diabetology

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Review

Old Target with New Vision: In Search of New Therapeutics for Diabetic Retinopathy by Selective Modulation of Aldose Reductase

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Abstract

Aldose Reductase (AR; AKR1B1) is an enzyme that plays a key role in the metabolism of glucose and other carbonyl compounds, and whose hyperactivity contributes to oxidative stress and vascular dysfunction. Despite decades of investigation into this enzyme, inhibitors have failed to translate into clinical application for Diabetic Retinopathy (DR). We argue that these failures might arise from non-selective inhibition, considering the dual roles of AR, which contribute not only to DR pathology but also support retinal health, as AR is an important detoxifying enzyme for aldehydes produced during oxidative stress. Here, we discuss missing structural information, despite more than one hundred crystal structures of AR in complex with inhibitors. Our review bridges this gap by discussing how recent advances in structural biology, e.g., fragment-based drug discovery and MicroED, provide novel ways to selectively modulate AR functions, offering advantages for the detection of weak, allosteric, or conformation-dependent binding events. Despite past challenges, we suggest that therapeutic targeting of AR to find new-generation inhibitors will become more effective once we have a clearer understanding of the requirements for selective inhibition of AR, blocking its pathological impact while preserving its physiological functions. By integrating fragment screening and structural biology, we outline a strategy to reinvigorate AR modulation as a viable retina-specific approach for managing DR, with potentially broader relevance toward multiple diabetic microvascular complications.

Keywords: aldose reductase (AR); diabetic retinopathy (DR); fragment-based drug discovery (FBDD); microcrystal electron diffraction (MicroED)

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1. Introduction

Diabetes mellitus (DM) is a major and growing health burden, with rising prevalence and substantial mortality and healthcare costs. According to The Diabetes Atlas, 589 million adults (20–79 years old) worldwide are living with diabetes worldwide, and the number is predicted to rise to 853 million by 2050.

Persistent hyperglycemia can lead to severe secondary complications [1,2]. These are often grouped into macrovascular (cardio- and cerebrovascular) and microvascular diseases affecting nerves (neuropathy), kidneys (nephropathy), and the eye (diabetic retinopathy, DR) [3,4].

DR is a leading cause of visual impairment in working-age adults, and its prevalence increases with diabetes duration [5,6]. Clinically, DR progression reflects a combination of

vascular endothelial dysfunction, breakdown of the blood–retinal barrier, microvascular occlusion, and ultimately retinal ischemia and neovascularization [5,7–12].

Multiple, interlinked biochemical routes connect hyperglycemia to microvascular injury [13,14]. A widely used framework highlights (1) increased flux through the polyol pathway, (2) oxidative stress, (3) formation of advanced glycation end-products (AGEs), and (4) the activation of protein kinase C (PKC) [11,14,15]. These mechanisms are not independent; shared metabolites and redox cofactors couple their activity [14].

Among these, the polyol pathway (an alternative two-step glucose metabolic pathway that converts glucose into fructose via sorbitol), is initiated by the enzyme aldo–keto reductase family 1, member B1 (AKR1B1), better known as Aldose Reductase (AR), the first enzyme involved in the polyol pathway, and plays an important role in the pathogenesis of DR and other diabetes related complications [16]. AR is a broad range oxidoreductase that catalyzes the reduction of aldehydes, including glucose, to their corresponding alcohols. It should be noted, however, that the framework based on the four pathways linking hyperglycemia to microvascular disease is useful, but one needs to bear in mind that the relative contributions and causal ordering in the human retina remain incompletely resolved [12,17]. Therefore, in this review, we will distinguish established biochemical consequences of AR activation (as an upstream, druggable node with a long clinical track record yet unresolved translational hurdles) from hypotheses about downstream drivers in DR, and will highlight where stage-stratified intervention and retina-directed delivery could affect translatability [18,19].

AR activity towards glucose is relatively low normally because of its high Km, but when glucose levels are chronically elevated, the increased substrate availability, together with AR altered expression/activity, can increase polyol flux [20–22] (Figure 1). This results in accumulation of sorbitol (osmotic stress) and altered NADPH/NADP balance, which can amplify oxidative and inflammatory signalling [23–25].

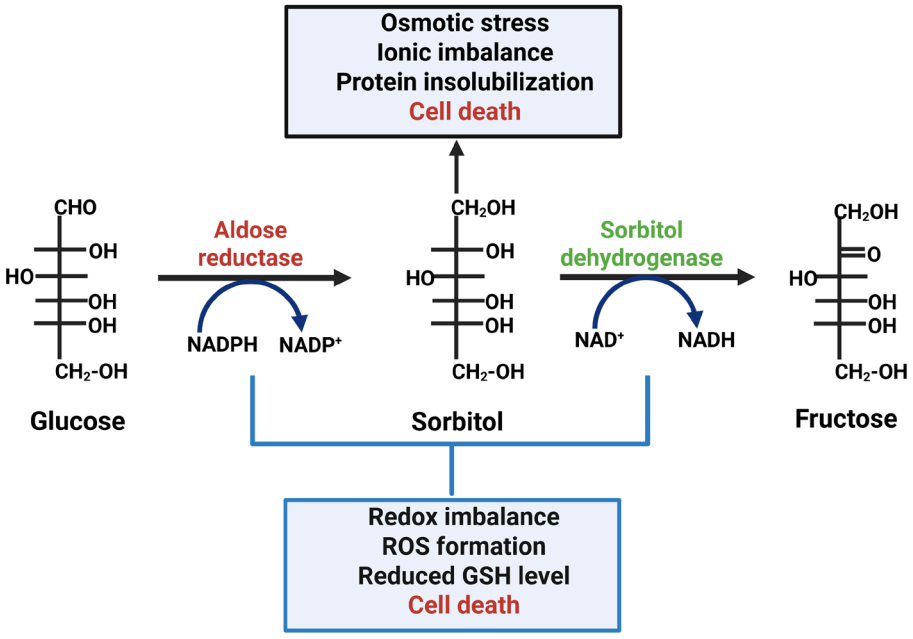


Figure 1. The polyol pathway. Under conditions of hyperglycemia, as occurs in diabetes, excess glucose is diverted to the polyol pathway, whereby it is converted to fructose through the consecutive action of Aldose Reductase (AR) and sorbitol dehydrogenase (SDH).

Because the retina is metabolically demanding and sensitive to osmotic and redox imbalance, AR has long been considered a therapeutic target in DR [5,7,9,11,21,26]. Nu-

merous AR inhibitors (ARIs) were developed over four decades [27–34], although with limited clinical efficacy; Epalrestat remains the only marketed ARI approved for diabetic neuropathy in some countries [35]. Available clinical and preclinical data suggest that efficacy can depend on disease stage, tissue exposure, and dosing, motivating renewed strategies to improve selectivity and delivery [36–38].

Prior attempts to inhibit AR have been hindered by a combination of factors, including (i) dose-limiting adverse effects and off-target inhibition within the aldo–keto reductase AKR family, (ii) suboptimal pharmacokinetics and limited tissue access (including retinal exposure across the blood–retinal barrier), and (iii) trial design issues such as patient heterogeneity, disease stage at enrolment, and endpoint sensitivity. Off-target inhibition is facilitated by high sequence identity within the human AKR1 family (>40%), with AKR1A1 and AKR1B10 sharing 65 and 70% sequence identity with AR, respectively [39]. Preclinical evidence reinforces the relevance of AR as a retina-specific target [40–42]. For instance, genetic deletion of AR prevents early diabetes-induced damage in neural, glial, and vascular cells of the retina [41], while ARIs can mitigate a spectrum of retinal abnormalities more effectively than other drug classes in animal models [42]. However, clinical translation has been inconsistent. For example, in the negative Sorbinil Retinopathy Trial [43], the dose used in humans was ~20-fold lower than that shown to prevent retinal polyol pathway activation and the development of retinopathy in diabetic rats [44,45], highlighting challenges related to exposure, dosing, tissue penetration, and toxicity-limited escalation. A contributing factor to tolerability may be limited selectivity over aldehyde reductase (AKR1A1), a closely related detoxifying enzyme [46]. Despite these setbacks, recent advances in structural biology, pharmacology, drug discovery, and drug delivery offer new opportunities to revisit AR modulation [47,48]. Given the limitations of classic ARIs, this review discusses a Fragment-Based Drug Discovery (FBDD) strategy to pursue improved selectivity and exposure, coupled to Microcrystal Electron Diffraction (MicroED) for rapid complementary structural readout, with the goal of capturing weak, allosteric, and conformational-dependent binding modes that are often missed by conventional approaches.

