, 81.
(69) Inagaki, K.; Miwa, I.; Yashiro T.; Okuda, J. *Chem. Pharm. Bull.* **1982**, *30*, 3244.
(70) Okuda, J.; Miwa, I.; Inakagi, K.; Horie, T.; Nakayama, M. *Chem. Pharm. Bull.* **1984**, *32*, 767.
(71) Kador, P. F.; Sharpless, N. E. *Mol. Pharmacol.* **1983**, *24*, 521.
(72) Doughty, C. C.; Lee, S.-M.; Conrad, S.; Schade, S. *Prog. Clin. Biol. Res.* **1982**, *114*, 223.
(73) Stribling, D.; Mirrless, D. J.; Harrison, H. E.; Earl, D. C. N. *Metabolism.*, in press.
(74) Sestanj, K.; Bellini, F.; Fung, S.; Nedumparambil, A.; Treasurywala, A.; Humber, L.; Simard-Duquesne, N.; Dvornik, D. *J. Med. Chem.* **1984**, *27*, 255.
(75) Terashima, H.; Hama, K.; Yamamoto, R.; Tsuboshima, M., Kikkawa, R.; et al. *J. Pharmacol. Exp. Ther.* **1984**, *229*, 226.
(76) Fauran, F.; Fenion, C.; Mosser, J.; Prat, G. *Eur. J. Med. Chem.* **1978**, *13*, 503.
(77) Chaudhry, P. S.; Cabrera, J.; Juliani, H. R.; Varma, S. D. *Biochem. Pharmacol.* **1983**, *32*, 1995.

3-rhamnoside quercitrin (8), 2-(3,4-dihydroxyphenyl)-6-hydroxy-5,7,8-trimethoxy-4-oxo-4*H*-chromene (9), 2-(4-hydroxyphenyl)-5,6,7,8-tetramethoxy-4-oxo-4*H*-chromene (10), and 2-(3,4-dihydroxyphenyl)-5,6,7,8-tetramethoxy-4-oxo-4*H*-chromene (11). Limited SAR studies of flavonoids suggest that the presence of 7-OH and 4'-OH groups enhance the inhibitory activity of certain flavones and that this enhancement is retained by the substitution of ethereal sulfate groups.[64,71,77] Little effect is seen by methylation at C-8 and the inhibitory potency is not decreased by the introduction of a bulky benzyl substituent at C-8, suggesting a tolerance for steric bulk at this position.[70] With 3-substituted sugars, inhibitory potency has been linked with increased lipophilicity resulting from the introduction of sugar acetals.[76]

Flavonoids and Chromones





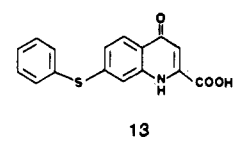
12

	R	3	5	6	7	8
7	OH	OH	OH	H	OH	H
8	OH	ORhamnose	OH	H	OH	H
9	OH	H	OMe	OH	OMe	OMe
10	H	H	OMe	OMe	OMe	OMe
11	OH	H	OMe	OMe	OMe	OMe

Inhibitory activity was retained by replacement of the aromatic 2-phenyl substituents of flavonoids with a nonaromatic carboxyl group, indicating that only the 4-oxo-4*H*-chromene ring system of flavonoids appeared to be necessary for inhibitory activity.[68] Structural similarities between 2-chromonecarboxylic acid **12** and the antiallergy agonist disodium cromoglycate resulted in an examination of a variety of other classes of antiallergy agonists as potential ARIs. These led to the observation that many antiallergy compounds including the quinolone **13**, coumarin **14**, oxanilic ester **15**, xanthone **16**, 11-oxo-11*H*-pyrido[2,1-*b*]quinazoline **17**, 1,4,6,9-tetrahydro-4,6-dioxopyrido[3,2-*g*]quinoline **18**, 1,6-dihydro-6-oxo-2-phenylpyrimidine **19**, and 3,4-dihydro-4-oxothieno[2,3-*d*]pyrimidine **20**, and others that contained the structural fragment **21** can also inhibit aldose reductase.[62,78,79] However, no correlation has been observed between aldose reductase inhibitory activity and antiallergy activity.

