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Aldose reductase structures: implications for mechanism and inhibition

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Abstract. During chronic hyperglycaemia, elevated vascular glucose level causes increased flux through the polyol pathway, which induces functional and morphological changes associated with secondary diabetic complications. Inhibitors of aldose reductase (ARIs) have been widely investigated as potential therapeutic agents, but to date only epalrestat is successfully marketed for treatment of diabetic neuropathy, in Japan. Promising compounds during in vitro studies or in trials with animal models have failed to proceed beyond clinical trials and to everyday use, due to a lack of efficacy or adverse side effects attributed to lack of inhibitor specificity and likely inhibition of the related aldehyde reductase (ALR1). Knowledge of the catalytic mechanism and structures of the current inhibitors complexed with ALR2 are means by which more specific and tightly bound inhibitors can be discovered. This review will provide an overview of the proposed catalytic mechanism and the current state of structure-based drug design.

Key words. Aldose reductase; mechanism; inhibitors; drug design; aldehyde reductase.

Introduction

Both insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) cause some form of chronic complications. The organs affected include the eye, kidney and central nervous system, exhibiting complications such as nephropathy, retinopathy, neuropathy and angiopathy [1–4]. The use of insulin treatment and strict glycaemic control has been shown to delay or prevent the onset of these complications [5–7]. However, blood glucose levels cannot be maintained at fully normal levels, and with the added risk of hypoglycaemia, it is important to search for alternative treatments for these complications.

Hyperglycaemia is regarded as the primary instigator for the pathogenesis of diabetic complications. To explain the relationship between hyperglycaemia and the resulting complications, four factors have been postulated [8]. Briefly, these factors are formation of reactive oxygen species (ROS), elevated activity of aldose reductase, activation of protein kinase C (PKC) isoforms and increased formation of advanced glycation end products. Each of these mechanisms has been targeted for therapeutic intervention [9–12], but this review will concentrate on efforts targeting the elevated activity of aldose reductase.

Aldose reductase (ALR2; EC 1.1.1.21), which is the first enzyme in the polyol pathway, converts glucose to sorbitol in the presence of NADPH as cofactor. The pathway is completed by the second enzyme, sorbitol dehydrogenase, which converts sorbitol to fructose with NAD^+ as cofactor. During a hyperglycaemic event, the elevated glucose level enhances the activity of aldose reductase by directly increasing the glucose flux through this pathway and indirectly by formation of ROS, which activates ALR2 [10]. The increased activity of ALR2 results in the decrease in the $NADPH/NADP^+$ ratio and impacts other NADPH-dependent enzymes, such as nitric oxide (NO) synthase and glutathione reductase [13–15]. The reduction of NO levels decreases nerve conduction and causes microvascular derangement. The retarded activity of the antioxidative enzyme glutathione reductase causes oxidative stress under diabetic conditions. In the peripheral nerve, reduced activity of these enzymes could lead to lower blood flow, tissue hypoxia and ultimately functional and structural lesions. Increased sorbitol flux through the polyol pathway compounds the problem by

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causing an increase in the NADH/NAD^+ ratio, which blocks the glycolytic pathway at the triose phosphate level. Consequently, PKC is activated, and formation of advanced glycation end products is increased. Advanced glycation end products are toxic, cause pathological changes by disrupting protein function and interfering with cellular receptors, and have been implicated in the etiology of diabetic complications [12].
Inhibitors against enzymes, such aldose reductase and protein kinase C, and compounds, such as aminoguanidine, that prevent the formation of advanced glycation end products are thought to be beneficial in alleviating or averting diabetic complications. Considerable research efforts have been expended to discover such compounds, which have been extensively reviewed in the literature [9, 16–19], particularly inhibitors against aldose reductase. This review will summarise the various types of ALR2 inhibitors (ARIs) and will examine in depth the use of drug design for developing more potent and specific ARIs. It is worth noting, however, that certain ARIs may not just function as intended, as inhibitors of ALR2, but may also function as inhibitors of phosphodiesterase, in a process that could have a positive impact on nerve conduction velocity [20]. This would be of significant interest, as increased activity in the polyol pathway does not appear to be the primary cause for diabetic neuropathy, which in part would account for the lack of efficacy of inhibitors in clinical trials.
Over nearly 2 decades, a number of promising ARIs have been discovered that showed significant activity in vitro and in animal models [9, 21, 22], but only one, epalrestat, has made it to clinical use. All the other compounds, for example sorbinil and ponalrestat, which have reached the stage of human clinical trials have been withdrawn because they have poor pharmacokinetic properties, resulting in the lack of efficacy, or they have demonstrated adverse side effects [22–25]. It is now widely believed that inhibition of the closely related aldehyde reductase (ALR1; EC 1.1.1.2), which also belongs to the same aldo-keto reductase (AKR) superfamily as ALR2, may play an important role in the development of adverse side effects [26–27]. ALR1 is responsible for the reduction of many aldehydes and metabolises compounds such as methyl glyoxal and 3-deoxyglucosone, which are intermediates in the formation of advanced glycation end products. Many ARIs have also been shown to be inhibitors of ALR1 [26], consequently impeding the proper functioning of the latter. The lack of inhibitor selectivity may be addressed through combinatorial chemistry and high-throughput screening. It may also be addressed by the use of structure-based drug design, principally by designing inhibitors that will bind specifically to ALR2 but not the homologous ALR1.
An understanding of the catalytic mechanism is an important preamble to the development of ARIs. This would involve determination of residues involved in enzymatic catalysis, which in turn would elucidate the residues that interact with inhibitor molecules within the active site. Armed with information from these analyses, the probability of a successful inhibitor design protocol would be greatly enhanced. The following sections will provide the current state of understanding of the catalytic mechanism, the various categories of currently available ARIs, the site of inhibitor binding and the residues that are involved, and the use of structure-based and de novo inhibitor design in the drug development strategy.

Identification of the residues involved in the enzymatic mechanism

Prior to the determination of the ALR2 crystal structure, the roles of different residues were studied by site-directed mutagenesis. A 66-fold increase in the value of K_m and a change in k_{cat} that resulted from a mutation of Lys262 to Met led Bohren et al. [28] to conclude that the catalytic action was affected by changes in the cofactor binding site. In 1992, Petrash et al. [29] carried out site-directed mutagenesis of cysteine residues to serine, which showed a big change in the catalytic efficiency of the C298S mutant with sorbinil but not tolrestat. Furthermore, three other solvent-accessible cysteines were found to be not essential for catalytic activity. The authors proposed the existence of two binding sites, with sorbinil bound to a site near Cys298, while tolrestat was bound to the other site. Recent ultra-high-resolution studies [30] have shown that there were two possible conformations of Cys298 with H-bonds to the nicotinamide ring of $NADP^+$, but only one with NADPH. The effect of a Cys298 mutation on the catalytic efficiency is rationalised in terms of the stabilisation of the $NADP^+$ through the extra H-bond to the C4 (hydrogen donor).
Also in 1992, the first two ALR2 structures were published. Wilson et al. [31] published the structure of human placenta ALR2 (refined to 1.65 Å, PDB entry: 1ads), and Rondeau et al. [32] the one of porcine ALR2. This was followed in 1995 by porcine ALR1 [33]. These structures were composed of similar α/β barrels, which are still the most prevalent motif for enzymes, with a core of eight parallel β strands connected by peripheral α helices. The $NADP^+$ coenzyme molecule was bound to the carboxyl terminus of the β barrel in an extended conformation. The nicotinamide ring was centred in the active site cavity. This highly hydrophobic active site pocket is formed by aromatic residues (Trp20, Tyr48, Trp79, Trp111, Phe121, Phe122 and Trp219); apolar residues (Val47, Pro218, Leu300 and Leu301) and polar residues (Gln49, Cys298 and His110). This cavity did not bind sugars with any high degree of specificity since aromatic compounds were better substrates. Wilson et al. [31] identified three possible

proton donors, which were Tyr48, His110 and Cys298. His110 had neighbouring hydrophobic residues and was nonprotonated; thus it could not be a proton donor. Cys298 was not conserved in the whole enzyme superfamily and hence omitted. Tyr48 was the favoured candidate due to its interaction with Lys77, which in turn is salt linked to Asp43, which could help in the proton donation.