Importantly, the proposed explanation that ARI failures primarily reflect loss of AR's endogenous detoxification, together with or in addition to the inhibition of related AKRs, remains plausible but has limited direct clinical support to date. Preferential suppression of glucose (and glutathionyl-4-hydroxynonenal, GS-HNE) turnover while sparing the reduction in lipid-derived aldehydes, such as 4-hydroxynonenal (4-HNE), is currently supported mainly by in vitro and in silico analysis [47,49], and remains to be rigorously validated in vivo under physiologically relevant substrate conditions [47,50]. We therefore frame differential inhibition as a testable strategy rather than a settled explanation for historical ARI failures.

2. Aldose Reductase: Catalytic Activity, and Pathological and Physiological Functions

Aldose Reductase (EC 1.1.1.21) is a cytosolic, monomeric member of aldo-keto reductase (AKR) superfamily, that catalyzes NADPH-dependent reduction of carbonyl substrates, including sugars and aldehyde products derived from lipid peroxidation [51].

AR was initially identified in the eye lens, and it is also present in various other tissues, with levels varying widely between organs [52–54]. Under normal physiological conditions, AR exhibits low catalytic activity toward glucose due to its high Km, reflecting its low affinity for glucose as a substrate. However, when intracellular glucose concentrations rise, a larger fraction of glucose molecules can be diverted towards the polyol pathway [55,56]. The physiological roles of AR are vast and involved in detoxifica-

tion of aldehyde compounds such as 4-hydroxynonenal, and osmoregulation. Therefore, AR therapeutic inhibition must balance suppression of pathological glucose flux with preservation of its protective functions [47,57–65].

The biochemical steps of the polyol pathway are well defined, and AR-dependent phenotypes are robust in the lens and the peripheral nerve [18,66,67]. In DR, much evidence for AR involvement comes from animal models (including streptozotocin diabetes and galactosemia) and from genetic/pharmacologic manipulation [44,45,68,69]. However, direct quantification of pathway flux and compartment-specific redox changes in human retina remains challenging; available data suggest heterogeneity across retinal cell types and disease stages [12,18,23,44].

2.1. The Polyol Pathway

The polyol pathway is a two-step route converting glucose into fructose via sorbitol (by AR, then sorbitol dehydrogenase (SDH) activities) [70] (Figure 1). Under normal glycemic conditions, only a small fraction of glucose enters this pathway, but hyperglycemia can markedly increase flux, consuming NADPH and generating NADH, thereby perturbing redox homeostasis [24,27,51,70,71].

Two consequences are particularly relevant to microvascular tissues: (i) sorbitol is poorly membrane permeable, so net accumulation can produce osmotic stress, especially where SDH activity is low; and (ii) altered NADPH/NADH balance ("pseudohypoxia") can influence oxidative stress, poly(ADP ribose) polymerase (PARP) activation, lipid signalling, and PKC activity [20,70,72,73]. For clarity, we discuss these distinct downstream consequences separately in Sections 2.1.1–2.1.4.

While polyol-driven redox and osmotic stress are well supported mechanistically, their quantitative contribution to human DR relative to other hyperglycemia-activated pathways remains actively discussed [14,18]. This uncertainty is one reason ARI efficacy has not translated cleanly from animal models to heterogeneous patient populations [18,32,43].

2.1.1. Polyol Pathway Activation and Sorbitol Accumulation

Sorbitol is a polyhydroxylated alcohol that diffuses poorly across membranes and can accumulate intracellularly when AR flux exceeds downstream clearance [18,74]. In the retina, AR has been detected in multiple cell types implicated in early DR (such as pericytes, retinal endothelial cells, ganglion cells, Muller cells, retinal pigment epithelial cells, and neurons) [41,44,75–78]. Osmotic stress from sorbitol accumulation is most clearly demonstrated in tissues with limited SDH activity and has been linked to cellular swelling, dysfunction, and vulnerability in experimental models. In humans, association studies and transcript/protein measurements support a role for AR in subsets of patients, although results vary between cohorts and endpoints [79–84].

2.1.2. Oxidative Stress Generation

AR consumes NADPH, and increased polyol flux can compete with antioxidant systems (such as glutathione reductase (GR)) for the cellular NADPH pool, lowering reduced glutathione (GSH) regeneration capacity and sensitizing cells to oxidative damage [24,27,85]. Redox imbalance can also increase mitochondrial superoxide production and stimulate NADH/NADPH oxidase activity [29,86–90]. Importantly, oxidative stress in DR is multifactorial; AR-dependent NADPH depletion is one contributor, but the relative importance of this route compared with other reactive oxidative species (ROS) sources appears to depend on cell type, metabolic state, and disease stage [12,86,90].

2.1.3. Formation of Advanced Glycation End Products (AGEs)

AGEs form through nonenzymatic glycation and through the "carbonyl stress" pathway involving dicarbonyl intermediates [91,92]. Hyperglycemia promotes AGE accumulation, and the polyol pathway can contribute indirectly by generating fructose and dicarbonyl precursors and by amplifying oxidative stress [85,93,94]. AGE accumulation alters extracellular matrix properties and activates receptor-mediated inflammatory signalling; downstream matrix remodelling enzymes (including matrix metalloproteases (MMPs)) and tight junction disruption have been reported in DR, consistent with blood–retinal barrier compromise [13–15,95–101].

2.1.4. Activation of Protein Kinase C (PKC)

PKC activation, often via increased diacyglycerol (DAG), is a well-studied hyperglycemia-responsive signalling axis in DR [30,102,103]. Polyol-linked redox changes and lipid metabolism (such as GS-HNE/GS-DHN) have been linked to the nuclear factor κB (NF-κB)-dependent inflammatory signalling that intersects with PKC pathways [15,25,49,104–108]. However, the causal chain from AR activity to specific PKC-dependent retinal endpoints is complex and likely involves multiple parallel inputs (oxidative stress, AGEs, and cytokine signalling) [103,109,110].

2.2. Clinical Manifestations

AR has been implicated in diabetic eye disease, particularly early pericyte degeneration and microvascular dysfunction, based largely on animal/galactosemia models where AR inhibition prevents pericyte loss and retinal lesions [68,69,111–113]. Human genetic association studies suggest some AR polymorphisms correlate with DR risk, although results are inconsistent across cohorts [40,66,78,114–116]. AR is also central to diabetic cataract formation in lens-AR overexpression models and may contribute via osmotic and oxidative signalling mechanisms [67,117–120].

AR is also expressed in the kidney and induces hyperglycemia; AR-deficient mice reduce features of diabetic nephropathy, supporting a contributory role, although downstream mediators (PKC, AGEs, ROS, TGF-β) overlap with other glucose-responsive pathways [54,121–123].

2.3. Physiological Function of AR

As compared to a significant number of studies on AR focusing on its pathological role in glucose toxicity, less attention has been paid to the physiological function of AR in normal and glycemic conditions. These roles are central to explaining both past ARI liabilities and the rationale for different AR modulation.

Physiologically, AR participates in osmoregulation in the kidney and in fructose production in the male genital tract [124–126]. Another important role of AR is in aldehyde reduction/detoxification of reactive aldehydes generated by lipid peroxidation and glycose (such as 4-HNE, acrolein, methylglyoxal, and related dicarbonyls), often with much lower Km than for glucose, supporting a protective role under oxidative stress [16,25,27,49,124]. AR can also contribute to the metabolism of steroid-derived aldehydes in adrenal and reproductive tissues [124,127].

These dual roles motivate the concept of differential (functional) selectivity: an ideal AR modulator would suppress pathological glucose (and possible GS-HNE-linked pro-inflammation) reactions while sparing detoxification of lipid-derived aldehydes [47,49,128]. Notably, AR expression and activity are context dependent and vary across tissues and disease stage [22,79,121], suggesting that state-, tissue-, and substrate-specific effects, rather than expression alone, may determine therapeutic windows.

Inhibitors also need to be selective across AKR homologues (such as for AKR1B10 and AKR1A1) that participate in carbonyl detoxification and lipid/retinoid metabolism. Broad inhibition could be counterproductive by impairing the clearance of reactive carbonyl species and perturbing stress-response signalling [129–131].