Combining the chroman system with a hydantoin ring has resulted in the formation of potent spirohydantoin ARIs. Of these compounds, sorbinil [(*S*)-6-fluorospiro-[chroman-4,5'-imidazolidine]-2',4'-dione, **22**, CP 45,634] was the first in vivo effective oral inhibitor capable of preventing the entire cataractogenic process when administered to either galactosemic or diabetic rats.[10,1180] Resolution of this sterically constrained spirohydantoin indicated that aldose reductase could differentiate stereochemically between inhibitors with the activity residing predominantly with the *S* enantiomer.[82,83] Reported SAR

Anti-allergy Agents



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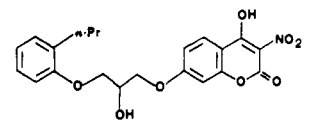

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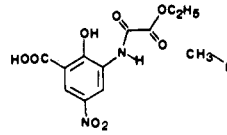

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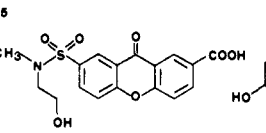

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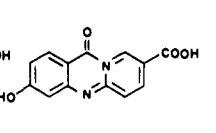

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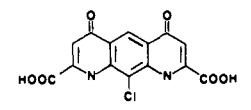

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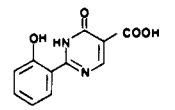

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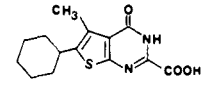

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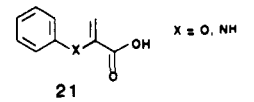
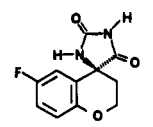


21

studies of sorbinil indicate that activity is improved through halo substitution of the aromatic ring while replacement of the 1-nitrogen with oxygen yields a spirooxazolidine with no improved inhibitory activity.[80,81] Today, sorbinil is considered the benchmark by which the potency and effectiveness of other aldose reductase inhibitors is assessed.

Hydantoins and Related Compounds



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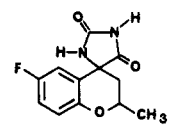
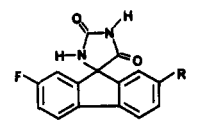
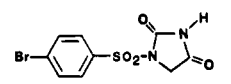


23



24 R = H

25 R = F



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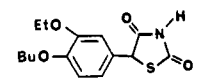


27

Sorbinil in turn has led to the development of several other spirohydantoins and related cyclic amides. Additon of a 2-methyl substituent has resulted in the development of **23** (M79175), a compound with apparently similar biological properties.[84] Replacement of the chroman ring system with a planar fluorene ring produced the spirohydantoin **24** with increased inhibitory activity.[85] This activity can be further increased through difluoro substitution to the nonchiral **25**. Another series of inhibitors has evolved from 1-substitution of the hydantoin ring. This series includes 1-[(*p*-bromophenyl)sulfonyl]-imidazolidine-2,4-dione (**26**), a compound reported to be essentially equipotent with sorbinil.[69] A series of 5-arylthiazolidine-2,4-diones possessing hypoglycemic activity have also been screened for their ability to inhibit aldose reductase.[86] Pronounced inhibitory activity was displayed

(78) Kador, P. F.; Sharpless, N. E.; Goosey, J. D. *Prog. Clin. Biol. Res.* **1982**, *114*, 243.
(79) Pfister, J. R.; Waterbury, L. D. *J. Med. Chem.* **1980**, *23*, 1264.
(80) Sarges, R. German Patent 27 46 244, 1977.
(81) Schnur, R. C.; Sarges, R.; Peterson, M. J. *J. Med. Chem.* **1982**, *25*, 1451.
(82) Sarges, R.; Belletire, J. L.; Schnur, R. C.; Peterson, "Abstracts of Papers", 177th National Meeting of the American Chemical Society, Honolulu, March 1979; American Chemical Society: Washington, DC, 1979; MED 16.
(83) Kador, P. F.; Goosey, J. D.; Sharpless, N. E.; Kolish, J.; Miller, D. D. *Eur. J. Med. Chem.* **1981**, *16*, 293.
(84) Ono, H.; Nozawa, Y.; Hayano, S. *Nippon Ganka Gakki Zasshi* **1980**, *86*, 1343.
(85) York, B. M. *European* Patent Application 0 092 385, 1983.
(86) Sohda, T.; et al. *Chem. Pharm. Bull.* **1982**, *30*, 3601.