In 1993, the structure of the ALR2-zopolrestat complex was solved at 1.8 Å resolution [34] (PDB entry: 1mar). Comparison with the holoenzyme structure [31] showed conformational changes in residues 121–135 and 298–305 to facilitate the binding of the inhibitor. When this ARI was complexed, the solvent-accessible area of the ALR2-ARI complex was only 6.4% of that available to ALR2 with the unbound zopolrestat. Most of the van der Waals contacts in the ALR2-ARI complex were between carbon atoms that showed hydrophobic characteristics. The residue with the most number of contacts was Trp111. The benzothiazole ring of zopolrestat was located between this residue and Leu300. The phthalazinyl ring was positioned between Trp20 and Phe122. These four residues comprised half of all the enzyme-inhibitor contacts, which explained the preference of ALR2 for hydrophobic substrates. The authors concluded that Tyr48 (2.65 Å distance to the acceptor oxygen of the ARI) was the residue in the better position for proton donation than His110 (2.89 Å from the ARI carboxylate oxygen that may mimic the carbonyl oxygen of a substrate). The extended configuration of NADPH, as previously reported [28], showed that Lys262 was involved in contacts with the phosphate groups of NADPH. However, the interaction between Cys298 and the nicotinamide ring of the coenzyme was not mentioned. Bohren et al. [35] then performed site-directed mutagenesis studies by preparing Y48F, Y48H, Y48S, H110Q, H110A and K77M mutants. All mutants experienced a significant loss in activity, with the His mutants affected the least. However, the His mutant also showed different substrate specificity, which suggested that this residue was necessary for the recognition of polar substrates. Furthermore, mutation of this residue resulted in a loss of stereoselectivity between D-xylose and L-xylose or between D-xylose and D-lyxose, which led the authors to conclude that His110 played an important role in directing the stereochemical orientation of polar substrates and was thus not the proton donor. The Y48F mutant was found to be inactive, while the Y48H mutant showed a several orders of magnitude loss in catalytic efficiency. A structure of the Y48H mutant, obtained at 1.76 Å resolution using citrate as the substrate, showed no differences with the structure of wild-type protein. The residual activity could be rationalised in terms of the proton donor being a water molecule that occupied the space of the hydroxyl group of the Tyr48 residue in the wild-type enzyme. Based on these arguments, Bohren et al. [35] concluded that Tyr48 was the proton donor.

In 1997, crystal structures of porcine ALR2-tolrestat and ALR2-sorbinil complexes showed both inhibitors bound in the same active site with the approximate dimensions of 4 Å × 15 Å × 15 Å [36]. The existence of a specificity pocket that involved the residues Trp111, Phe122 and Leu300 was established. This pocket was shown to be closed in the ALR2-sorbinil complex but opened in the ALR2-tolrestat complex, which allowed tolrestat to bind within this pocket, albeit in a different conformation to the ALR2-zopolrestat structure [34]. The W111A mutant decreased the inhibition by 400-fold for zopolrestat but the W111Y mutant had no effect, which suggested that the effects of the Ala mutation were due to changes in the van der Waals interactions of the ALR2-ARI complex [37]. The most significant change in activity was observed for the W20A mutant, even though the number of eliminated van der Waals contacts was less than that for the W111A mutation. The same results were found with other inhibitors (tolrestat, ponalrestat, sorbinil and imirestat), which demonstrated that binding activity was not governed by the number of contacts.

In 1998, Lee et al. [38] analysed the binding modes of ARIs to the active site by means of computer calculations. Starting with the 1.8 Å-resolved structure of Wilson et al. [34] and the assumption of a double-protonated His110, different inhibitors were manually docked into the active site where the ionised group of the ARI could form H-bonds with Tyr48 and His110. Minimisation of the system was then carried out using the program CHARMM [39]. The authors concluded that the ARIs must have an ionised portion that interacted with Tyr48, His110 and Trp111, along with one of two aromatic portions that interacted with Trp20, Trp111 and Phe122, whereas the second was placed between the Trp111 and Leu300 residues of the specificity pocket. Based on the same assumption of a double-protonated His110, with hydrogen atoms at the Nδ1 and Nε2 positions, the catalytic mechanism was studied with a combination of quantum mechanics (QM) and molecular mechanics (MM) techniques [40] using the programs GAMESS [41] and CHARMM. In the minimised structures, His110 Nε2 as the proton donor was favoured not only by a shorter distance to the substrate acceptor oxygen but also by 8.7 kcal/mol in the energetics of transition states to product formation, when compared with Tyr48 as the proton donor. This difference was attributed to the unfavourable formation of the tyrosinate anion. The authors ascribed the complete loss of enzymatic activity when Tyr48 or Lys77 were mutated to the breaking of the crucial hydrogen bond network involving these residues and the substrate.

In 1998, Schlegel et al. [42] proposed a 'push-pull' mechanism applicable to all the aldo-keto reductase family members. The similar K_d values for NADPH in all cases of wild-type enzyme and mutants (Y55F, Y55S, K84M,

K84R, D50N, D50E, H117A) implied that cofactor binding was not affected by different mutations. Only the His117 mutant was active in the oxidation study, while other mutants were inactive in both oxidation and reduction studies. Accordingly, the authors assigned dual roles of a general acid and a general base to Tyr55, which donated a proton via His117 in the oxidation direction, while Lys84 facilitated proton removal in the reduction direction (fig. 1).

In 1999, Varnai et al. [43] performed another QM-MM study, using CHARMM followed by exploration of the potential energy surface using the restrained distance (RESD) method, coupled with a grid calculation. For both Tyr48 and His110, the case where an initial hydride transfer preceded the proton donation required significantly lower energy than the case that involved a proton donation before the hydride transfer. The lowest-energy pathway involved a hydride transfer followed by a proton donation from Tyr48.

Bohren et al. [44] reported stopped-flow kinetic data, which showed that the negatively charged sorbinil was bound to the ALR2-NADP$^+$ but not the ALR2-NADPH complexes. This result disagreed with those obtained from previous fluorescence studies [45].

Singh et al. [46] studied the role of Trp20 and Trp111 by constructing mutants (W20A, W20Y, W111A and W111Y) based on a refined structure of the ALR2-zopolrestat complex of Wilson et al. [34]. Using the AMBER 4.0 program [47], the minimised structures were docked into the active site, and free-energy calculations carried out. Energetic differences for the W20Y, W111Y, W20A and W111A mutants (1.0, 4.0, 10.5 and 14.0 kcal/mol, respectively) agreed with experimental energetic differences. The changes in the carbon atom surface area, determined using the IMPACT program [48], and the calculated average interaction energies, correlated well with the IC_{50} (concentration of the inhibitor that produced half-maximal effect) values of wild-type and mutant ALR2-zopolrestat complexes. The W20 mutation affected inhibitor affinity and substrate binding, while the W111 mutation caused loss of interaction between the benzothiazole ring of zopolrestat and the indole ring of tryptophan, which showed the importance of the specificity pocket for inhibitor binding.

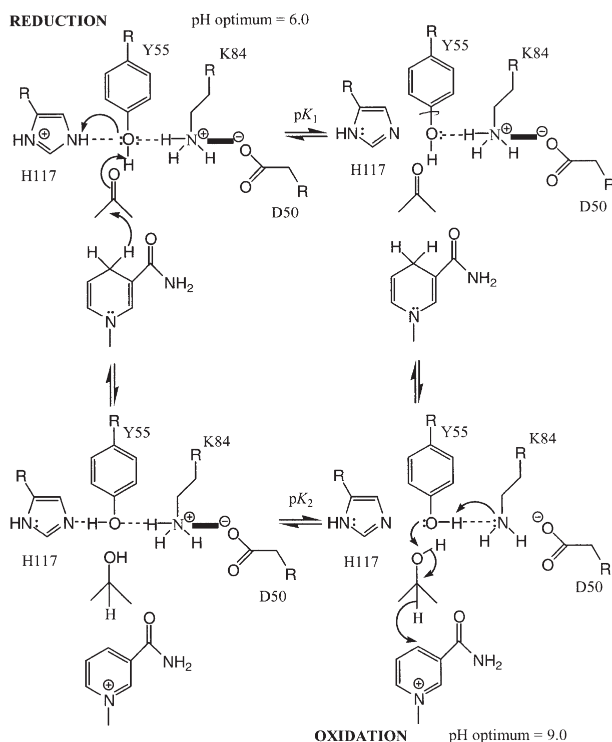

Figure 1. 'Push-pull' mechanism proposed by Schlegel et al. [42]. Enzymatic activity in both directions is summarised.

