Molecular Modeling and Structure-based Drug Discovery
Studies of Aldose Reductase Inhibitors

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Abstract

Aldose reductase has been implicated in the etiology of diabetic complications. A variety of compounds have been observed to inhibit aldose reductase and effective, orally active inhibitors of the enzyme have been investigated for many years. Although several of these compounds have progressed to the clinical level, only one such drug is currently on the market. Due to the limited number of available drugs for the treatment of diabetic complications, a number of rational approaches for the discovery of aldose reductase inhibitors have been taken since the determination of the 3-dimensional structure of the enzyme. In this review, rational approaches such as the molecular modeling of aldose reductase and its complex structure and structure-based drug discovery are presented, following a short summary of known inhibitors.

Key Words: molecular modeling, structure-based drug discovery, aldose reductase, inhibitor, diabetic complication

Area of Interest: Molecular Recognition (Review)

1. Aldose Reductase and Diabetic Complications

The estimated prevalence of diabetes mellitus among adults worldwide was 4.0% in 1995 and is expected to double by 2025 [1]. In spite of insulin treatment, most diabetic patients eventually experience long-term diabetic complications, such as retinopathy, neuropathy, cataract, and angiopathy. Although there is still no definite pathogenic link between hyperglycaemia and diabetic complications, several mechanisms seem to be involved in the toxic effects caused by excess glucose (Figure 1) [2,3]. Among well-examined factors are the activation of protein kinase C [4,5], enhanced protein glycation with the formation of advanced glycated end products (AGEs) [6,7], rise of oxidative stress [8,9], and activation of the polyol pathway [10].
The polyol pathway was first implicated in the etiology of secondary complications of diabetes [11]. Aldose reductase (AR) is the first enzyme of this pathway and is widely distributed in mammalian tissues [12]. In the presence of NADPH, the enzyme converts glucose to sorbitol, which is only slowly metabolized to fructose by sorbitol dehydrogenase, the other enzyme in the pathway, with concurrent reduction of NAD\(^+\). The activation of the polyol pathway, which occurs during hyperglycaemia, brings about various metabolic imbalances in tissues that undergo insulin-independent uptake of glucose. In the ocular lens, hyperosmotic swelling is caused by the accumulation of sorbitol. In other tissues, the depletion of the cofactor NADPH used in the pathway results in the deactivation of glutathione reductase and nitric oxide synthase, leading to an increased susceptibility to oxidative stress, vascular derangement and a decrease in nerve conduction velocity. It has been shown that the oxidation of sorbitol catalyzed by sorbitol dehydrogenase increases the ratio of NADH:NAD\(^+\), resulting in an increased lactate:pyruvate ratio and pseudohypoxia [13].

![Figure 1](image_url)

Figure 1. A schematic interaction diagram of factors related to the polyol pathway among pathogenic mechanisms of diabetic complications. AR: Aldose reductase; SDH: Sorbitol dehydrogenase; NO: Nitric oxide; GSH: Glutathione; AGE: Advanced glycated end product. (Reproduced with permission from Ashley Publications Ltd., Ref. 17)

There exist a variety of structurally diverse aldose reductase inhibitors (ARIs) (Figure 2). These compounds can be divided into two general classes, those containing a carboxylic acid moiety and those having a cyclic imide represented by a spirohydantoin or related ring system [14,15,16,17]. Recently, however, arylsulphonylnitromethanes have emerged as a new class. Although several ARIs have been tested in clinical trials on diabetic patients for more than 20 years, they still remain to be proven sufficiently effective [18]. Tolrestat (1), which was launched in 1989, was withdrawn in 1996, principally due to its low efficacy. Of the newer compounds, zopolrestat (2) [19] and zenarestat (3) were withdrawn from clinical trials. At present, only epalrestat (4), which was developed by Ono and launched into the Japanese market in 1992, is still available [20].
Figure 2. Chemical structures of known ARIs.
Several crystal structures of AR have been solved by X-ray crystallography. Since the discovery of the 3D structure of AR, studies using molecular modeling techniques have been conducted. X-ray crystal structures and molecular modeling studies have given us useful insight into the structure of the enzyme-bound inhibitor at the atomic level. Furthermore, these studies together with various discovery approaches have led to structure-based drug discovery (SBDD).