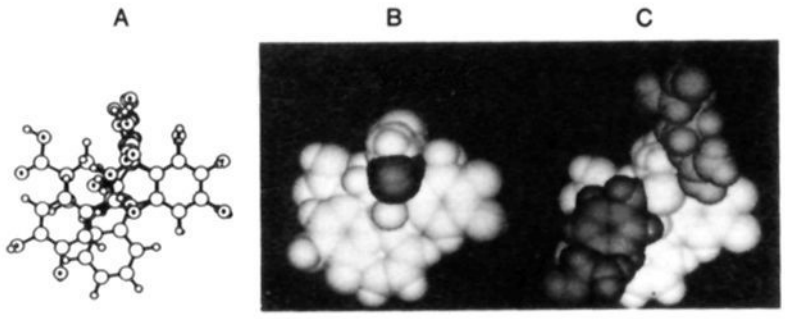
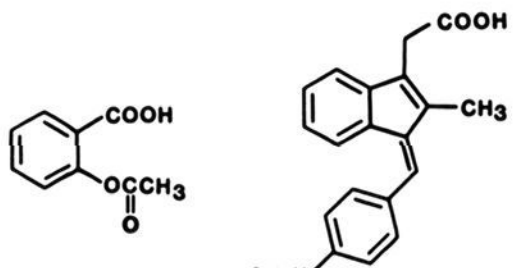


Figure 2. Common molecular conglomerate depicted as a ball and stick model (A) and CPK space-filling model (B) formed by superimposition of the aromatic A rings (shaped in A) of selected ARIs. The dark region in B indicates areas of apparent overlap of the common carbonyl regions. C illustrates the potential ability of the common carbonyl regions to serve as "charge-transfer" bridges between a tyrosine hydroxyl group (left) and an acidic imminium hydrogen of arginine (right).

by 5-(3,4-dialkoxyphenyl)thiazolidine-2,4-dione **27** of this series.

Several nonsteroidal antiinflammatory agents have also been reported to inhibit aldose reductase.[77,87,88] These include salicylic acid (**28**), sulindac (**29**), and its human sulfide metabolite **30**. These agents, however, display only weak inhibitory activity in animal studies.

Nonsteroidal Anti-inflammatory Agents



28 **29** X = S
30 X = SO

Common Molecular Features of ARIs. Although aldose reductase inhibitors appear to be diverse, certain common electronic and steric features have been elucidated with the aid of computer modeling and molecular orbital calculations. Through the use of simple Huckel and CNDO/2 molecular orbital calculations, it has been suggested that ARIs inhibit aldose reductase through a charge-transfer interaction.[68,71,78] These calculations indicate that ARIs generally contain a group (such as carbonyl, thiocarbonyl, etc.) that is capable of undergoing reversible nucleophilic attack. Moreover, a common molecular conglomerate can be formed by superimposing specific aromatic residues common to most inhibitors. This consists of a generally planar structure with two hydrophobic (aromatic) regions and a common region which is susceptible to reversible nucleophilic attack (Figures 2A,B and 3B).

Kinetic and competition studies with purified enzyme indicate that ARIs reversibly interact with aldose reductase at a common site independent of either the substrate or nucleotide cofactor fold. This site appears to be stereospecific and contains a nucleophilic residue.[81,71] Moreover, the steric requirements of this site may vary with enzyme from different tissues and species.

Proposed Inhibitor Site Model. From the ARI conglomerate, it has been proposed that ARIs may bind to the

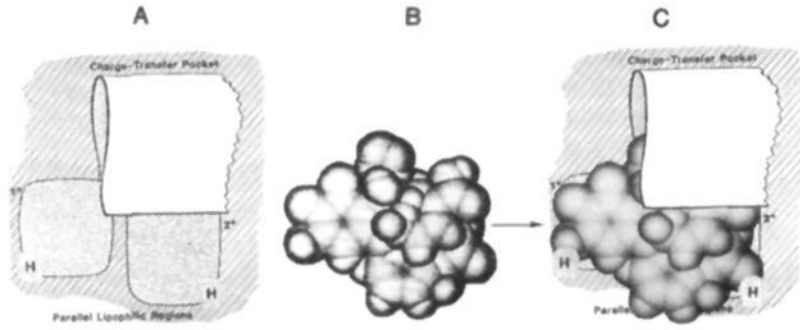


Figure 3. Schematic diagram of the proposed aldose reductase inhibitor site (A) and the common molecular conglomerate formed by selected ARIs as viewed from side opposite from Figure 2B (B). C illustrates the possible "interaction" between this conglomerate and the proposed site.