In 2000, Varnai and Warshel [49] carried out a study with computer simulations, using the structure of the holoenzyme [31]. The pKa and free energy obtained for His110 (1 and 8.7 kcal/mol, respectively) showed that His110 was not protonated at the active site and was also not the proton donor. Conversely, the values for Tyr48 (8.5 and –2.2 kcal/mol, respectively) showed that Tyr48 was the proton donor, as the tyrosinate anion was stabilised in the protein environment, where Lys77 could be involved. A 3.6 kcal/mol destabilisation of the tyrosinate anion and a 5.8 kcal/mol increase in the energetic barrier of proton transfer for the K77M mutant supported this involvement. The empirical valence bond (EVB) method [50], coupled with free energy perturbation (FEP) technique [51], was used to obtain the energy surface of the reaction. In considering a hydride transfer preceding a proton donation, the reaction in the protein showed a 3 kcal/mol reduction in the initial energetic barrier, but the intermediate was less stable by 8 kcal/mol than in water. This finding was consistent with its role in catalysing the nonspecific reduction of a wide range of carbonyl compounds.

A very high resolution structure, refined to 0.66 Å [30], contained 54% of all H-atoms in the complex, which allowed the hydrogen bond network in the active site to be determined. At pH 5.0, where the crystals were obtained, a singly protonated His110 at the Nε2 position made a hydrogen bond with the carboxylate of the ligand (IDD594). This orientation disagreed with the push-pull model, in which this hydrogen had to point to Tyr48 [42]. The Tyr48 hydroxy group was polarised by Lys77 as pointed out by Wilson et al. [31]. Different carbon atoms of NADP$^+$ hydrogen bonded with the two conformations of Cys298, one of which was involved in the hydride donation step of the enzymatic mechanism. The loss of catalytic activity found by Petrash et al. [29] was due to the interaction between the cysteine and the cofactor, not with the substrate.

Cachau et al. [30] published the first model of the catalytic mechanism using high-resolution crystallographic data.

The salient point, a singly protonated His110 at the Nε2 position, which was directed towards the carboxylate group of the ARI, was consistent with experimental findings. A new reaction path was obtained, in which the proton source was the Tyr48 residue but the His110 acted as a proton shuttle (fig. 2). After the donation from Tyr48, the $His110^+$ intermediate formed then released the proton to the substrate along with a hydride transfer from NADPH. The flexibility of His110 was necessary for this mechanism and agreed with the greatest experimental RMSD value of 1.2 Å, compared with the RMSD value of 0.45 Å for Tyr48. A water bridge and correlation of movements between His110 and Lys77 oriented the lysine that polarized Tyr48, which explained the loss of the catalytic activity in Lys77 mutants. The importance of Asp43 was evident as a salt bridge replaced the initial interaction of Lys77 with $NADP^+$ after the reaction. The resulting deprotonated Lys77 was unstable; the reprotonation could then be possible through the water channel that ended at Asp43. This recharging process could be involved in the ejection of the $NADP^+$ from the reactive centre.

Recently, a new step in the understanding of ALR2 enzymatic activity was achieved with crystals of IDD552 that were obtained from pH 8 at IGBMC [A. Podjarny, personal communication]. They showed a new conformation in which Tyr48 (with its oxygen located at 2.56 Å from the carboxyl oxygen of the inhibitor) was in a better position for proton donation.

Classes of ARIs

A list of the most commonly available inhibitors is shown in figure 3. Generally, these inhibitors contain either a cyclic imide group, such as a spirohydantoin group [52] or a spirosuccinimide group [53], or an acetic acid moiety [54]. The best known of the spirohydantoin-containing compounds, sorbinil, has been thoroughly analysed by structural analysis of the ternary structure with ALR2 and NADPH, and several clinical studies. Other examples of this group include fidarestat (SNK 860) and its stereoisomers. Minalrestat (WAY-509) and AS-3201 are examples of spirosuccinimide-based compounds. There are numerous compounds that incorporate the acetic acid moiety, and they include tolrestat, ponalrestat (statil) and zopolrestat. It is noted that the cyclic imide or acetic acid moieties bind to an essentially hydrophilic area of the active site of ALR2, which contains the Tyr48, His110 and Trp111 residues.

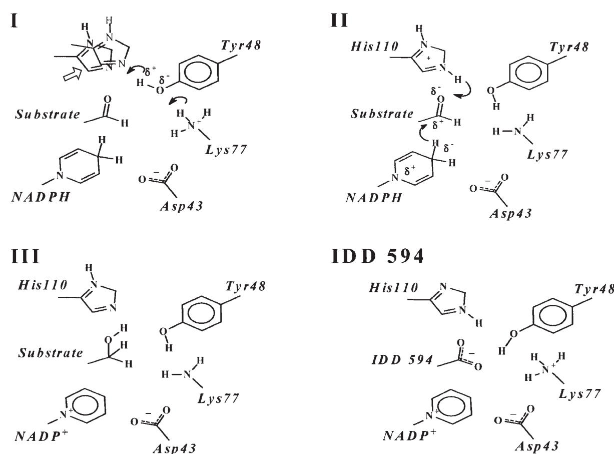

Figure 2. Proposed enzymatic mechanism by Cachau et al. [30]. The observed position of His110 is indicated by the arrow.

Another common feature among the various inhibitors is the presence of one or more aromatic groups, which may include a phthalazinyl group (ponalrestat and zopolrestat), a naphthyl group (tolrestat), a benzothiazole group (zopolrestat), a 2-thioxo-1,3-thiazolan-4-one group (epalrestat) and a halogenated benzyl group (ponalrestat). These aromatic groups bind in the hydrophobic pocket of ALR2, bordered by the Trp111, Phe122 and Leu300 residues, either through hydrogen bonding or hydrophobic contact. This would constitute a second important pharmacophoric requirement for inhibitor binding to ALR2.

Inhibitors containing the cyclic imide or carboxylic groups exhibit similar in vitro but different in vivo activities [7, 55, 56]. The carboxylic acid-containing inhibitors have lower in vivo activity, which has been attributed to relatively lower pK_a values, thus causing ionisation at physiological pH and an inability to traverse cell membranes. Conversely, cyclic imides have higher pK_a and are only partially ionised at physiological pH, thus able to pass through cell membranes and have better pharmacokinetic properties. Sorbinil possessed all these artributes, but its development as a therapeutic agent was halted due to a hypersensitive reaction [57].

The flavonoids (2-phenyl-4*H*-1-benzopyran-4-one) are also good inhibitors of ALR2 [58, 59] but do not contain either the carboxylic acid or cyclic imide moieties. This class of inhibitors, both naturally occurring and synthetic, has higher pK_a values than the carboxylic acids [60] and also has antioxidant properties [61], which prevents 4-hydroxy-2,3-*trans*-nonenal (HNE)-induced cataract formation, since HNE is produced in larger amount from lipid peroxidation during hyperglycaemia. A series of 1-benzopyran-4-one, based on quercetin, were synthesised by Costantino et al. [60] with compound **1**, 7-acetyl-2-(4′-hydroxybenzyl)-4H-1-benzopyran-4-one, having an IC_{50} value of 1.3 µM and a 99-fold selectivity over ALR1. A series of 35 flavonoid derivatives, primarily with the chalcone template, were synthesised by Lim et al. [62] and evaluated for inhibitory activity against rat lens ALR2, for antioxidant effects and also for accumulation of sorbitol in various tissues. The authors concluded that 3,4,2′,4′-tetrahydroxychacone has the best potential for treatment of diabetic complications.