Therefore, a key limitation of conventional AR inhibitors is their broad spectrum of activity. While they suppress the polyol flux, many also inhibit AR's detoxification function and cross-react with related AKRs, potentially exacerbating oxidative stress and offsetting the benefits. Evidence for substrate-dependent ("differential") inhibition that preserves lipid-aldehyde detoxification while blocking glucose reduction is currently strongest in vitro/in silico [47,104] and has not been demonstrated robustly in vivo. This paradox motivates the development of selective or differential inhibitors that block pathological glucose flux without impairing protective detoxifying activity [5,21,49,57,131–133]. The conceptual difference between non-selective AR inhibition and selective/differential AR modulation is summarized in Figure 2.

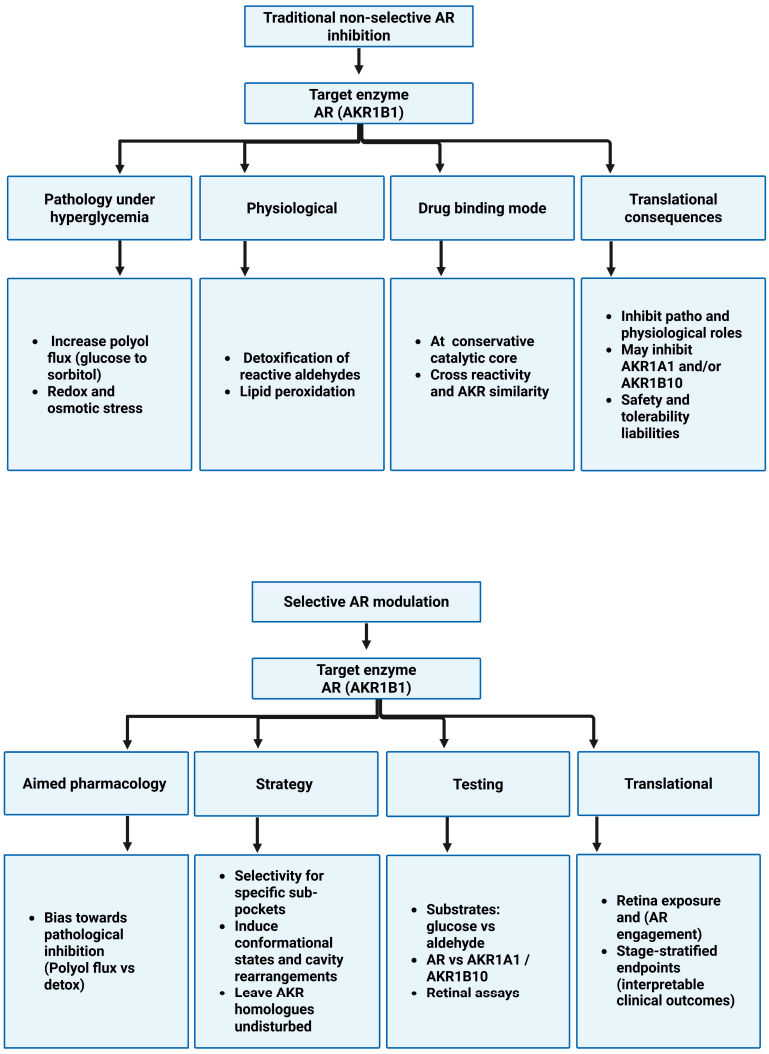


Figure 2. Conceptual distinction between non-selective AR inhibition and selective/differential AR modulation. Legacy ARIs often block both (i) hyperglycemia-driven polyol flux and (ii) AR's protective carbonyl detox function, and may inhibit closely related AKRs (such as AKR1B10 and AKR1A1). A next-generation strategy aims to bias inhibition towards the pathological flux while preserving detox capacity and sparing homologs, coupled to retina exposure and target engagement readouts.

3. Structural Characteristics of Aldose Reductase

To date, 176 X-ray crystal structures of AR have been reported in pursuit of understanding its detailed structure and mechanism of action. The first structures were published in 1992 and were heterologous in nature, being of recombinantly expressed human AR protein and of pig lens AR [134–136]. Over the years, more detailed structures of AR have been described in the apo-protein as well as of its complexes with various ligands, especially inhibitors and the NADPH co-enzyme (or analogues) [137]. These studies have given us the opportunity to look more deeply into the catalytic mechanism as well as the binding and interaction modes of the ligands [137].

Human AR is 316 amino acids long and has been reported at 34 kDa. The enzyme is monomeric in nature and is composed of an eight-sheeted β/α barrel with the N terminus composed of two short antiparallel β-strands capping the bottom of the barrel. Parallel β-strands, with each alternating with α-helical segments that run anti-parallel to the β-sheet [137], providing a rigid catalytic scaffold, while some loops between the α-helices and β-strands are the mobile and sequence diverse elements that shape the active site cleft. The substrate binding site is in the C-terminal region of the barrel and gives a large and deep cleft in appearance. The pyridine NADP+ acts as a cofactor and is localized at the C-terminal end of the parallel strands of the barrel [134–136], in an extended form and with the nicotinamide ring positioned at the centre of the barrel, contributing to the active-site machinery, and the pyrophosphate straddling the lip of the barrel [136–138]. The active-site-relevant loops are named A, B, and C and correspond to residues 109 to 137 in between β4 and α4, residues 209 to 230 in between β7 and α7, and the C-terminal residues, respectively, protruding from the top of the barrel [134–136,139]. Two additional α helices, namely, H1 and H2, are instrumental in anchoring the loops. The H1 helix anchors the cofactor binding loop, while the H2 helix helps in anchoring the C-terminus loop [140].

The region that helps to retain the cofactor is known as the 'safety-belt' loop and is composed of residues Gly213 to Ser226, which can be traced to the loop B [136,138]. While the cofactor is bound to the loop, it becomes partially covered by it and thus referred to as the "closed" conformation; the structure in the absence of the cofactor is termed the "open" conformation [138]. This safety belt is responsible for mediating the conformational changes in the enzyme and for the release and binding of the cofactor. The "closed" and "open" conformations are thus central to the working of the enzyme [138]. Within the safety belt, Gly213, Ser214, and Ser226 are the hinge points and take part in the conversion of these closed and open states, while Trp219 acts as the "latch" bridge between Cys298 and Arg293 (via sulfur-aromatic or ring-stacking interactions, respectively) to stabilize the whole conformation as well as interactions with ligands [140].

The active site of AR has been described in slightly different ways across the literature (Figure 3), but most structural studies agree on two principal regions: a rigid anion-binding pocket and a region with a more flexible specificity pocket (induced cavity) that enables ligand diversity [27,32,141]. According to Maccari and Ottanà (2015), the anion-binding pocket is a polar, hydrogen-bonding subsite formed by residues, such as Asp43, Tyr48, Lys77, His110, Ser159, Asn160, Gln183, and Tyr209 [32]. This pocket anchors polar groups and ensures catalytic precision [142,143]; Tyr48 serves as a proton donor to the carbonyl oxygen atom of the substrate during the second step of the reduction, whereas His110 helps maintain substrate orientation. This subsite can serve as a polar recognition region able to anchor anionic and/or hydrogen-bond acceptor groups of various substrates and inhibitors [32]. Urzhumtsev and colleagues (1997) similarly highlighted these residues as central to a hydrophilic network dominated by hydrogen bonds, van der Waals forces, and water-mediated interactions [141]. Additional residues, including Trp20, Val47, and Trp111, also contribute to substrate stabilization at the catalytic site. That study highlighted that the

anion-binding pocket, lined by residues such as Tyr48, His110, Trp20, Val47, and Trp111, anchors the catalytic machinery by stabilizing the substrate identified via hydrogen bonds and water-mediated contacts. Urzhumtsev and colleagues also indicated the hydrophobic substrate-binding cleft consisting of Trp20, Trp79, Trp111, Phe122, Pro218, Trp219, Cys298, Leu300, and Val47. Of those, Trp20, Phe122, and Trp219 fully face the active site and are thus expected to make the major contacts with a potential substrate. The study further mentioned the existence of a specificity pocket, the residues of which are also in the second group of cleft-lining residues, and include Thr113, Phe115, Val130, Ser302, and Cys303. Residues Trp111, Phe122, and Leu300 are shared by both the active site and the specificity pockets [141]. This cavity can assume a variety of flexible states and adopt conformations differently to accommodate different ligands accordingly [142,143].