enzyme at a site as schematically presented in Figure 3.[71] According to this model, inhibitor site attachment should result from a combination of hydrophobic bonding and a reversible charge-transfer reaction. Assuming that a compound with higher affinity for a receptor is more potent, the inhibitory potency of an ARI would be expected to increase with either the addition of selective lipophilic substituents which could enhance overall hydrophobic interaction or by the addition of groups which could increase charge-transfer capabilities. This lipophilic factor has been observed in the SAR of various ARIs, including alrestatin (**3**), sorbinil (**22**), and various flavonoids.[76,80,89] Hydrogen bonding sites located adjacent to the two lipophilic regions also appear to help orient an inhibitor containing hydroxyl groups onto this site. These sites correspond to regions encompassed by hydroxyl groups at the 7-position on the 4-oxo-4*H*-chromene ring and the 2-(4-hydroxyphenyl) position and methylation of these hydroxyl groups results in decreased inhibitory potency.[64,71] The potential importance of these groups in inhibitor orientation is further illustrated in Table I in which compounds containing the specified hydroxyl groups lose their ability to inhibit purified human placental aldose reductase less rapidly than those inhibitors which primarily interact through hydrophobic binding. A possible explanation for this observation assumes destabilization during purification of the aldose reductase protein with some unfolding of the lipophilic regions at the inhibitor site while the adjacent hydrogen bonding regions are unaffected. As illustrated in Figure 4, for the rigid spirohydantoin sorbinil which binds through hydrophobic interaction (A), such an unfolding could result in altered charge-transfer geometry (B) whereas this geometry could be maintained for compounds such as quercetin, which possesses the appropriate hydroxyl groups (C, D).

Depending upon the concentration of inhibitor available, ARIs inhibit either uncompetitively or noncompetitively. Therefore, one can assume that the interaction between ARI and enzyme could result either in a reversible conformational change, before or after the binding of substrate and nucleotide, or in steric interference of the catalytic site produced by partial overlapping of bound inhibitor with the catalytic site. Both possibilities can involve charge transfer. What, however, is the possible nature of the charge-transfer interaction?

Simple ionic interaction between a negatively charged region of the ARI and the aldose reductase enzyme could account for such a charge-transfer interaction since a majority of ARIs possess either carboxylic acid functions or are 5-substituted hydantoins which are negatively charged at physiological pH. If that is the case, then esterification of the acidic moiety should result in de-

(87) Jacobson, M.; Sharma, Y. R.; Cotlier, E.; Hollander, J. D. *Invest. Ophthalmol. Visual Sci.* **1983**, *24*, 1426.
(88) Dvornik, D. M.; Simard-Duquesne, N. Belgium Patent BE 885368, 1981.
(89) Sestanj, K. U.S. Patent 27 46 244, 1981.