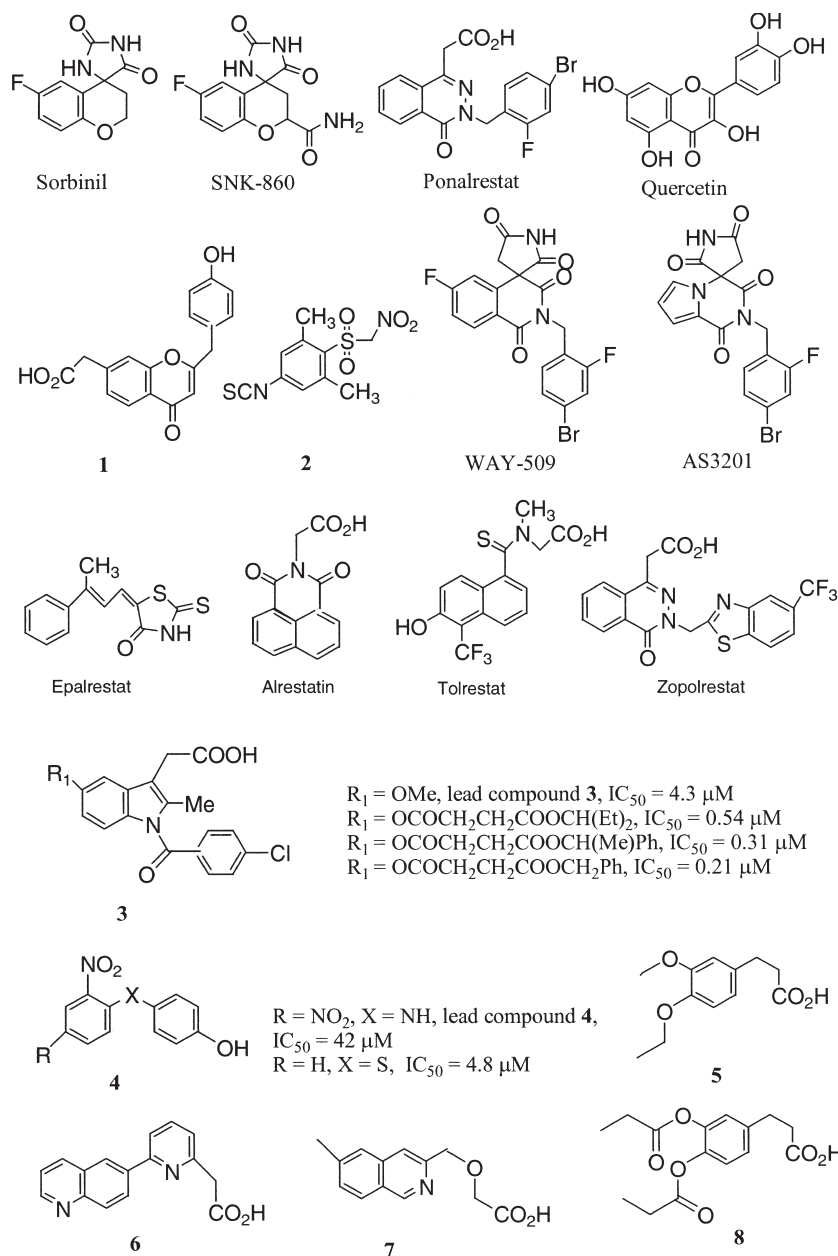

Figure 3. Structures of various types of ARIs.

Rakowitz et al. [63] synthesised diphenylmethyleneaminooxycarboxylic acids as novel ARIs and then prepared ester derivatives of the most active analogue (IC_{50} = 33 µM) for evaluation as prodrugs; various esters tested showed different propensity for stability or ease of cleavage and hydrolysis.

A newer class of ALR2 inhibitors is the phenylsulfonylnitromethanes [64], which exhibited potent activity against ALR2 and some of which also showed irreversible inhibition. Compound **2** had an IC_{50} value of 0.13 µM and almost total irreversibility at 100 µM [64].

Significant interest has also been shown in the 2,4-thiazolidinedione derivatives, since these can be regarded as hydantoin bioisosters and have shown inhibitory activity against ALR2 and as antihyperglycaemic agents [56, 65–67].

Newer types of inhibitors, which included compounds with aurone, chalcone and biphenyl backbones, including those that have been patented, were discussed in a recent review by Miyamoto [68].

Inhibitor design based on active site residues

The departure of the $NADP^+$ from the enzyme is believed to be the rate-determining step, and this is when current ALR2 inhibitors inhibit enzyme action. Recent crystallographic and modelling studies with both ALR1 and ALR2 have shown that inhibitors specific to ALR2 interact with the C-terminal residues by binding to the same subsite, termed the 'specificity' pocket [34, 36–38, 52, 69–71]. The hydrogen-bonding interactions between the inhibitor and active site residues (Tyr50, His113, Trp114 in ALR1 and Tyr48, His110, Trp111 in ALR2) are conserved, and the inhibitor is similarly oriented in the active sites of these enzymes. However, the binding of the inhibitor to ALR1 induces a conformational change in the side chain of the nonconserved C-terminal loop residue Arg312, creating a space for the inhibitor molecule to bind to the active site. Studies have suggested that differences in enzyme-inhibitor interactions at this C-terminal loop between these enzymes have a significant effect on inhibitor potency [72, 73].

When an inhibitor binding-induced conformational change occurs, significant differences in inhibitor potency are observed between ALR1 and ALR2. This was exemplified in cases with tolrestat and zopolrestat, where inhibition of ALR2 over ALR1 was increased by 72-fold and 450-fold, respectively [36]. El-Kabbani et al. [73] reported that tolrestat and zopolrestat were more potent inhibitors of the R312A mutant than the wild-type porcine ALR1 by 130- and 24-fold, respectively. Barski et al. [72] reported that tolrestat and zopolrestat were more potent inhibitors of the R311A mutant than the wild-type human ALR1 by 29- and 5-fold, respectively. Arg312 in the C-terminal loop is an important residue contributing to the less potent inhibition of ALR1 by ARIs than ALR2. The binding of tolrestat involves movement apart of the side chains of Leu300 and Phe122 in the hydrophobic pocket, whereas the binding of zopolrestat involves movement of the loops 121–135 away from Trp111. No conformational changes occurred with the binding of sorbinil, which showed only a 2.7-fold greater inhibition for ALR2. It was shown here that the inhibitors that bind to this hydrophobic pocket were better ARIs. Such knowledge has been utilised in the design of potent and specific inhibitors of ALR2.

Overview of structure-based and de novo design of ARIs

The basis of structure-based drug design is utilisation of the three-dimensional (3D) structure of ALR2 and its interaction with small molecules to discover potential inhibitors. The two main strategies involves either the use of a 3D search of the existing databases or a strictly de novo process whereby the inhibitor is constructed from atoms and fragments. In the former, the docking of each suitable candidate into the active site of the enzyme follows the 3D search. Programs that may be used for this task include DOCK [74], AutoDock [75], ADAM & EVE [76] and EUDOC [77]. During the procedure, nonbinding ligands and those with bad contacts are eliminated, while the ligands with good interactions are manoeuvred into optimal position for binding. Several crystal structure coordinates of the ALR2 holoenzyme and ternary complexes are readily available from the Protein Database (PDB) [78]. One of the more popular search models was the 1.8 Å-resolved structure of Wilson et al. [34], with the PDB code of 1mar. Other structures that were used included the 1.76 Å-resolved structure of Harrison et al. [79], with the PDB code of 2acq, and the human ALR2-IDD384 inhibitor complex of Calderone et al. [80], solved at 1.7 Å with the PDB code of 1el3. A more recent structure is that of the human ALR2-zenarestat inhibitor complex, which was solved at 2.5 Å resolution by Kinoshita et al. [81] and has a PDB code of 1iei.

The subsequent search and design processes will undoubtedly be highly influenced by the choice of the ALR2 structure. The initial 3D search and subsequent docking of ligands are then carried out using structures of ligands available from databases, such as the Available Chemicals Directory (ACD3D) [82], the National Cancer Institute (NCI-3-D) database [83] and the Cambridge Structure database [84]. The second strategy starts with a dictionary of common chemical fragments and a program that constructs a ligand within the active site of the enzyme through the maximisation of the ligand-enzyme interactions. Programs that may be used for this task include LUDI [85], LigBuilder [86] and LEGEND [87]. The optimal binding mode of each ligand can be independently obtained by either of these two methods.

The series of ligands that are docked or constructed into the active site of ALR2 can then be subjected to a preliminary ranking with the programs AutoDock and SCORE [88]. This process will select the ligands with the most favourable interactions as templates either for restarting the iterative process of ligand building, through producing a new database or new fragments, or for more advanced molecular modelling techniques. These techniques, which include FEP, Delphi reaction field (DRF), molecular mechanics interactions (MMI) and linear interaction energies (LIE), will provide a clearer, more detailed picture of ligand-enzyme interaction. On the basis of these studies, ligands may be synthesised and biological activities evaluated. The structure-based de novo design processes are summarised in the following flowchart (fig. 4). In the subsequent sections, a more detailed look at specific examples of this process will be presented.

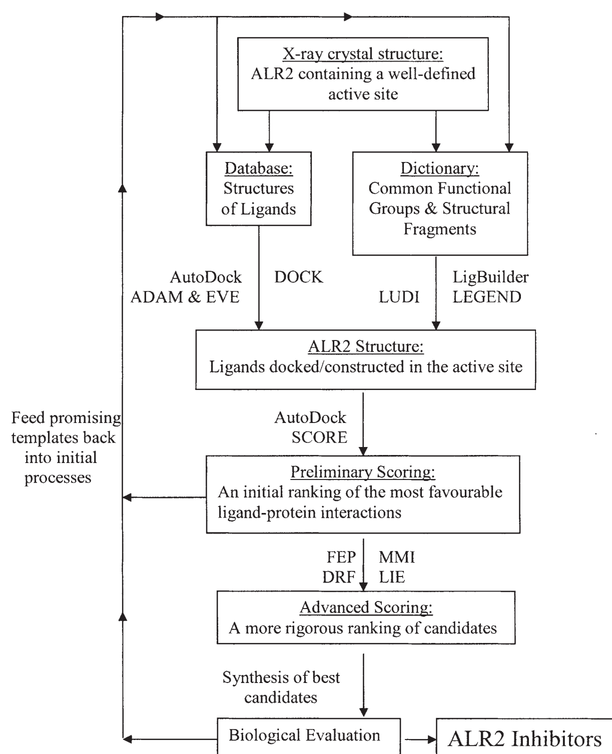

Figure 4. Flowchart of structure-based de novo design of ARIs.