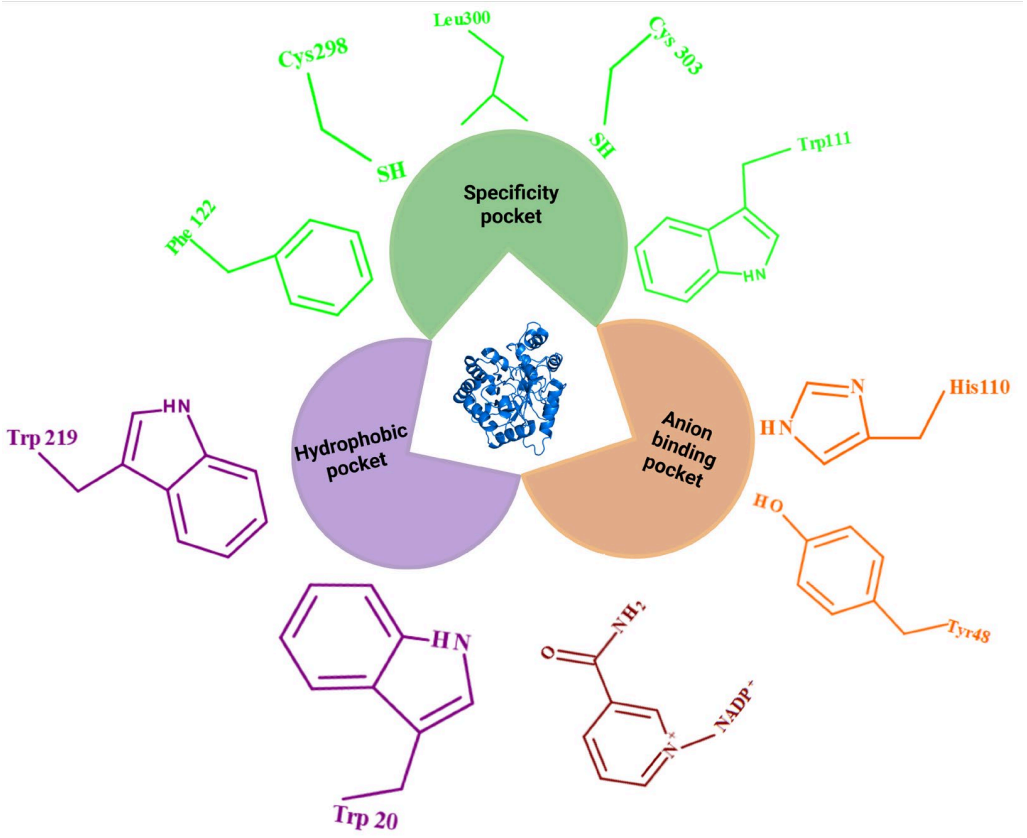


Figure 3. Schematic view of the binding pocket of Aldose Reductase (AR). The central insert depicts the AR structure, as a cartoon representation (PDB 3S3G).

The second region of the AR active site is a hydrophobic cleft situated above the coenzyme. This "specificity pocket" (or "induced cavity") is lined by residues such as Trp20, Trp79, Trp111, Phe122, Pro218, Trp219, Cys298, and Leu300 [32]. It is highly flexible and capable of adopting different conformations depending on the size and chemistry of the ligand. While Maccari and Ottanà (2015) emphasize slightly different residue sets for the cavity (namely, Trp111, Thr113, Phe122, Ala299, Leu300, Ser302, and Cys303) [32]. In contrast, Balestri and colleagues (2022) stated that the specificity pocket (also referred to as the induced cavity) consists of Thr113, Phe115, Phe122, Cys303, and Tyr309, and contributes to ligand adaptability by undergoing conformational rearrangements (residue numbers and names are taken from [27]). Several residues, including Trp111, Phe122, and Leu300, are shared by both the anion-binding and specificity pockets, underscoring their dual role in substrate recognition [141].

With respect to the enzymatic reactions, it is known that AR follows an ordered reaction mechanism. NADPH binds first and induces conformational changes that accommodate binding of the substrate, its conversion to the alcohol, and its release. Then, while NADP+ is still bound to the protein, there is a second conformation change in the protein, facilitating the release of NADP+ [27,137,138]. Although there have been different structural models proposed for the AR protein, reflecting the resolution of structural analysis, the rigid anion-binding region and the flexible specificity pocket remain the central determinants for substrate recognition and inhibitor selectivity. Importantly, many fragment-based and classical inhibitors exploit induced conformations of the specificity pocket, making its flexibility a key target for rational drug design.

3.1. Conformational Flexibility

When AR inhibitors such as minalrestat, zopolrestat, and tolrestat bind to the enzyme, they induce conformational changes in loops containing the residues Phe122 and Leu300, opening the normally closed specificity pocket (apo state) and allowing bulky hydrophobic groups (such as 4-bromo-2-fluorophenyl, benzothiazole, or naphthalene moieties) to bind [141,144,145]. Leu300 acts as a gate-keeper residue and controls the pocket opening/accessibility, influencing nearby residues, including Phe122, Trp219, and Cys303 [142,146]. By contrast, sorbinil lacks a suitable hydrophobic group and does not access the specificity pocket; the pocket remains closed in this complex [141]. Importantly, residues within the specificity pocket are generally less conserved across related enzymes than catalytic residues such as Tyr48 and His110, providing a potential structural basis for isoform selectivity. The flexibility of the specificity pocket, however, complicates co-crystallization and can enable multiple binding conformations for ligands and substrates with either hydrophilic (for example, glyceraldehyde and aldoses) or hydrophobic (for example, HNE) moieties [141,142,147].

Structural Homology and Challenges

The challenge in designing an effective ARI lies in the fact that AR shares more than 80% sequence homology with aldehyde reductase (AKR1A1), one of the most important aldo–keto reductases. Additionally, AKR1B10 (aldo–keto reductase family 1, member B10) also shares high sequence and structural homology with AR. The homology between AKR1B10 and AR is 69% [139,148,149]. These two enzymes also share similarities in the architecture of their active sites. As common features of AKRs, AKR1B1 and AKR1B10 have a $(\alpha/\beta)8$-barrel core motif and a highly conserved catalytic tetrad in the active site, which is composed of residues Asp43, Tyr48, Lys77, and His110, and the active site, the neighbouring Trp111 is Trp112 in AKR1B10 (AKR1B1 numbering) [139,150]. This homology provides a challenge for inhibitors to selectively distinguish these two enzymes by the inhibitors. Aldehyde reductase is involved in the reduction of highly reactive toxic 2-oxalodehyde, for example, 3-deoxyglucosone and methylglyoxal. Under hyperglycemic conditions, these aldehydes are produced in high quantities, resulting in tissue and vascular damage if gone unchecked [151,152]. Thus, the similarity in the active sites presents a high challenge to the inhibitors, since the inhibition of aldehyde reductase instead of or along with AR would hamper the reduction in these harmful byproducts, which could ultimately result in hepatotoxicity, one of the most observed side-effects of the ARIs in clinical trials.

A concise comparison of AKR1B1 (AR), AKR1B10, and AKR1A1 relevant to inhibitor selectivity is provided in Table 1.

Table 1. Comparative summary of AKR1B1 (aldose reductase, AR), AKR1B10, and AKR1A1 (aldehyde reductase), highlighting features most relevant to inhibitor selectivity.