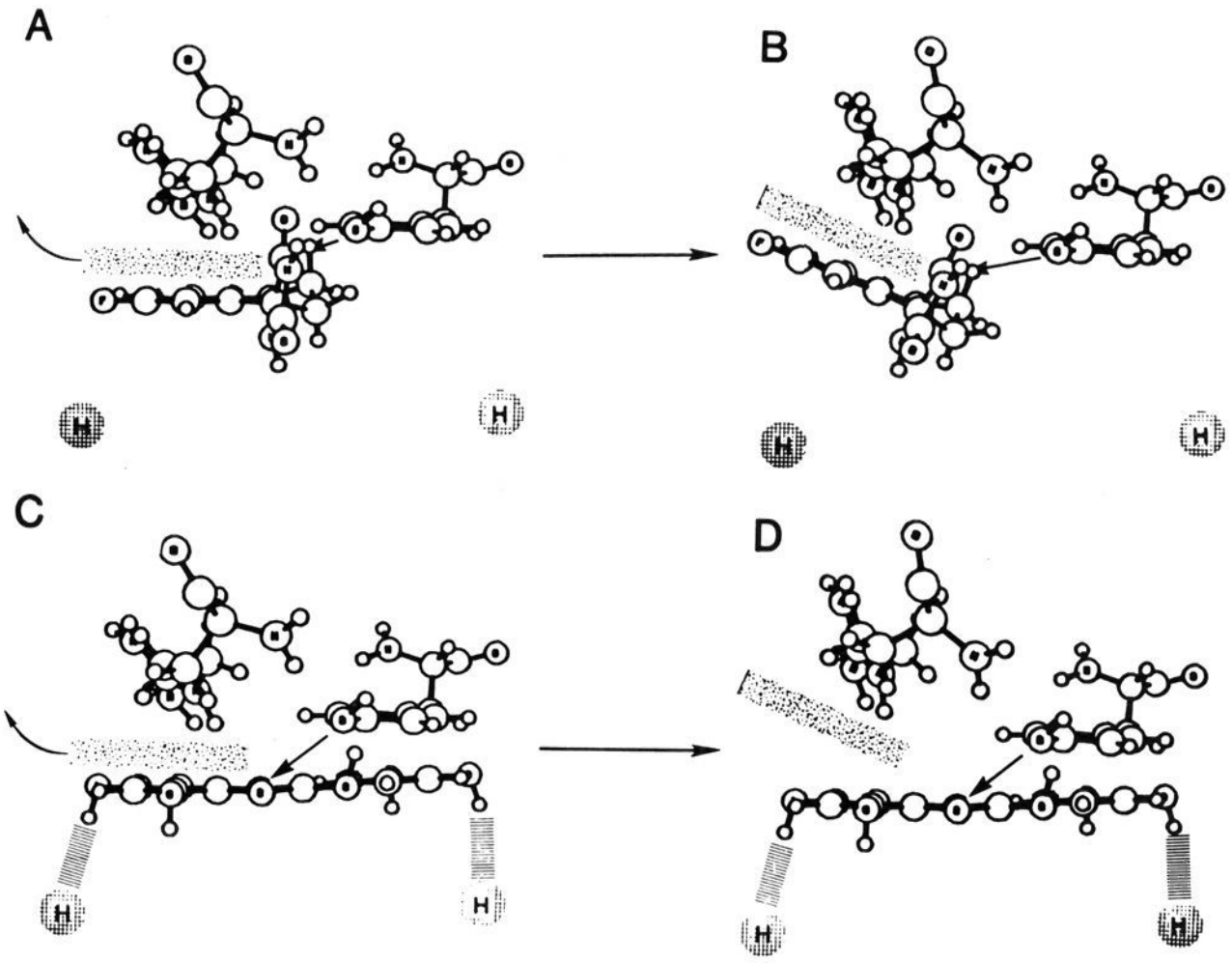
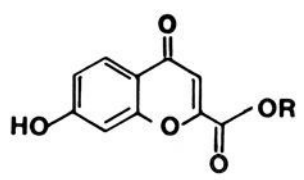


Figure 4. Schematic representation of the posential effect of conformational changes on the binding of ARIs to the proposed inhibitor site (Figure 3). For compounds such as the rigid spirohydantoin sorbinil (**22**) (A), which binds chiefly through lipophilic interaction to the primary hydrophobic region, slight perturbation of this site during enzyme purification can result in a significant change in the geometry required for "charge transfer". Inhibitors such as quercetin (**7**), which can be oriented onto the inhibitor site through hydrogen bonding of its 4′- and 7-hydroxyl groups with two distinct regions near this site, may not be affected by such a perturbation.

Table IV. Inhibitory Activity of 7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic Acid and Its Ethy, Isopropyl, and Benzyl Ester[a]



R	IC_{50} μM	
	RLAR	HPAR
H	2.2	1.8
Et	2.3	5.0
iPr	3.1	4.3
Bzl	2.5	3.2

[a] Evaluated in vitro against rat lens aldose reductase (RLAR) and human placental aldose reductase (HPAR).