Crystallographic and modelling studies of ALR2-inhibitor complexes

Utilising a potent and currently available inhibitor, the enzyme-inhibitor complex may first be crystallised, the structural data collected by X-ray diffraction analysis and the structure solved by molecular replacement using the refined structure of the native enzyme. The advantage of this method is that favourable steric and electrostatic interactions imbued by a potent inhibitor can then be readily examined and optimised. The disadvantage of this approach is that only a limited number of models can be investigated at any one time.

The knowledge acquired from an investigation of these modelled enzyme-inhibitor complexes can then be utilised to design more potent and specific inhibitors of the enzyme. Using a 2.1 Å-resolved crystal structure of human aldose reductase holoenzyme in complex with the acetic acid-based inhibitor statil (fig. 5), El-Kabbani et al. [89] designed potential inhibitors of human aldose reductase with enhanced binding energies. The program GRID [90] was utilised to evaluate various probes that could be attached to the statil backbone in regions of favoured interactions with specific residues on the enzyme. The maximum interactions were found in the vicinity of the fluorine atom in statil. Because of these interactions with nonconserved residues from the C-terminus, improved specificity for ALR2 was anticipated. The fluorine atom was replaced with selected probes to give compounds that were docked into the active site of ALR2. Energy minimisation and molecular dynamics calculations were then performed on these compounds, resulting in both enhanced and retarded binding energies of these complexes in relation to statil. Replacement of the fluorine atom with a carboxylic acid group was shown to be the most favourable, with a 33% enhancement in the binding energy of the complex. The interaction of this additional carboxylic group with the enzyme residue Leu300 is also illustrated (fig. 6). Similar investigations of ALR2 with the compounds SNK860 (fidarestat) and WAY509 (minalrestat) as the search models have also been completed [O. El-Kabbani et al., unpublished observations].

3D database screening and molecular docking

This method utilises the many thousands of structures of compounds and ligands available from online databases. These ligands are then docked into the active site of the enzyme. There are various crystal coordinates of ALR2 complexes available to be accessed from the PDB. Kuntz [74] demonstrated the success of this 3D-search approach using the DOCK program, as have others [38, 91, 92].

Iwata et al. [93] used the ADAM & EVE program [76] to search the ACD3D database [82] using an ALR2 ternary structure that included the cofactor NADPH as well as glucose-6-phosphate. They also carried out a preliminary screen of the enzyme at the catalytic and glucose-6-phosphate binding sites to evaluate the electrostatic and van der Waals interactions as well as hydrogen bond energies using an in-house program, CALGRID. Using the grid data from this preliminary search, the authors searched the approximately 120,000 structures in the ACD3D database and also simultaneously performed a conformational search during docking of the ligands. They positioned the ligands over two to three hydrogen bond sites in the ligand binding pocket and then used the ADAM & EVE program to optimise the ligand conformation. Interactions with Nε2 of His110, Oη of Tyr48, Nε1 of Trp111, Sγ of Cys298 and the N atom of $NADP^+$ were used as criteria for selection. The authors performed further selection, based on total interaction energies and visual examination by computer graphics, to yield a total of 179 compounds. They acquired 36 of these compounds of which 10 were found to be active. The most potent compound, with an IC_{50} value of 4.3 µM, was used for lead optimisation using the ADAM & EVE program. Analogues of this compound were synthesised by the authors, with most showing increased potency in inhibition of ALR2. They also showed a loss of inhibitory activity with a *t*-butyl protected carboxylate group. The structure and IC_{50} values of the lead compound **3** and the 3 most potent analogues are shown in figure 3.

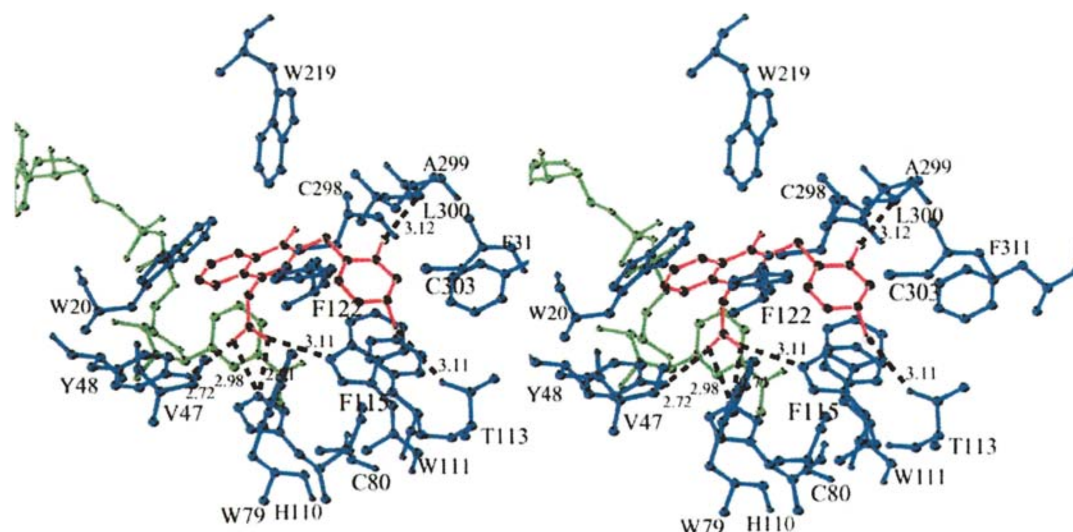

Figure 5. Stereoview of ALR2 with the bound inhibitor statil [89]; only the active site residues are shown.

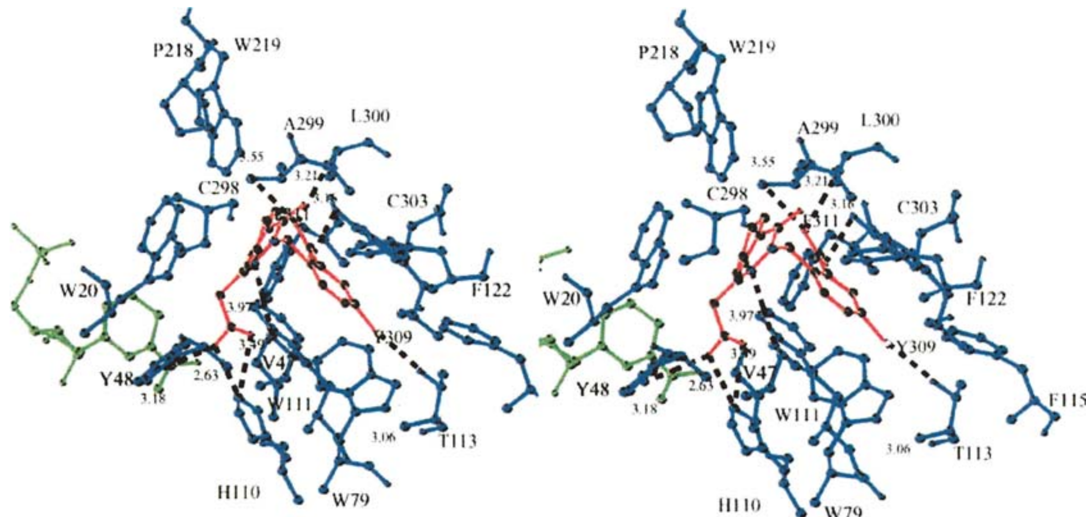

Figure 6. Stereoview of ALR2 with bound carboxylate analogue of statil [89]; only the active site residues are shown.

The authors also claimed a newly inferred hydrophobic subsite, based on the complex structure models of the most potent compounds, which could be useful in the design of more specific ARIs.

Rastelli et al. [94] favoured a complex of ALR2 with an inhibitor bearing both a carboxylic acid and benzothiazole moieties as the enzyme of choice, because of an open additional hydrophobic pocket. They used the program DOCK [74] to dock 127,000 structures of organic compounds from the NCI-3-D database [83] into the enzyme search model. Different families of inhibitors were proposed from this exercise, and these include carboxylic acid, sulfonic acid, nitro, sulfonamide and carbonyl derivatives. Five of 25 representative compounds from the different inhibitor classes were determined to have IC_{50} values in the 0.6–42 µM range. Some selectivity of ALR2 over ALR1 was also obtained for these compounds. The authors selected the nitro derivative as the lead compound for optimisation, in spite of it having the highest IC_{50} value at 42 µM. They reasoned that this compound has a simple structure and does not undergo ionisation at physiological pH, which is the case for the phenylsulfonyl nitromethanes, sulfonic acid and carboxylic acid derivatives. The authors synthesised six derivatives of this compound, based on the binding modes proposed by DOCK. The most potent of the derivatives, which also has a 10-fold selectivity for ALR2 with respect to ALR1, is shown in figure 3 along with the lead compound **4**.