2. Representative Known ARIs

2.1 Cyclic imides and related compounds

Since the discovery of sorbinil (5) by Pfizer in 1978, several compounds with a spirohydantoin group or closely related skeleton have been developed. From the structure-activity relationship studies, it is recognized that the AR inhibitory activity almost entirely depends on the C-4S enantiomer, and this has also been explained by a theoretical study on AS-3201 (6) [21]. Unfortunately, in the early weeks of therapy, hypersensitivity reactions were induced by sorbinil (5), which is oxidatively metabolized to a potentially toxic intermediate [22]. Nonetheless, several compounds belonging to this class of cyclic imides have entered clinical trials in the past several years. One of them is fidarestat (7, SNK 860) [23], a derivative of 5 with ten-fold higher potency. It is developed by Sanwa Kagaku and is awaiting approval as a drug for diabetic neuropathy in Japan.

Furthermore, not only the spirocyclic compounds described above exhibit AR inhibitory activity but also simple imides or their bioisoters do so as well. For example, a 2,4-thiazolidinedione moiety of risarestat (8, CT-112) can be considered to be a hydantoin bioisoster. After the discovery of 2,4-thiazolidinedione derivatives as hypoglycaemic agents, a number of compounds in this class (general formula 9) were recognized as both antihyperglycaemic and ARI agents. This series of compounds act on peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$), improving glucose utilization without stimulating insulin release [24]. The thiazolidinedione or rhodanine moiety can be regarded as bioisosters of hydantoins.

2.2 Carboxylic acids

A large number of carboxylic acids were synthesized and evaluated as ARIs in the past. Besides epalrestat (4) several carboxylic acids have been tested in clinical trials, e.g., ponalrestat (10), zopolrestat (2), and zenarestat (3). Although no clinically important adverse effect was observed for 10, its beneficial effect was neither obvious [25].

Alrestatin (11) is one of the compounds that were in clinical testing several years ago. It was reported that NZ-314 (12) increased the motor nerve conduction velocity and the sciatic nerve blood flow compared to diabetic controls in STZ-induced rats. A 3-thiazolidineacetic acid derivative (13), which can be regarded as a cyclic derivative of 4, has been reported to be a potent ARI [26]. Recently, an indole derivative (14) with a benzothiazole moiety and its related compounds were patented as ARIs, showing excellent selectivity for AR over aldehyde reductase.

2.3 Other compounds

Phenylsulphonylnitromethanes, such as 15 and structurally similar nitromethylketone derivatives were recently shown to have AR inhibitory activities. The IC$_{50}$ values of these two kinds of compounds were in the low nanomolar range.
3. 3D Structure of Aldose Reductase

AR consists of a single polypeptide chain with 315 residues. Several crystal structures of the enzyme have been solved by X-ray crystallography. It folds into a $\beta/\alpha$ barrel with a core of eight parallel $\beta$ strands [27]. The ligand binding site is a large, deep, elliptical pocket with the nicotinamide ring of the NADPH cofactor at the base. In Figure 3, the complex crystal structure of AR and tolrestat (1) is shown [28]. Although a definite catalytic mechanism has not yet been determined, the crystal structures suggest the involvement of Tyr48 and Lys77 as well as the nicotinamide ring in the catalytic reaction [29]. A potential anion binding site delineated by the nicotinamide ring, Tyr48, and His110 at the bottom of the cavity is also suggested based on the crystallographic studies (Figure 3).

The naphthalene group of tolrestat (1) and the benzothiazole moiety of zopolrestat (2) were found to fit into the hydrophobic pocket of AR by X-ray crystallography [28,30] (Figure 3). Interestingly, this pocket emerges after conformational changes in a loop (residues 121–135) as well as in a short segment (residues 298–303). This conformation change of the enzyme provides the ligand specificity towards AR over aldehyde reductase. Moreover, the residues in the opening of the hydrophobic pocket are not conserved between the two related enzymes (65% sequence identity), which coexist in most tissues [31]. Compounds with benzothiazole, naphthalene, or comparable moieties that bind to this specificity pocket generally have much higher selectivity for AR as compared with aldehyde reductase.