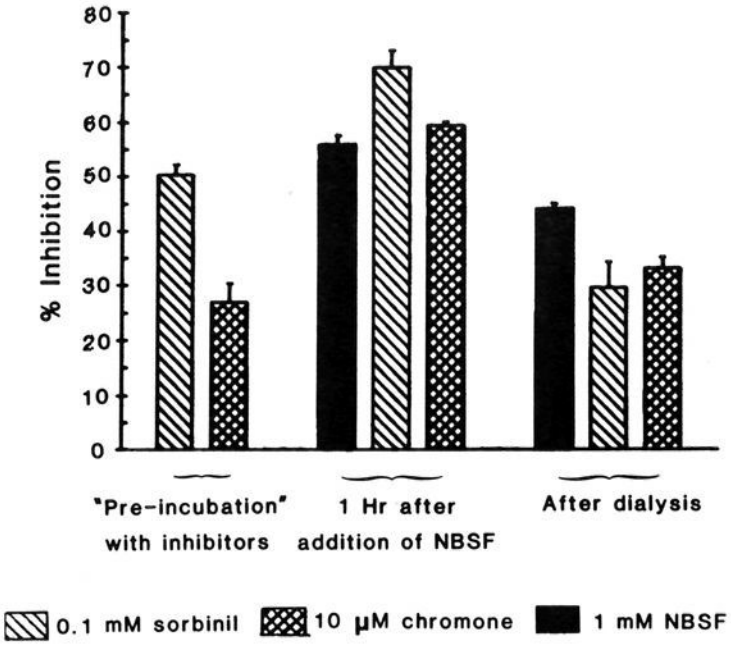
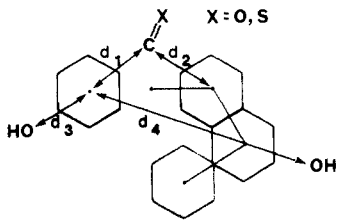


Figure 5. Competition studies of 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (**12**), sorbinil (**22**), and 4-nitrobenzenesulfonyl fluoride (NBSF, Pierce Chemical Co.) with purified human placental aldose reductase. The enzyme, purified by affinity chromatography,[52] was dialyzed against 0.1 M Na,K–phosphate buffer (pH 7.2) and separated into four groups. To two groups was added 0.1 mM sorbinil and 10 μM chromone followed after 30 min by addition of 1 mM NBSF in 5% Me_2SO. To the third group only 1 mM NBSF in 5% Me_2SO was added while 5% Me_2SO was added to the fourth, control group. After 60 min, the solutions were dialyzed through Sephadex G-25M (Pharmacia Fine Chemicals). Enzyme activities were spectrophotometrically assayed with use of glyceraldehyde as substrate.[68] All points represent the means of four to six determinations.

creased charge-transfer interaction and decreased inhibitory activity. This is not observed in the in vitro inhibition of aldose reductase with esters of 7-hydroxychromone-2-carboxylic acid (Table IV). On the other hand, molecular orbital calculations of charge distributions in the ground state, combined with molecular modeling, indicate the presence of a certain common region corresponding to the 4-carbonyl of the chromone ring system and the 4-carbonyl of 5-substituted hydantoins which is susceptible to reversible nucleophilic attack (Figure 2B).[71] In 5-substituted hydantoins the reactivity of this 4-carbonyl to nucleophilic base attack is increased with ionization of the 3-position imide.[90] The potential importance of such a carbonyl group has been further demonstrated in a series of oxazole derivatives where it was concluded that the presence of a carbonyl group appears to be necessary for the interaction of inhibitor with enzyme. In this series, inhibitory activity was influenced by the distance between the aromatic oxazole nucleus and

(90) Aspelund, H.; Waselius, P. *Acta Acad. Abo., Ser. B* **1967**, *27*, 18.



Approximate Distances

d_1	2.8–3.8 Å
d_2	2.8–6.1 Å
d_3	2.8–3.8 Å
d_4	8.0–9.3 Å

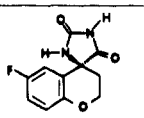
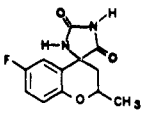
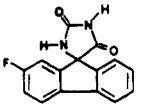
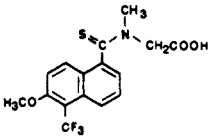
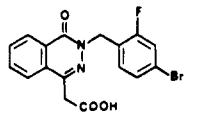
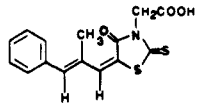
Figure 6. Proposed pharmacophor requirements of ARIs.

carbonyl substituent at the C-2 position.[91] This common carbonyl region appears to interact with a nucleophilic amino acid, tentatively identified as tyrosine, which has been detected at the common inhibitor site through studies with protein modification agents.[71] Competition studies in Figure 5 illustrate that this nucleophilic residue can be irreversibly alkylated with the tyrosine-specific reagent 4-nitrobenzenesulfonyl fluoride (NBSF) and that this alkylation is decreased by preincubation with the reversible ARIs sorbinil (22) or 7-hydroxychromone-2-carboxylic acid (12). Thus all three appear to compete for the same nucleophilic residue.