Features	AKR1B1 (Aldose Reductase; AR)	AKR1B10	AKR1A1 (Aldehyde Reductase)
Protein information			
a. UniProt id b. Length (amino acids) c. Molecular weight (kDa)	P15121 316 35.9	O60218 316 36	P14550 325 36.6
Subcellular localization and expression (high levels)	Cytosol; broad expression; high in lens/peripheral nerve and present in retinal neurovascular unit (cellular and stage dependent) [39,44,45,77,111,124]	Cytosol, and also secreted from the Lysosome; higher in liver/intestine; inducible in multiple pathologies [129,131]	Cytosol; Broad detox enzyme (liver, kidney, and other tissues); relevant mainly as systemic off-target to preserve detox capacity [46,133]
Molecular function	Oxidoreductase	Oxidoreductase	Oxidoreductase
Sequence/structural relation	Canonical AKR1B family enzyme implicated in diabetic complications; drug design target in DR [39,44,45,139]	Closest homologue (71%/83% sequence identity/similarity to AR), frequently cross-inhibited by ARIs [65,132,139]	More distance AKR1A subfamily enzyme (49%/69% sequence identity/similarity to AR); off-target risk for some ARIs (detox function) [39,46,133]
Main physiological role/main substrate	Lipid peroxidation-derived aldehydes (e.g., 4-HNE); glucose (under hyperglycemia) [16,18,104,105]	Endogenous carbonyl substrates; lipid metabolism (including steroids) [65,153]	2-oxoaldehydes (e.g., 3-deoxyglucosone) [113,151,152]
Active site characteristics	The architecture of the active sites also shares similarities between these two enzymes (although with subtle pocket/loop differences). As common features of AKRs, AKR1B1, and AKR1B10 show a (α/β)8-barrel core motif and a highly conserved catalytic tetrad in the active site, which is composed of residues Asp43, Tyr48, Lys77, and His110 and the active site, the neighbouring Trp111 is Trp112 in AKR1B10 (AKR1B1 numbering) [132,139,150]		AKR1A1 presents notable differences with respect to AKR1Bs: (i) it lacks the hyper-reactive active site cysteine (Cys298 in AR) and the Nε of the imidazole ring of the active site histidine interacts with the amide side chain of the nicotinamide ring of NADPH; (ii) the size of loop C is nine residues longer than that of AKR1Bs, determining a rather distinct substrate specificity and inhibitor selectivity [46,146]
Implication on ARI	Need to balance inhibition of pathological polyol flux with preservation of detox capacity; functional triage recommended (glucose vs. aldehyde substrates) [16,47,128]	Major cross-target in selectivity panels; counter-screening and structure-guided optimization are essential [30,132,148]	Safety-relevant detox off-target; include in selectivity/safety profiling to avoid impairing aldehyde clearance [39,46,133]

Note: Entries summarize commonly discussed trends in the AR/AKR literature; quantitative differences depend on assay substrates/conditions and should be confirmed in matched enzyme panels.

Understanding the three-dimensional structure and catalytic landscape of AKRs is crucial for the rational design of selective inhibitors, especially in the case of AR (as promptly realized by the initial structures from 1992 and the immediate mention of rational design of specific inhibitors by Wilson and colleagues [136]), and its split between the invariant (anion-binding pocket) and its more flexible part ("specificity" pocket) [142,143]

(Figure 3). The clinical failure of classical ARIs has often been attributed, at least in part, to limited selectivity, specificity, and off-target interactions due to the conserved nature of the AKR active site. However, exposure/tolerability constraints and trial design also likely contributed [27,43]. Taking into account the flexibility of the protein and the different binding modes for distinct ligands, selective inhibitors are still plausible and merit further research [27,49]. The following section reviews the limitations of current ARIs and explores how emerging strategies like fragment-based design may overcome these barriers.

3.2. Current AR Inhibitors

Due to the involvement of AR in various diseases, substantial effort over the last fifty years has aimed to design and synthesize molecules that inhibit pathological AR activity. Proposed inhibitors span multiple chemotypes, but some features are constant. ARIs usually present a polar moiety that interacts with the anion binding pocket and a hydrophobic moiety that interacts with the non-polar region/induced cavity of the active site [31,141,145,154–156]. Most ARIs are divided into three broad classes: compounds that contain cyclic imides, carboxylic-acid derivatives, and polyphenol compounds, and these have been nicely reviewed in recent publications [27–29,31,157], and include well-studied representatives such as Sorbinil and Fidarestat (cyclic imides), Epalrestat and Tolrestat (carboxylic acid derivatives), and Curcumin and Quercetin (polyphenols) [27] (Table 2). Several synthetic ARIs have been developed since the 1970s, starting with alrestatin and sorbinil, but most were discontinued due to toxicity or limited efficacy [158,159]. The only ARI that remains in clinical use is epalrestat, approved in Japan since 1992 [160–162]. More recently, second- and third-generation ARIs such as fidarestat, ranirestat, and govorestat have been evaluated, though none have yet gained global approval [163–166].

Table 2. Representative aldose reductase inhibitors (ARIs) and key development attributes relevant to selectivity, safety, and clinical translation.

Drug Name (Class/Scaffold Representative)	Trial Phases	Main Indications Studied	Trial Period (Approx)	Key Outcomes	Development Status
Alrestatin (Carboxylic acid derivative)	Phase I/II	Diabetic neuropathy	~1978–1983	Hepatotoxicity, rash	Terminated
	Trial References: [158,167–169]				
Sorbinil (Cyclic imide)	Phase I–III	Diabetic retinopathy and neuropathy	1981–1985	Hypersensitivity (7–10%), limited efficacy	Discontinued
	Trial References/NCT IDs: [159,170–172], NCT00000159 (ClinicalTrials ID)				
Tolrestat (Carboxylic acid derivative)	Phase III	Diabetic neuropathy and retinopathy	Mid-1980s	Initial efficacy, severe liver toxicity	Approved then Withdrawn (1997)
	Trial References: [173–177]				
Epalrestat (Carboxylic acid derivative)	Phase II/III/IV	Diabetic neuropathy	1990s–ongoing	Safe, effective for long-term use	Approved (Japan, India, others)
	Trial References: [36,160–162,178–181], NCT03244358 and NCT04925960 (ClinicalTrials ID)				
Fidarestat (SNK-860) (Cyclic imide)	Phase II	Diabetic neuropathy	Late 1990s–early 2000s	Improved nerve conduction, discontinued further dev	Not approved
	Trial References: [163,164]				

Table 2. *Cont.*

Drug Name (Class/Scaffold Representative)	Trial Phases	Main Indications Studied	Trial Period (Approx)	Key Outcomes	Development Status
Ranirestat (AS-3201) (Fluobenzenes)	Phase III	Diabetic sensorimotor polyneuropathy	2005–2012	Improved motor nerve conduction; sensory effect negative	Discontinued
	Trial References/NCT IDs: [165,182], NCT00101426 (ClinicalTrials ID)				
AT-007/AT-001 (Govorestat/Caficrestat) (Fused ring heterocyclic)	Phase I/II/III	Diabetic cardiomyopathy (not neuropathy/retinopathy)	2018–2024	Subgroup benefit (Phase III), primary endpoint unmet	Phase III readout complete
	Trial References/NCT IDs: [166], NCT04083339 (ClinicalTrials ID)				

Note: "Selectivity" is summarized qualitatively from reported biochemical/structural and clinical literature; quantitative selectivity depends on assay conditions and comparator enzymes (namely, AKR1A1 and AKR1B10) and should be confirmed in matched panels and, ideally, in vivo target-engagement studies.

A comparative overview of representative AR inhibitors (selectivity, safety/tolerability, and clinical development status) is provided in Table 2 to contextualize historical limitations and highlight design constraints for next-generation, retina-directed AR modulation.

3.2.1. Major Limitations of ARIs

Currently, the best therapies available for DR focus on antagonists of the vascular endothelial growth factor (VEGF) signalling pathway and on laser photocoagulation that closes microaneurysms. These therapies address later stages of disease; therefore, paradigm-shifting DR therapies will need to tackle early changes before outright microvascular lesions, and thus AR remains a worthy target [7]. Despite considerable efforts, virtually all synthesized ARIs failed as drugs for the treatment of diabetic complications; Epalrestat remains the only marketed ARI in routine clinical use (approved in Japan) [160–162]. Potential reasons for failure include poor bioavailability, undesirable pharmacokinetics, and the occurrence of adverse side effects, such as hepatotoxicity [27]. Furthermore, AR shares overlapping substrate specificities, and the challenge of achieving selectivity is compounded by the high sequence homology with other aldo–keto reductases [183]. Structural studies illustrate this complexity: In AR holoenzyme, Trp111 is located between the anion binding pocket and the specificity pocket, with its side chain always oriented toward the anion binding site and stabilized by hydrophobic interactions with Leu300, whereas in AKR1B10, the corresponding Trp112 can adopt an alternative orientation stabilized by a hydrogen bond network centred on the Gln114 [132]. This increases the likelihood of cross-reactivity. Importantly, off-target inhibition of AKR1B10 (and AKR1A1) may be problematic because these enzymes contribute to detoxification of reactive carbonyls and lipid metabolism, including retinoid and isoprenoid homeostasis under hyperglycemic conditions [128,133]. These limitations underscore the need for novel inhibitor design strategies that can overcome the twin challenges of selectivity and functional preservation of AR physiological roles, without disturbing the conserved catalytic core shared across the AKR family, and that will look into more than just inhibitory potency for the production of sorbitol [128]. From a translational perspective, this argues for early demonstration of target engagement in relevant tissues and for optimization of compound reach ocular compartments, alongside careful monitoring of detox pathways that could be compromised by broad AKR inhibition. This is particularly important when considering chronic administration in preventive settings [184].