De novo inhibitor design

Iwata et al. [95] had also undertaken an investigation whereby they utilised the same crystal coordinates as above [93] to undertake an atom-by-atom construction of 3D molecular structures using the LEGEND program [87]. They used the Nε2 of His110 and the Oη of Tyr48 as the building points of structures sited at the bottom of the binding pocket. 200 chemical structures were designed to bind into this ligand-binding site. Geometry optimisation was then carried out and the interaction energies for these compounds were calculated. A combination of familiar compounds and novel ones was generated, although most of the latter encompasses the usual aromatic and carboxylate moieties. All four compounds **5–8** that were selected for synthesis (fig. 3) gave reasonable IC_{50} values in the range 17–91 µM.
Although the ratio of 4 compounds synthesised is small, compared with the 200 initially designed, the authors emphasised that the reasonable IC_{50} values coupled with the structural novelty of all 4 compounds represent a first successful generation of nonpeptide lead compounds using a rational de novo design approach.

More advanced molecular modelling techniques

One of the techniques listed under this category is an FEP study. Rastelli et al. [96] carried out such a study on a class of inhibitors, 7-hydroxy-2-substituted-4*H*-1-benzopyran-4-one, which they had previously reported to be potent and selective inhibitors of ALR2 [54]. The relative binding energies of three analogues of 7-hydroxy-2-benzyl-4*H*-1-benzopyran-4-one were predicted by FEP simulations. 7-hydroxy-2-(4′-hydroxybenzyl)-4*H*-1-benzopyran-4-one was then selected to be the lead compound for investigation into the role of hydrogen bonding at the active site of ALR2. The authors converted the 4′-hydroxy to a methyl and a trifluoromethyl, and additionally introduced a hydroxy at position 8. They found that the compounds displayed K_i values that were in good agreement with the calculated relative free energies of binding and provide a rationale to the differences in binding affinities. They emphasised the relative importance of hydrogen bonding with Thr113 and with Trp111 and cofactor. Rastelli et al. [97] also undertook an earlier study on a series of methoxylated analogues of the 5,6-dihydrobenzo(h)cinnolin-3(2*H*)one-2-acetic acid to develop an explanation for differences in inhibitory activities.

Site-directed mutagenesis and other structural studies

Hohman et al. [37] had previously investigated the inhibitor-binding site with site-directed mutagenesis. The replacement of Trp20, Trp79, Trp111 and Phe115 residues with either alanine or tyrosine decreased the potential of van der Waals interactions. They found that the inhibitors bind through hydrophobic interactions at the active site of the enzyme. Srivastava et al. [98] had modified aldose reductase by S-nitrosothiols, notably the active site residue Cys298. They found an enzyme with enhanced catalytic rate and K_m and a desensitisation towards inhibition by sorbinil. Newly designed inhibitors that bind to these residues or to residues that may be similarly modified can then be studied as a means to confirm specific interactions proposed in inhibitor design and optimisation processes.
Structure activity relationships can also be used to study the interactions of inhibitors and active site residues. This can be accomplished by modifying specific areas on the inhibitors that have been shown by molecular modelling studies to have interactions with the enzyme active site residues. Costantino et al. [99] synthesised several derivatives of 7-hydroxy-2-(4′-hydroxybenzyl)-4*H*-1-benzopyran-4-one modified at position 2 and tested them with bovine and human ALR2. They found that substitution of the methylene bridge with an isosteric sulfur group gives an active derivative, while substitution with a polar NH group reduces inhibitory activity. The finding is in accordance with the methylene linker being adjacent to the hydrophobic Leu300 residue. Further replacement at the 4 position favours derivatives with polar substituents in this position, presumably acting as hydrogen bond donors to the Thr113 residue of the enzyme.

1 Kinoshita J. H. and Nishimura C. (1988) The involvement of aldose reductase in diabetic complications. Diabetes Metab. Rev. **4:** 323–337
2 Pugliese G., Tilton G. T. and Williamson J. R. (1991) Glucose-induced metabolic imbalances in the pathogenesis of diabetic vascular diseases. Diabetes Metab. Rev. **7:** 35–59
3 Dunlop M. (2000) Aldose reductase and the role of the polyol pathway in diabetic nephropathy. Kidney International **77:** S3–S12
4 Van den Enden M. K., Nyengaard J. R., Ostrow E., Burgan J. H. and Williamson J. R. (1995) Elevated glucose levels increase retinal glycolysis and sorbitol pathway metabolism. Invest. Ophthalmol. Vis. Sci. **36:** 1674–1685
5 Diabetes Control and Complications Trial Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. New Engl. J. Med. **329:** 977–986
6 Ohkubo Y., Kishikawa H., Araki E., Miyata T., Isami S., Motoyoshi S. et al. (1995) Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: randomised prospective 6-year study. Diabetes Res. Clin. Pract. **28:** 103–117
7 Sarges R. and Oates P. J. (1993) Aldose reductase inhibitors: recent developments. Prog. Drug Res. **40:** 99–161
8 Nishikawa T., Edelstein D. and Brownlee M. (2000) The missing link: a single unifying mechanism for diabetic complications. Kidney International **58:** S26–S30