Such a nucleophilic attack by the hydroxyl group of tyrosine could simply result in the formation of a reversible tetrahedral intermediate. Alternatively, the hydroxyl group could form a reversible "charge-transfer" bridge between the tyrosine moiety and an "acidic" residue so that long-distance interaction leading to conformational perturbation can occur. This is illustrated in Figure 2C for tyrosine and the "acidic" iminium hydrogen of arginine, both of which were arbitrarily positioned in real time on the Evans and Sutherland system. In such a position, the aromatic ring of tyrosine could account for hydrophobic binding attributed to the secondary lipophilic bonding site. No evidence currently exists for such an interaction between tyrosine and arginine and its inclusion here is only for illustration of the charge-transfer concept. Arginine, however, has been identified near the nucleotide fold where it is believed to aid in the alignment of the NADPH cofactor.[71,72] For example, slight perturbation of this arginine could conceivably result in misalignment of the NADPH cofactor and subsequent inhibition. More specific studies are required in order to elucidate the charge-transfer mechanism.

From the location of the lipophilic binding regions and the charge-transfer pocket, stereochemical recognition of the inhibitor site is possible. Moreover, the concept of bulk tolerance has been introduced into this model through a sterically constrained charge-transfer pocket. From this model the minimum pharmacophor requirements for an aldose reductase inhibitor have been proposed to consist of a planar lipophilic (aromatic) region separated by approximately 2.8–3.8 Å (center to center) from a group susceptible to nucleophilic attack (Figure 6). Introduction of a broad secondary hydrophobic/lipophilic region, coplanar with the primary lipophilic region, located 2.8–6.1 Å from the group susceptible to nucleophilic attack, enhances hydrophobic binding to the aldose reductase inhibitor site. Similarly the location of two hydroxyl groups 2.8–3.8 and 8.9 and 9.3 Å from the center of the primary lipophilic region can enhance binding to this site. Since this initial description two new ARIs, tolrestat (4) and alconil (24), have been reported. Both of these compounds conform to the apparent pharmacophor requirements as outlined above. In addition, the recently reported crystal structures of *N*-[[6-methoxy-5-[(trifluoromethyl)thio]-1-naphthalenyl]thioxomethyl]-*N*-methylglycine, an analogue of tolrestat (4), and sorbinil also closely correspond the above requirements.[92,93] Much more work, however, is required in order to define the specific spatial and functional requirements for an ARI.

Table V. ARIs Currently Undergoind Clinical Trials

structure	name	company
	CP 45,634 Sorbinil	Pfizer
	M 79,175	Eisai
	AL 1567 Alconil	Alcon
	AY 27,773 Tolrestat	Ayerst
	ICI 128,436 Statil	I.C.I. Ltd.
	ONO 2235	ONO

ARI Therapy for Diabetic Pathology—Intervention or Prevention?

With the rapid development of ARIs several compounds are now undergoing clinical trials (Table V). Unlike the development of other drugs, however, animal studies have not unequivocally demonstrated the potential efficacy of ARIs against specific diabetic complications such as retinopathy, neuropathy, microangiopathy, or possibly nephropathy. This is due in part to the long onset of many of these complications, the lack of appropriate or convenient animal models, and the lack of understanding of the specific role that the polyol plays in the pathogenesis of many of these diabetic complications. Clinical observations during these trials are, however, providing impetus for more basic studies and for the development of new animal models and pharmacological tests. For example, the favorable effect of ARIs on motor nerve conduction velocities (MNCV) was clinically demonstrated prior to the demostration of a similar effect in rats.[13,94]

(91) Tanimoto, T.; et al. *Chem. Pharm. Bull.* **1982**, *32*, 1032.

(92) Varughese K. I.; Przyblska, M.; Sestanj, K.; Bellini, F.; Humber, L. G. *Can. J. Chem.* **1983**, *61*, 2137.

(93) Kissinger, C. R.; Adman, E. T.; Clark, J. I.; Stenkamp, R. E. American Crystallographic Association Meeting, Lexington, KY, 1984, Paper PC22, p 55.