Beyond target biology, several factors likely contributed to limited efficacy and/or failed translation of historical ARIs in DR, and should be treated as explicitly designed constraints for next-generation programmes, namely (i) retinal exposure constraints: systemic dosing may not achieve sustained free drug levels across the blood–retinal barrier and within the relevant retinal compartments [19]; (ii) dose/tolerability ceilings: hypersensitivity and other adverse events limited achievable exposure for several ARIs, restricting the therapeutic window even when biochemical potency was adequate [27,155,156,184]; (iii) disease stage and endpoints: many trials target established microvascular disease, where upstream metabolic modulation may be too late, and clinical endpoints may have been insensitive to early disease modifications [9,12,18]; (iv) patient heterogeneity and background therapy: variable polyol-pathway contribution, concomitant cardiometabolic drugs, and lack of stratification may have diluted the signal in unselected cohorts [7,9,12,184]; and (v) limited pharmacodynamics confirmation in ocular tissue: robust target-engagement or pathway-suppression biomarkers in the retina were rarely integrated, making it difficult to distinguish target failure from exposure or design failure [19,21,184].

3.2.2. Translational Positioning of Selective/Differential AR Modulation in Current DR Care

Current DR management is centred on systemic metabolic control and on local, late-stage interventions (anti-VEGF therapy for diabetic macular edema and proliferative disease, and laser photocoagulation), which address microvascular consequences but do not directly modulate early metabolic stressors [7,9,12]. A selective/differential AR modulator would therefore be most logically positioned as an early-stage, disease-modifying strategy aimed at slowing progression from mild-moderate non-proliferative diabetic retinopathy (NPDR), or as an adjunct to standard care in patients with residual progression risk despite optimized control [7,12].

From a development standpoint, this implies (i) prioritizing endpoints and imaging readouts sensitive to early retinal dysfunction, and (ii) embedding ocular pharmacokinetics and retinal target engagement into first-in-humans and proof of concept designs, given the blood–retinal barrier and the compatibility of relevant cell types [19]. Where feasibility, pathway-suppression biomarkers (e.g., sorbitol/fructose flux proxies in ocular fluids or surrogate tissue) and stage-aware patient stratification should be incorporated to avoid repeating historical trial-design limitations [9,18,19,184].

Finally, combination positioning should be considered explicitly: metabolic modulation may synergize with vascular-target agents by reducing upstream inflammatory/oxidative pressure, but concomitant cardiometabolic therapies can also confound subgroup responses, reinforcing the need for prospective stratification and exposure-response analyses [12,184].

3.2.3. New Strategies for Aldose Reductase Inhibitor Design

Several approaches have been used for ARIs or drug design, such as High-Throughput Screening (HTS), structure-based or ligand-based drug design (SBDD, LBDD), machine learning (ML) models, and screening of natural compounds. Since the discovery of the 3D structure of AR, molecular modelling studies such as SBDD have been reported. Molecular modelling studies are useful to give insightful information into the structure of the enzyme-bound inhibitor and its inhibition mechanisms. For instance, Wang and colleagues (2013) adopted a structure-based virtual screening strategy using several crystal structures of AR to represent its flexible active site [185]. By docking compound libraries against multiple receptor conformations, they identified several novel inhibitors with IC_{50} values in the low micromolar range and demonstrated favourable selectivity over the related enzyme AKR1A1. While this multi-conformational approach accounted for the enzyme's

dynamic pocket architecture, the identified compounds still fell short of progressing to clinical application, often limited by bioavailability, metabolic instability, or lack of efficacy in vivo [185]. Similarly, the study done by Michael Eisenmann and colleagues in 2009 used a structure-based drug-design approach to optimize the novel ARIs identified through virtual screening [186]. They integrated both computational and experimental methods to enhance the potency and selectivity of ARIs to develop novel candidates for the treatment of diabetic complications. They employed ultrahigh-resolution crystal structures of AR complexed with inhibitors such as IDD-594 to guide structure-based optimization. Using these structural insights, they conducted virtual screening to identify new scaffolds with low micromolar to nanomolar IC_{50} values. The initial hits were refined by simplifying chemical structures and synthesizing derivatives, with X-ray crystallography confirming their improved binding modes. Despite this rational and targeted approach, none of these candidates progressed successfully through preclinical development [186], maybe partially because the many conformations determined or generated still do not capture the full breadth of AR flexibility and adaptation to different ligands, significantly complicating structure-based design of inhibitors.

Similarly, other studies using combinatorial libraries, natural product screening, and rational design approaches have yielded promising leads in vitro but failed in later stages of development. This lack of clinical success, often due to off-target and toxicity effects, underscores the need for alternative strategies (such as FBDD and differential inhibition approaches) that aim to preserve the physiological function of AR while selectively inhibiting its pathological activity [47]. Unlike HTS, which relies on large, complex molecules, FBDD begins with small fragments that interact in an "atom-efficient" manner, enabling the detection of subtle but exploitable structural differences. Such differences, including those in sub-pocket plasticity and residue orientations (e.g., Trp111/Trp112 positioning), can be used during fragment elaboration to achieve differential inhibition of AR, while sparing homologous enzymes like AKR1A1 and AKR1B10. This strategy not only reduces chemical complexity and cost but also enhances the likelihood of achieving selective and physiologically compatible inhibition. FBDD has been very successful in discovering several drugs where traditional high-throughput screening methods have limitations [187,188]. In this review article, we propose a new strategy to screen the inhibitors with the FBDD approach, along with further structural validation of the proposed inhibitor with Microcrystal Electron Diffraction (MicroED). MicroED can resolve high-resolution conformational states of AR-fragment complexes that are difficult to capture through conventional crystallography, thereby guiding rational fragment growing, merging, or linking to achieve inhibitors with both selectivity and clinical translatability.

4. Emerging Tools in Rational Drug Design: Fragment-Based Drug Discovery and MicroED

To address the limitations of traditional strategies for AR inhibitor development (most notably incomplete selectivity, uncertain retinal exposure, and limited structural feedback during lead optimization), below we explore two emerging techniques that may overcome the previous challenges: fragment-based drug discovery (FBDD) and microcrystal electron diffraction (MicroED). We distinguish what is already demonstrated from what remains prospective for AR, and we outline a practical workflow that keeps selectivity and retinal translation as explicit design constraints.

Rather than reiterating generic methods overviews, we emphasize AR-specific decision points: which binding modes provide realistic selectivity vectors (beyond conserved-site potency), how to triage weak fragment hits with orthogonal assays, and how rapid structural readouts can shorten optimization cycles.

MicroED has already resolved protein-ligand complexes from microcrystals in other systems (such as acetazolamide soaked into carbonic anhydrase II microcrystals, and bevirimat to an HIV-1 Gag CTD-SP1 construct), demonstrating that ligand placement can be recovered from crystals far smaller than those typically used for X-ray crystallography [189,190]. To our knowledge, MicroED has not yet been reported for AR-ligand complexes; for AR, it should therefore be viewed as a complementary option when crystallization yields only microcrystals or when soaking compromises larger crystals, preserving structure-guided iterations rather than delaying it [191,192].

4.1. Fragment-Based Drug Discovery (FBDD)

FBDD usually starts with the screening of a relatively small compound library comprising compounds with low molecular weights, up to 300 Da, called fragments, that efficiently sample binding hot spots; validated hits are then elaborated by fragment growing, linking, or merging guided by structure and ligand efficiency (Figure 4) [187,188,193,194]. For AR, this approach is attractive because the enzyme is biochemically robust and crystallographically tractable, and presents inducible subpockets adjacent to the conserved catalytic site that can be exploited for selectivity [141,143,195].