9 Yabe-Nishimura C. (1998) Aldose reductase in glucose toxicity: a potential target for the prevention of diabetic complications. Pharmacol. Rev. **50:** 21–33
10 King G. L. and Brownlee M. B. (1996) The cellular and molecular mechanisms of diabetic complications. In: Endocrinology and Metabolism Clinics of North America, vol. 25, pp. 255–270, Brownlee M. B. and King G. L. (eds), WB Saunders, Philadelphia
11 Koya D. and King G. L. (1998) Protein kinase C activation and the development of diabetic complications. Diabetes **47:** 859–866
12 Friedman E. A. (1992) Advanced glycosylated end products and hyperglycaemia in the pathogenesis of diabetic complications. Diabetes Care **22:** B65–B71
13 Cameron N. E., Cotter M. A. and Hohman T. C. (1996) Interactions between essential fatty acid, prostanoid, polyol pathway and nitric oxide mechanisms in the neurovascular deficit of diabetic rats. Diabetologia **39:** 172–182
14 Lee A. Y. W. and Chung S. S. M. (1999) Contributions of polyol pathway to oxidative stress in diabetic cataract. FASEB **13:** 23–30
15 Chan N. N., Vallance P. and Colhun H. M. (2000) Nitric oxide and vascular responses in Type 1 Diabetes. Diabetologia **43:** 137–147
16 Costantino L., Rastelli G., Vianello P., Cignarella G. and Barlocco D. (1999) Diabetes complications and their potential prevention: aldose reductase inhibition and other approaches. Med. Res. Rev. **19:** 3–23
17 Oka M. and Kato N. (2001) Aldose reductase inhibitors. Journal of Enzyme Inhibition **16:** 465–473
18 Kaul C. L. and Ramarao P. (2001) The role of aldose reductase inhibitors in diabetic complications: recent trends. Methods Find. Exp. Clin. Pharmacol **23:** 465–475
19 El-Kabbani O., Wilson D. K., Petrash J. M. and Quiocho F. A. (1998) Structural features of the aldose reductase and aldehyde reductase inhibitor-binding sites. Mol. Vis. **4:** 19–26
20 Zopolrestat. (1997) Drugs Fut. **22:** 97
21 Mealy N. and Castaner J. (1996) SNK860. Drugs Fut. **21:** 261
22 Oates P. J. and Mylari B. L. (1999) Aldose reductase inhibitors: therapeutic implications for diabetic complications. Exp. Opin. Invest. Drugs **8:** 1–25
23 Pfeifer M. A., Schumer M. P. and Gelber D. A. (1997) Aldose reductase inhibitors: the end of an era or the need for different trial design. Diabetes **46:** S82–S89
24 Sorbinil Retinopathy Trial Research Group (1990) A randomized trial of sorbinil, an aldose reductase inhibitor, in diabetic retinopathy. Arch. Ophthalmol. **108:** 1234–1244
25 Foppiano, M. and Lombardo, G. (1990) Worldwide pharmacovigilance systems and tolrestat withdrawal. Lancet **349:** 399–400
26 Sato S. and Kador P. F. (1990) Inhibition of aldehyde reductase by aldose reductase inhibitors. Biochem. Pharmacol. **40:** 1033–1042
27 Vander Jagt D. L., Torres J. E., Hunsaker L. A. Deck L. M. and Royer R. E. (1996) Physiological substrates of human aldose and aldehyde reductases. Adv. Exp. Med. Biol. **414:** 491–497
28 Bohren K. M., Page J. L., Shankar R., Henry S. P. and Gabbay K. H. (1991) Expression of human aldose and aldehyde reductases. Site-directed mutagenesis of a critical lysine 262. J. Biol. Chem. **262:** 24031–24037
29 Petrash J. M., Harter T. M., Devine C. S, Olins P. O., Bhatnagar A., Liu S. et al. (1992) Involvement of cysteine residues in catalysis and inhibition of human aldose reductase. Site-directed mutagenesis of Cys-80, -292, -303. J. Biol. Chem. **267:** 24833–24840
30 Cachau R., Howard E., Barth P., Mitschler A., Chevrier B., Lamour V. et al. (2000) Model of the catalytic mechanism of human aldose reductase based on quantum chemical calculations. Journal de Physique IV **10:** 3–13
31 Wilson D. K., Bohren K. M., Gabbay K. H. and Quiocho F. A. (1992) An unlikely sugar substrate site in the 1.65 Å structure of the human aldose reductase holoenzyme implicated in diabetic complications. Science **257:** 81–84
32 Rondeau J. M., Tête-Favier F., Podjarny A., Reymann J. M., Barth P., Biellman J. F. et al. (1992) NADPH-binding domain revealed by the crystal structure of aldose reductase. Nature **355:** 469–472
33 El-Kabbani O., Judge K., Ginell S.., Myles D. A., DeLucas L. J. and Flynn T. G. (1995) Structure of the porcine aldehyde reductase holoenzyme. Nat. Struct. Biol. **2:** 687–692
34 Wilson D. K., Tarle I., Petrash J. M. and Quiocho A. (1993) Refined 1.8 Å structure of human aldose reductase complexed with the potent inhibitor zopolrestat. Proc. Natl. Acad. Sci. USA **90:** 9847–9851
35 Bohren K. M., Grimshaw C. E., Lai C. J., Harrison D. H., Ringe D., Petsko G. A. et al. (1994) Tyrosine-48 is the proton donor and histidine-110 directs substrate stereochemical selectivity in the reduction reaction of human aldose reductase: enzyme kinetics and crystal structure of the Y48H mutant enzyme. Biochemistry **33:** 2021–2032
36 Urzhumtsev A., Tête-Favier F., Mitschler A., Barbanton J., Barth P., Urzhumtseva L. et al. (1997) A 'specificity' pocket inferred from the crystal structures of the complexes of aldose reductase with the pharmaceutically important inhibitors of tolrestat and sorbinil. Structure **5:** 601–612
37 Hohman T., El-Kabbani O., Malamas M., Lai K., Putilina T., McGowan M. et al. (1998) Probing the inhibitor-binding site of aldose reductase with site-directed mutagenesis. Eur. J. Biochem. **256:** 310–316
38 Lee Y. S., Chen Z. and Kador P. F. (1998) Molecular modeling studies of the binding modes of aldose reductase inhibitors at the active site of human aldose reductase. Bioorg. Med. Chem. **6:** 1811–1819
39 Brooks B. R., Bruccoleri R. E., Olafson B. D., States D. J., Swaminathan S. and Karplus M. (1983) CHARMM: a program for macromolecular energy, minimisation and dynamics calculations. J. Comput. Chem. **4:** 187–217
40 Lee Y., Hodoscek M., Brooks B. and Kador P. (1998) Catalytic mechanism of aldose reductase studied by the combined potentials of quantum mechanics and molecular mechanics. Biophys. Chem. **70:** 203–216
41 Schmidt M. W., Baldridge K. K., Boatz J. A., Elbert S. T., Gordon M. S., Jensen J. J. et al. (1993) General atomic and molecular electronic structure system. J. Comput. Chem. **14:** 1347–1363
42 Schlegel B. P., Jez J. M. and Penning T. M. (1998) Mutagenesis of 3-alpha-hydroxysteroid dehydrogenase reveals a push-pull mechanism for proton transfer in aldo-keto reductases. Biochemistry **37:** 3538–3548
43 Varnai P., Richards W. G. and Lyne P. D. (1999) Modelling the catalytic reaction in human aldose reductase. Proteins Struct. Funct. Genet. **37:** 218–227
44 Bohren K. and Grimshaw C. (2000) The sorbinil trap: a predicted dead-end complex confirms the mechanism of aldose reductase inhibition. Biochemistry **39:** 9967–9974
45 Nakano T. and Petrash J. M. (2000) Kinetic and spectroscopic evidence for active site inhibition of human aldose reductase. Biochemistry **35:** 11196–11202
46 Singh S., Malamas M., Hohman T., Nilakantan R., Carper D. and Kitchen D. (2000) Molecular modeling of the aldose reductase-inhibitor complex based on the X-ray crystal structure and studies with single-site-directed mutants. J. Med. Chem. **43:** 1062–1070
47 Pearlman D. A., Case D. A., Caldwell J. C., Seibel G. L., Singh U. C., Weiner P. et al. (1991) Amber (4.0), University of California, San Francisco
48 Kitchen D. B., Hirata F., Westbrook J. D., Levy R., Kofke D. and. Yarmush M. (1990) Conserving energy during molecular