From a practical standpoint, current clinical trials are evaluating the ability of ARIs either to prevent the progression or reverse the presence of clinically observed pathology. A major anticipated problem with this approach, however, is that the timing of intervention may not be optimal. Checking the progress of diabetic pathology may not be possible once the pathological process has progressed to a certain point. Moreover, the dosage of inhibitor at that point may not be adequate. Thus, despite the fact that aldose reductase may be involved in the initiation of the pathology, the clinical trials with ARIs may not demonstrate this point. Intervention studies conducted on cataract formation in galactosemic rats suggests such a possibility.[36] While cataract formation can be essentially prevented through administration of ARI concomitant with the galactose diet, the "reversal" of the cataractogenic process can only be accomplished through either the removal of the high galactose diet or the administration of an adequate dose of ARI prior to the sixth day of galactose feeding. After the sixth day a point of no return was reached in the reversal of the cataractous process with the vacuolar stage continuing on to the dense nuclear opacity.

The galactose cataract study indicates the required administration of ARI at the initial onset of the pathogenic process, and to date, most animal studies with ARIs have stressed prevention of diabetic pathology rather than intervention. These animal studies suggest that ARIs should eventually be clinically administered at the onset of diagnosed diabetes on a prophylactic basis. For such long-term administration ARIs with extremely low toxicity and no side effects will be needed.

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Registry No. Aldose reductase, 9028-31-3.

(94) Judzewitsch, R.; et al. *Diabetes* 1981, *30* (suppl 1), 30A.

Articles

A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules

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The interaction of a probe group with a protein of known structure is computed at sample positions throughout and around the macromolecule, giving an array of energy values. The probes include water, the methyl group, amine nitrogen, carboxy oxygen, and hydroxyl. Contour surfaces at appropriate energy levels are calculated for each probe and displayed by computer graphics together with the protein structure. Contours at negative energy levels delineate regions of attraction between probe and protein and are found at known ligand binding clefts in particular. The contours also enable other regions of attraction to be identified and facilitate the interpretation of protein–ligand energetics. They may, therefore, be of value for drug design.

Several attempts have been made to use the atomic structures of biological macromolecules in order to discover novel therapeutic agents.[1] The novel molecules are designed so that they should fit onto an appropriate protein whose structure has been previously determined by X-ray crystallography. However, this fitting process is not just a matter of simple geometry because the chemical properties of the individual atoms are critically important, and the goodness of fit may be assessed by predicting the energetics of the drug–protein system instead of its shape.[2] A method is now described in which energy contour surfaces are displayed in three dimensions on a computer graphics system together with the macromolecular structure, so that energy and shape can be considered simultaneously when designing drugs.

The idea of small hard atoms goes back to Democritus and Lucretius, but it was van der Waals who used the Joule–Thompson effect to show that there are attractive forces between atoms. The combination of attractive with repulsive forces has led to a number of simple functions[3,4] that can display energy minima corresponding to preferred nonbonded arrangements of atoms and molecules, and the (12,6) Lennard–Jones potential (Figure 1) is such a function:

$$E_{lj} = A/d^{12} - B/d^{6} \quad (1)$$

In this equation d is the distance between a pair of nonbonded atoms whose Lennard–Jones energy E_{lj} is described by the parameters A and B. When d is small, the A/d^{12} term generates a dominating repulsion corresponding to a large positive value of E_{lj}. This effectively defines a minimum separation that can be apportioned between the atoms, giving each of them a nominal radius, and the threshold step function established in this way can be extended to larger systems, thus determining a molecular surface that may be measured[5] or displayed by computer graphics.[6] Alternatively, a somewhat bigger value of d may be used, corresponding to the minimum value of E_{lj} at the

(1) Goodford, P. J. *J. Med. Chem.* 1984, *27*, 557.
(2) Richards, W. G. "Quantum Pharmacology", 2nd ed.; Butterworths: London, 1983.
(3) Buckingham, R. A. *Proc. R. Soc. London, A* 1938, *168*, 264.
(4) Jones, J. E. *Proc. R. Soc. London, A* 1924, *106*, 463.
(5) Lee, B.; Richards, F. M. *J. Mol. Biol.* 1971, *55*, 379.
(6) Blaney, J. M.; Jorgensen, E. C.; Connolly, M. L. Ferrin, T. C.; Langridge, R.; Oatley, S. J.; Burridge, J. M.; Blake, C. C. F. *J. Med. Chem.* 1982, *25*, 785.

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