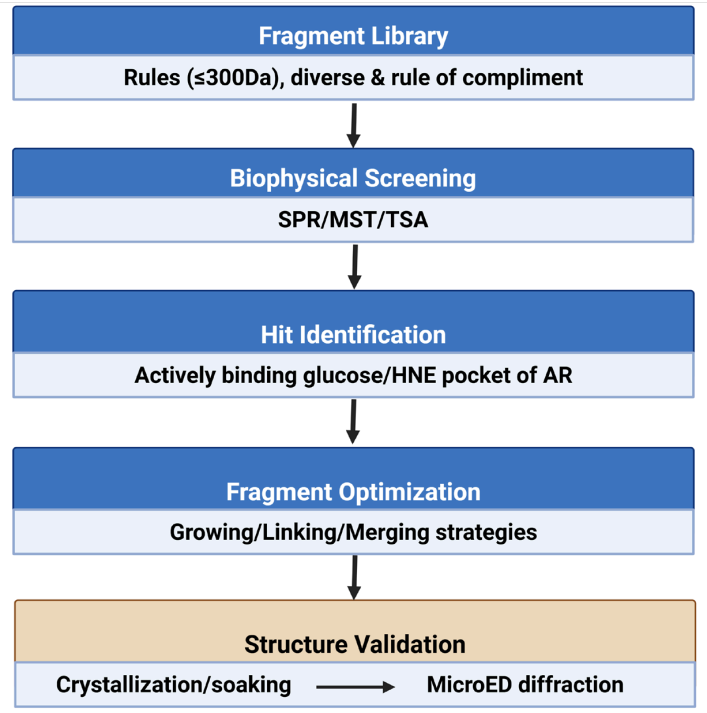


Figure 4. Workflow of FBDD and structural validation.

After fragment library selection, hits are screened in parallel (biophysical plus functional assays), prioritizing by orthogonal confirmation, and advanced to structural follow-up [188,196–198]. Because fragment binding can be weak and artefact-prone, early triage against aggregation and assays interface is essential before medicinal-chemistry iteration [199–202]; we also recommend early counter-screening against relevant AKRs (as explained in previous sections, AKR1B10 and AKR1A1) once a tractable series emerges.

Virtual screening (VS), a computer-based method for predicting the binding compounds from a compound library to a target protein, which includes docking, virtual fragment screening, and hotspot mapping, can help prioritize fragments and suggest growth vectors [203,204]. However, for AR, it should be treated as hypothesis generation

and validated experimentally, particularly for highly polar chemotypes and the influence of the NADPH/NADP+ cofactor state [135,141,185].

4.1.1. Fragment Screening Biophysical Detection

Fragment screening typically uses a combination of sensitive biophysical assays (such as Surface Plasmon Resonance (SPR), Thermal Shift Assay (TSA), and Microscale Thermophoresis (MST)), with an orthogonal functional readout and, when possible, structural confirmation. Below, we briefly summarize the strengths and limitations of these approaches for fragment triage in AR [196,205,206].

SPR provides real-time binding kinetics [207]. For AR, SPR is useful to rank fragment binders and thus prioritize hits [197]. However, since it is a surface method, it may suffer from assay-format artefacts; thus, orthogonal confirmation is necessary [208]. TSA is a reliable technique to measure the denaturation temperature of a protein and offers a low-material screen to identify stabilizing fragments. For AR, TSA is best used for triage because ΔTm values can be small and may not correlate linearly with affinity [209,210]. MST is a well-established biophysical technique used for any kind of biomolecular interactions, quantifying binding in solution with modest material needs [211,212]. For AR, SPR is useful to prioritize fragments and nonspecific binders, and MST can support early cross-isoform comparisons.

Macromolecular X-ray analysis is a key method for FBDD, as it remains the most direct way to confirm fragment poses and define growth vectors, including expansions into inducible pockets [213,214]. By this method, opportunities are given to conduct structure-based design studies to improve the ligand affinity efficiently [198,215,216]. The crystal can be achieved by two different methods: co-crystallization or soaking. Both methods are challenging but achievable, since not all proteins are easily crystallized, and some ligands can disrupt the crystal lattice [217]. For AR, generating a co-crystal, which is aimed to be small in size, allows MicroED to provide complementary structural information [191,192].

4.1.2. Why AR Is Particularly Well Suited for FBDD

AR is particularly well suited to fragment discovery because the catalytic site contains well-defined anchoring interactions (that support ligand-efficient fragment binding), which are in close proximity to a conformational plastic "specificity pocket" offering a route to isoform selectivity [141,145]. Moreover, extensive structural precedent across inhibitor classes provides multiple reference confirmations for pose interpretation and fragment-growth strategy [218,219].

From a design perspective, the key opportunity is to retain conserved-site anchoring needed for potency while using fragment exit vectors that probe inducible volume [141,220]. This helps avoid a common failure mode in which potency improves through interactions shared across AKRs without adding an explicitly determinate [188,194].

At the pocket level, fragments that anchor in the catalytic region can be ranked by whether they stabilize alternative conformations that open adjacent inducible volume and by whether their exit vectors point towards regions that differ across AKR isoforms [141,146]. In practice, for AR will be advantageous to prioritize series that (i) retain a consistent anchor pose across cofactor states, (ii) provide a clear growth vector into inducible volume, and (iii) can be elaborated without excessive polarity that would compromise cellular and retinal exposure.

Accordingly, fragments can be prioritized not only by affinity and ligand efficiency but also by vectors that probe inducible subpockets while preserving conserved-anchoring sites [221,222]. That will support a differential modulation strategy that aligns with an AR retina-focused translation.

4.2. Microcrystal Electron Diffraction (MicroED)

While X-ray crystallography remains the gold standard for structural-guided medicinal chemistry, Microcrystal Electron Diffraction (MicroED), which is a CryoEM diffraction method, has emerged as a powerful complementary technique, capable of resolving atomic details from extremely small crystals that are otherwise unusable for conventional X-ray methods [191,192,223]. In the drug discovery context, MicroED has demonstrated bound-ligand structure determination from microcrystals in other targets [189,190], supporting feasibility when only microcrystals are available or when larger crystals are fragile. For AR, MicroED could therefore help maintain continuous pose confirmation during fragment-to-lead optimization, particularly by using small macromolecular crystals, which may have fewer defects and lower mosaicity than larger crystals, and any external changes, such as ligand soaking or rapid flash freezing, can be easily and uniformly applied [224,225]. Moreover, such a solution also addresses a practical bottleneck in FBDD campaigns, where soaking or co-crystallization can readily produce many small crystals, but obtaining fewer, larger crystals suitable for conventional diffraction may require interactive optimization that slows medicinal-chemistry cycles [217,226]. Because MicroED can work with micro-crystals, it can preserve throughput for pose confirmation and growth-vector decisions, enabling rapid elimination of non-productive series and reducing the time spent optimizing crystal size rather than chemistry [227] (Figure 5).

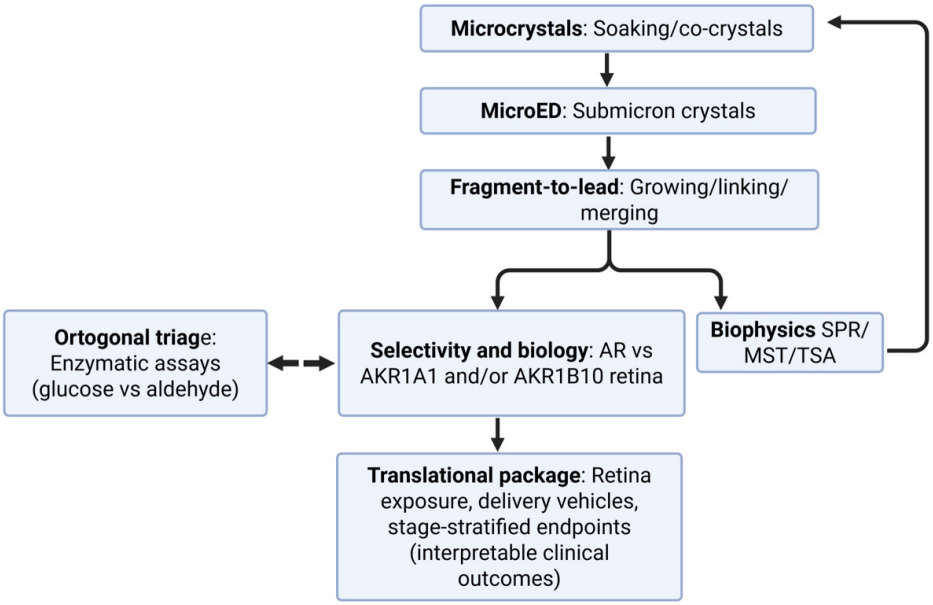


Figure 5. AR-tailored FBDD-MicroED workflow to develop selective/differential AR modulators. Fragments are first identified by orthogonal biophysical methods and then triaged by functional assays that compare glucose/polyol flux versus aldehyde-substrate turnover. Microcrystal soaking/co-crystallization followed by MicroED enables rapid structure determination from submicron crystals and captures ligand poses and conformational states. Interactive fragments growing/merging/linking are guided by structures and selectivity profiling against AKR1A1/AKR1B10 and are coupled to a translational package emphasizing retinal exposure, target engagement biomarkers, and stage-stratified endpoints.