The complex crystal structure of AR with fidarestat (7) revealed that its amide group interacts with Leu300 which is in the short segment susceptible to conformational change (Figure 4). This could explain the high selectivity of 7 against aldehyde reductase [32]. The complex structure of alrestatin (11) with AR was recently elucidated to have a unique binding mode whereby the two molecules are bound to the pocket in a ‘double-decker’ arrangement [33].

![Figure 3. Complex crystal structure model of AR (white) and tolrestat (1) (green) [30]. In this model, polar hydrogen atoms are added and oxygen, nitrogen and sulfur atoms are shown in red, blue, and yellow, respectively. Dashed lines represent hydrogen bonds.](image-url)
4. Structure-based Approaches

Since the discovery of the 3D structure of AR, molecular modeling studies as well as SBDD have been reported. Molecular modeling studies are useful to give us insight into the structure of the enzyme-bound inhibitor and their inhibition mechanisms. It is well recognized that rational methods such as SBDD are important in the R & D of drugs. In line with the current pace of advancements in X-ray crystallographic technologies as well as progress in the human genome project, the structure of a great number of macromolecules have been determined. SBDD has therefore become more and more important as a rational approach to the discovery of leads and their optimization [34,35].

One of the two representative methods of SBDD is the 3D-database search which retrieve chemical structures that fit into the ligand binding site of a target protein. Some successful results from 3D search using the DOCK program have been summarized by Kuntz [36]. Of the 100 to 200 best-scoring compounds, 10–50 were selected for biological testing and between 2 and 20% of the compounds tested showed inhibition in the micromolar range. Recently, Zhang et al. carried out a 3D search to look for protein tyrosine phosphatase inhibitors [37]. From the 2000 compounds identified by DOCK, 25 were further assayed. Seven of them exhibited inhibition in the range between 21 and 510 µM. Pang et al. reported on a virtual screening with the EUDOC program, identifying four farnesyltransferase inhibitors with IC50 values in the range of 25 to 100 µM [38].

The other of the two SBDD methods is the de novo design which builds chemical structures that fit into the ligand binding site of a target protein either atom-by-atom or fragment-by-fragment [39,40,41,42]. Although many successful application results were reported for the 3D search as shown above, few examples of de novo designed and synthesized nonpeptide compounds, which
showed expected activities, have yet been reported. In the following, molecular modeling studies of AR are first summarized and then SBDD studies are reviewed including our recent works.

4.1 Molecular modeling

A molecular modeling study performed by Itzstein et al. showed that Tyr48, His110 and Trp111 are involved in the hydrogen-bonding interactions with three substrates, D-xylene, L-xylene, and D-lyxose [43]. They also found that a good correlation between calculated interaction enthalpies and experimental log($K_m$) or log($k_{cat}/K_m$) values was obtained only when His110 was modeled with its Ne2 atom in the protonated form and Nδ1 atom in the unprotonated form. Molecular dynamics with the AMBER program [44] and Delphi algorithm [45] were used in the calculations of interaction enthalpies and solvation energies, respectively. Substrate binding was also explored by Kador et al. using quantum mechanics combined with molecular mechanics [46]. It was inferred that the reduction of D-glyceraldehyde to glycerol catalyzed by His110 was favored over the reduction process catalyzed by Tyr48. However, this conclusion was not supported by a point mutation study [47] and a recent computer simulation study that was performed by Warshel et al. [48] with the semi-microscopic protein dipole Langevin dipoles [49] and the empirical valence bond methods [50]. These studies showed that Tyr48 acts as the proton donor in the reduction, and that neutral His110 has a role in substrate binding during the catalysis.

A molecular modeling investigation of the pyridazinone carboxylic acid inhibitor (16; Figure 5) with an IC$_{50}$ value of 6.4 µM was reported by Barlocco et al. [51]. The molecular mechanics and molecular dynamics calculations of the complex gave indications of specific interaction site responsible for the binding. Molecular dynamics simulations of AR complexed with tolrestat (1) were carried out by Costantino et al. [52]. One of the resulting complex models was in good agreement with the crystal complex structure. Kador et al. presented a docking study in which some of the representative ARIs were extensively examined [53]. On the basis of the derived binding modes and inhibitory activities of those compounds, the pharmacophore requirements for ARIs were discussed.