dynamics simulations of water, proteins and proteins in water. J. Comput. Chem. **11:** 1169–1180
49 Varnai P. and Warshel A. (2000) Computer simulation studies of the catalytic mechanism of human aldose reductase. J. Am. Chem. Soc. **122:** 3849–3860
50 Warshel A. (1991) Computer modeling of chemical reactions in enzymes and solutions, Wiley, New York
51 Kollman P. (1993) Free energy calculations – applications to chemical and biochemical phenomena. Chem. Rev. **93:** 2395–2417
52 Oka M., Matsumoto Y., Sugiyama S., Tsuruta N. and Matsushima M. (2000) A potent aldose reductase inhibitor, (2S,4S)-6-fluoro-2′,5′-dioxyspiro[chroman-4,4′-imidazoline]-2-carboxamide (Fidarestat): its absolute configuration and interaction with aldose reductase by X-ray crystallography. J. Med. Chem. **43:** 2479–2483
53 Kurono M., Fujiwara I. and Yoshida K. (2001) Stereospecific interaction of a novel spirosuccinimide type aldose reductase inhibitor, AS-3201, with aldose reductase. Biochemistry **40:** 8216–8226
54 Da Settimo F., Primofiore G., Da Settimo A., La Motta C., Taliani S., Simorini F. et al. (2001) [1,2,4]Triazino[4,3-a]benzimidazole acetic acid derivatives: a new class of selective aldose reductase inhibitors. J. Med. Chem. **44:** 4359–4369
55 Eggler J. F., Larson E. R., Lipinski C. A., Mylari B. L. and Urban F. J. (1993) A perspective of aldose reductase inhibitors. In: Advances in Medicinal Chemistry, vol. 2, pp. 197–246, Jai Press, Greenwich, CT
56 Larson E. R., Lipinski C. A. and Sarges R. (1988) Medicinal chemistry of aldose reductase inhibitors. Med. Chem. Rev. **8:** 159–186
57 Spielberg S. P., Shear N. H., Cannon M., Hutton N. J. and Gunderson K. (1991) In vitro assessment of a hypersensitivity syndrome associated with sorbinil. Ann. Intern. Med. **114:** 720–724
58 Kador P. F., Kinoshita J. H. and Sharpless N. E. (1985) Aldose reductase inhibitors: a potential new class of agents for the pharmacological control of certain diabetic complications. J. Med. Chem. **28:** 841–849
59 Iinuma M., Tanaka T., Mizuno M., Katsuzaki T. and Okawa H. (1989) Structure-activity correlation of flavonoids for inhibition of bovine lens aldose reductase. Chem. Pharm. Bull. **37:** 1813–1815
60 Costantino L., Rastelli G., Gamberini M. C., Vinson J. A., Bose P., Iannone A. et al. (1999) 1-Benzopyran-4-one antioxidants as aldose reductase inhibitors. J. Med. Chem. **42:** 1881–1893
61 Middleton E. and Kandaswami C. (1994) The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: The Flavonoids, Advances in Research since 1986, pp. 619–652, Harborne J. B. (ed.), Chapman and Hall, London
62 Lim S. S., Jung S. H., Ji J., Shin K. H. and Keum S. R. (2001) Synthesis of flavonoids and their effects on aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rat tissues. J. Pharm. Pharmacol. **53:** 653–668
63 Rakowitz D., Matuszczak B., Gritsch S., Hofbauer P., Krassnigg A. and Costantino L. (2002) On the prodrug potential of novel aldose reductase inhibitors with diphenylmethyleneaminooxycarboxylic acid structure. Eur. J. Pharm. Sci. **15:** 11–20
64 Saab N. H., Donkor I. O., Rodriguez L., Kador P. F. and Miller D. D. (1999) Phenylsulfonylnitromethanes as potent irreversible inhibitors of aldose reductase. Eur. J. Med. Chem. **34:** 745–751
65 Costantino L., Rastelli G., Gamberini M. C. and Barlocco D. (2000) Pharmacological approaches to the treatment of diabetes complications. Exp. Opin. Ther. Patents **10:** 1245–1262
66 Fresneau P., Cussac M., Morand J.-M., Szymonski B., Tranqui D. and Leclerc G. (1998) Synthesis, activity and molecular modeling of new 2,4-dioxo-5-(naphthylene)-3-thiazolidine-acetic acids and 2-thioxo analogues as potent aldose reductase inhibitors. J. Med. Chem. **41:** 4706–4715
67 Bruno G., Costantino L., Curinga C., Maccari R., Monforte F., Nicolo F. et al. (2002) Synthesis and aldose reductase inhibitory activity of 5-arylidene-2,4-thiazolidinediones. Bioorg. Med. Chem. **10:** 1077–1084
68 Miyamoto S. (2002) Recent advances in aldose reductase inhibitors: potential agents for the treatment of diabetic complications. Expert Opin. Ther. Patents **12:** 621–631
69 Harrison D. H. T., Bohren K. M., Petsko G. A., Ringe D. and Gabbay K. H. (1997) The alrestatin double-decker: binding of two inhibitor molecules to human aldose reductase reveals a new specificity determinant. Biochemistry **36:** 16134–16140
70 Rogniaux H., Van Dorsselaer A., Barth P., Biellmann J.-F., Barbanton J., Van Zandt M. et al. (1999) Binding of aldose reductase inhibitors: correlation of crystallographic and mass spectrometric studies. J. Am. Soc. Mass Spectrom. **10:** 635–647
71 El-Kabbani O., Rogniaux H., Barth P., Chung R. P.-T., Fletcher E. V., Van Dorsselaer A. et al. (2000) Aldose and aldehyde reductases: correlation of molecular modeling and mass spectrometric studies on the binding of inhibitors to the active site. Proteins Struct. Funct. Genet. **41:** 407–414
72 Barski O. A., Gabbay K. H. and Bohren K. M. (1996) The C-terminal loop of aldehyde reductase determines the substrate and inhibitor specificity. Biochemistry **35:** 14276–14280
73 El-Kabbani O., Carper D. A., McGowan M. H., Devedjiev Y., Rees-Milton K. J. and Flynn T. G. (1997) Studies on the inhibitor-binding site of porcine aldehyde reductase: crystal structure of the holoenzyme-inhibitor ternary complex. Proteins Struct. Funct. Genet. **29:** 186–192
74 Kuntz I. D. (1992) Structure-based strategies for drug design and discovery. Science **257:** 1078–1082
75 Morris G. M., Goodsell D. S., Halliday R. S., Huey R., Hart W. E., Belew R. K. et al. (1998) Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. J. Comput. Chem. **19:** 1639–1662
76 Mizutani M. Y., Tomioka N. and Itai A. (1994) Rational automatic search method for stable docking models of protein and ligands. J. Mol. Biol. **243:** 310–326
77 Perola E., Xu K., Kollmeyer T. M., Kaufmann S. H., Prendergast F. G. and Pang Y.-P. (2000) Successful virtual screening of a chemical database for farnesyltransferase inhibitor leads. J. Med. Chem. **43:** 401–408
78 Berman H. M., Westbrook J., Feng Z., Gilliland G., Bhat T. N., Weissig H. et al. (2000) The protein data bank. Nucleic Acids Res. **28:** 235–242
79 Harrison D. H., Bohren K. M., Ringe D., Petsko G. A and Gabbay K. H. (1994) An anion binding site in human aldose reductase: mechanistic implications for the binding of citrate, cacodylate, and glucose 6-phosphate. Biochemistry **33:** 2011–2020
80 Calderone V., Chevrier B., Van Zandt M., Lamour V., Howard E., Poterszman A. et al. (2000) The structure of aldose reductase bound to the inhibitor IDD384. Acta Cryst. **D56:** 536–540
81 Kinoshita T., Miyake H., Fujii T., Takakura S. and Goto T. (2002) The structure of human aldose reductase complexed with the potent inhibitor zenarestat. Acta Cryst. **D58:** 622–626
82 Molecular Design Limited Information Systems, San Leandro, California (http://www.mdli.com)
83 National Cancer Institute (NCI-3-D) database (http://dtp.nci.nih.gov)
84 Allen F. G., Bellar S. A., Brice M. D., Cartwright B. A., Doubleday A., Higgs H. et al. (1979) The Cambridge Crystallographic Data Centre: computer-based search retrieval, analysis and display of information. Acta Cryst. **B35:** 2331–2339 (http://www.ccdc.cam.ac.uk)

85 Böhm H.-J. (1992) The computer program LUDI: a new method for the de novo design of enzyme inhibitors. J. Comp.-Aided Mol. Design **6:** 61–78
86 Wang R., Gao Y. and Lai L. (2000) Ligbuilder: a multiple-purpose program for structure-based drug design. J. Mol. Model. **6:** 498–516
87 Nishibata Y. and Itai A. (1993) Confirmation of usefulness of a structure construction program based on three-dimensional receptor structure for rational lead generation. J. Med. Chem. **36:** 2921–2928
88 Wang R., Liu L., Lai L. and Tang Y. (1998) SCORE: a new empirical method for estimating the binding affinity of a protein-ligand complex. J. Mol. Model. **4:** 379–394
89 El-Kabbani O., Ramsland P., Darmanin C., Chung R. P.-T. and Podjarny A. (2003) Structure of human aldose reductase holoenzyme in complex with statil: an approach to structure-based inhibitor design of the enzyme. Proteins Struct. Funct. Genet. **50:** 230–238
90 Goodford P. J. (1985) A computational procedure for determining energetically favourable binding sites on biologically important macromolecules. J. Med. Chem. **28:** 849–857
91 Rastelli G., Vianello P., Barlocco D., Costantino L., Corso A. and Mura U. (1997) Structure-based design of an inhibitor modeled at the substrate active of aldose reductase. Bioorg. Med. Chem. Lett. **7:** 1897–1902
92 Wilson D. K., Quiocho F. A. and Petrash J. M. (1997) Structural studies of aldose reductase inhibition. In: Structure-Based Drug Design, Veerapandian P. (ed.), pp. 229–246, Marcel Dekker, New York
93 Iwata Y., Arisawa M., Hamada R., Kita Y., Mizutani M. Y., Tomioka N. et al. (2001) Discovery of novel aldose reductase inhibitors using a protein structure-based approach: 3D-database search followed by design and synthesis. J. Med. Chem. **44:** 1718–1728
94 Rastelli G., Ferrari A. M., Costantino L. and Gamberini M. C. (2002) Discovery of new inhibitors of aldose reductase from molecular docking and database screening. Bioorg. Med. Chem. **10:** 1437–1450
95 Iwata Y., Naito S., Itai A. and Miyamoto S. (2001) Protein structure-based de novo design and synthesis of aldose reductase inhibitors. Drug Design Discov. **17:** 349–359
96 Rastelli G., Costantino L., Gamberini M. C., Del Corso A., Mura U., Petrash J. M. et al. (2002) Binding of 1-benzopyran-4-one derivatives to aldose reductase: a free energy perturbation study. Bioorg. Med. Chem. **10:** 1427–1436
97 Rastelli G., Costantino L., Vianello P. and Barlocco D. (1998) Free energy perturbation studies of the inhibitor 5,6-dihydrobenzo(h)cinnolin-3(2*H*)one-2-acetic acid and its methoxylated analogs to aldose reductase. Tetrahedron **54:** 9415–9428
98 Srivastava S., Dixit B. L., Ramana K. V., Chandra A., Chandra D., Zacarias A. et al. (2001) Structural and kinetic modifications of aldose reductase by S-nitrosothiols. Biochem. J. **358:** 111–118
99 Costantino L., Del Corso A., Rastelli G., Petrash J. M. and Mura U. (2001) 7-Hydroxy-2-substituted-4*H*-1-benzopyran-4-one derivatives as aldose reductase inhibitors. Eur. J. Med. Chem. **36:** 697–703

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