Two groups performed free energy perturbation studies with the AMBER program. Based on a series of methoxylated analogs of 16, Barlocco et al. provided a rationale for the observed differences in the inhibitory activities [54]. Singh et al. evaluated the effects of the site-directed mutations on residues within the active site to provide a binding model of zopolrestat (2) [55]. The model was then used to generate binding models for other ARIs such as 17 (Figure 5). On the grounds of carbon surface area change calculations, these models were found to correspond with their binding affinities.
4.2 Structure-based drug discovery

Based on the modeling study of 16 mentioned above [51], Barlocco et al. performed an SBDD and provided a new derivative (18, Figure 5) that had 100-fold higher inhibitory activity [56]. Compound 18 is, however, a composite of the known tricyclic pyridazinone skeleton and the benzothiazole ring of zopolrestat (2).

With respect to the discovery of ARIs by the 3D-database search, one of the first studies that were conducted was the one briefly reported by Petrash et al. [57]. Among the 30 highest scoring compounds derived by DOCK [36], several aromatic aldoximes with inhibition constants in the micromolar range, were found. Unfortunately, these were similar to known benzaldoximes with comparable inhibition constants [58]. Recently, Iwata et al. published successful results of a 3D search followed by design and synthesis [59]. With the ADAM&EVE program [60], the 3D database of the Available Chemicals Directory was searched and 179 candidate compounds were obtained. Out of 36 compounds that were further analyzed, 10 compounds (19-27, Figure 6) showed more than 40% inhibition of AR at a concentration of 15 µg/mL. In a subsequent lead optimization study based on the predicted docking mode, an approximately 20-fold increase in inhibitory activity (IC_{50} = 0.21 vs 4.3 µM) was achieved (28; Figure 6).

More recently, Iwata et al. carried out a structure-based de novo design and synthesis of ARIs [61]. With the LEGEND program [40], we have designed 200 chemical structures that fit into the ligand binding site of the crystal structure of the enzyme. After their visual inspection and assessment of synthetic feasibility, four compounds (35, 36, 38, and 39; Figure 7) were chosen and synthesized. The synthesized compounds were all found to have inhibitory activities (IC_{50} = 17–91 µM), indicating the first successful generation of nonpeptide drug leads obtained through a rational de novo design approach.

5. Concluding Remarks and Future Prospects

From the pioneering studies on sorbinil and alrestatin (11) to recent investigations on zopolrestat (2) and zenarestat (3), several compounds in clinical trials or on the market for the treatment of diabetic complications have been developed but withdrawn. Among others, lacks of improvement as well as occasional occurrences of side effects caused by ARIs appear to have led to their discontinuation. Some hurdles must be overcome before new drugs including novel ARIs can be used therapeutically. First is the issue of pharmacokinetics. As is often the case, predicting the in vivo activity from the in vitro activity is a tricky problem to resolve. While the activity of the cyclic imide derivatives and carboxylic acids is similar in vitro, the former is generally more potent than the latter in vivo, probably because of different pharmacokinetic effects. Second, toxic side effects, which may be caused by ARIs through the interaction with other enzymes, must be minimized. Thus, good selectivity should be ensured for ARIs.

Due to the limited number of currently available drugs for the treatment of diabetic complications, research for new aldose reductase inhibitors using molecular modeling studies as well as SBDD have been performed and are proving to be promising, as reviewed in this article. SBDD studies that seek substructures that fit into the specificity pocket might be useful for solving the selectivity problem. With respect to pharmacokinetics, experimental works (early ADME) may be effective when combined with molecular modeling studies to elucidate physicochemical parameters of candidate compounds. Hopefully, advances will be made through such studies, which will reduce diabetic complications for a great number of diabetes patients.
Figure 6. Chemical structures of ARIs discovered in the 3D search and an optimized ARI. IC₅₀ values in parentheses are from a second independent experiment.
Figure 7. Chemical structures of designed compounds and synthesized compounds. Compounds 29–37 are those originally designed by the LEGEND program. Compounds 35, 36, 38 and 39 were actually synthesized.

References