4.3. Proposed Strategy: Integrating FBDD and MicroED for Aldose Reductase Inhibitor Design

Drug discovery remains a critical aspect of structural biology and pharmaceutical development [228]. In the AR context, the goal is to translate structural insight into selective, retina-relevant modulation while minimizing liabilities that undermined prior ARI programmes (selectivity, exposure, and trial-design mismatches) [32,41,44,45]. This motivates

an AR-centred workflow with early orthogonal validation and continuous structure-guided iteration [188,196,206].

As described above, AR exhibits overlapping substrate specificity with related AKRs and contains both conserved interactions and inducible volumes that may be leveraged for selectivity [139,141,147]. We therefore propose an AR-focused workflow in which: (i) fragments are screened using at least two orthogonal biophysical assays plus an enzymatic readout; (ii) confirmed hits are triaged for tractable growth vectors that probe inducible subpockets; (iii) binding poses are solved by MicroED; and (iv) fragment growth, linking and merging is guided by explicit selectivity hypotheses and parallel profiling against relevant AKR isoforms.

Although aldose reductase is expressed in many tissues, its contribution to retinal pathology appears to be context dependent [18]. In the retina, elevated AR activity has been reported in multiple cell types, including retinal endothelial cells, pericytes, and Muller glial; cell populations that are particularly sensitive to metabolic and oxidative stress [12,22,112]. This cellular vulnerability, together with the retina's high metabolic demand and limited oxygen reserves, may render AR-driven metabolic disturbances disproportionately injurious in the eye. From a translational perspective, effective retinal AR inhibition is additionally constrained by ocular barriers to drug delivery: the blood-aqueous barrier, and for the posterior segment, the blood–retinal barriers, which restricts entry of administered agents [19,43,229,230]. Many historical ARIs were optimized for systemic use rather than sustained intra-retinal exposure and target engagement, raising the possibility that subtherapeutic retinal concentrations and systemic side effects contributed to limited efficacy in clinical trials [43,231,232]. These considerations suggest that the retinal relevance of AR may reflect selective cellular vulnerability and drug accessibility rather than retina-exclusive expression; within this framework, fragment-based drug design may provide a rational approach for developing AR inhibitors with physicochemical properties better aligned with retinal and durable intra-retinal target engagement.

To implement this workflow efficiently, we recommend a screen > orthogonal confirmation > structure determination > iterate cycle. Enzymatic assays help against assay interference, while structural methods drift into non-productive chemical space (Figure 6).

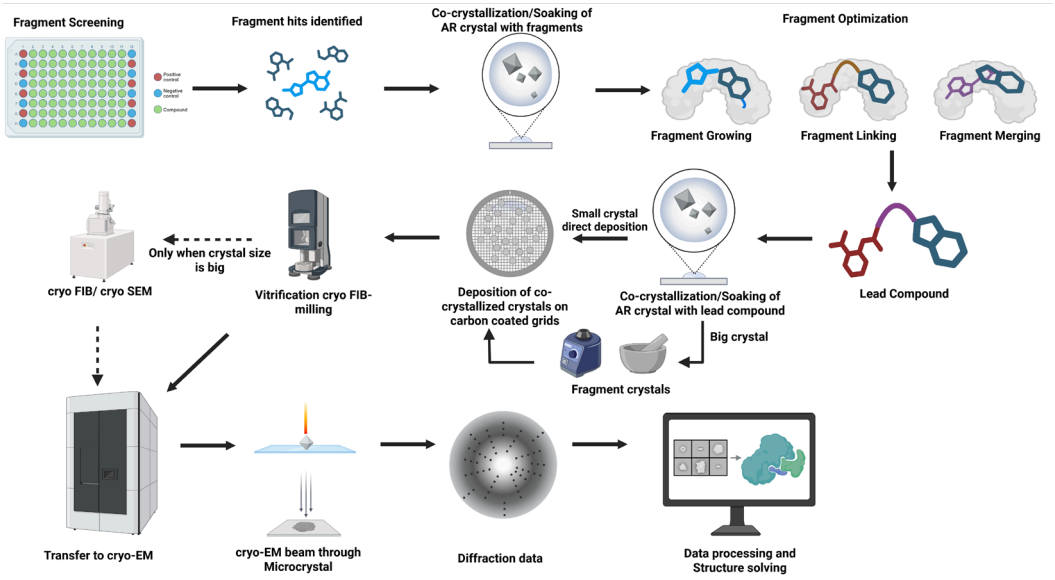


Figure 6. Schematic representation of fragment optimization to structure validation. Dotted arrow strategy is applicable only in case of big/large crystals.

4.4. Fragment Optimization Strategies for Selective Aldose Reductase Inhibition

Following the identification of fragment hits that differentially bind to AR (relative to closely related AKRs), the next critical step is fragment optimization. Canonical strategies include fragment growing, linking, and merging [188,233] (Figures 4 and 6). For AR, optimization should be driven by selectivity, thus requiring deliberate use of growth vectors that reach beyond the conserved catalytic site into inducible volumes [141,143,146], while controlling polarity and permeability for tissue exposure [234,235].

Fragment growing is often the most practical first step: substituents are added to validated anchor fragments to improve potency while preserving key interactions [188,233,236,237]. For AR, growth is informative when it tests vectors towards inducible subpockets (in particular the "specificity pocket"), because growth confined to the conserved site can increase potency without improving selectivity [27,141–143,146,155].

Fragment linking joins two non-competitive fragments (fragments which bind in two proximal sites) to gain affinity through additive interactions, but requires precise geometry and can rapidly increase size and polarity [188,234,238–240]. For AR, linking will be attractive when both fragments are structurally confirmed and the resulting scaffold will remain compatible with the active-site topology, and will need to be selectively tested against related AKRs in parallel with potency [141,146].

Fragment merging combines overlapping features from related fragments into a single scaffold that captures the best interactions while avoiding excessive size [188,222,236,241]. For AR, is particularly useful when multiple fragment series preserve the same anchor interactions but differ in how they sample adjacent inducible volume, allowing a rational merge that prioritizes selectivity vectors [132,141,195,219].

Overall, integrating growing, linking, and merging offers a versatile toolkit for designing differential AR inhibitors. Each step should be driven by structural validation and explicit selectivity hypotheses (which subpocket or conformation is being targeted), rather than potency optimization alone [146,188,195,198,206].

4.5. Structural Validation of Optimized Leads Using MicroED

Once fragment hits are optimized, confirming that medical-chemistry changes produce the intended binding mode is essential to avoid misleading structure–activity relationship (SAR) [242]. In AR, where conformational plasticity can alter accessibility of inducing subpockets, interactive structural checks help ensure that selectivity vectors remain engaged as potency increases [30,142,147].

Traditionally, X-ray crystallography has been the gold standard for structural validation, but it may be limited by crystal size, soaking tolerance, or crystallization bottlenecks [217,226,228,243]. Recent advances in microcrystallography [244], such as microfocus X-ray beams, serial femtosecond crystallography (SFX), and especially microcrystal electron diffraction (MicroED), have dramatically improved the ability to study small crystals [244,245]. MicroED, in particular, enables high-resolution structure determination from crystals as small as a few hundred nanometers, overcoming key barriers in traditional methods [223,246]. It also allows for rapid and uniform soaking of ligands or fragments, essential for validating differential binding modes in dynamic enzymes like AR. This advantage can also be explored in dedicated microfocus X-ray beamlines such as VMXm in Diamond Light Source [244,247], or taking full advantage of their VMXi beamline and Xchem platform, which couples it with in situ diffraction and fragment screening [248], but electron scattering is dictated by the electrostatic potential of electrons and nuclei, while X-ray photon scattering is dictated by electron clouds around individual atoms. Thus, for drug development, MicroED brings the added advantage of potentially resolving electrostatic potential maps and hydrogen positioning, revealing binding modes [244,249,250].

Additionally, MicroED's compatibility with routine cryo-EM instruments, which, with the spread of cryoEM instrumentation, increases its reach compared to microfocus-equipped synchrotrons [251], and its lower sample volume requirement (as electrons are charged particles and interact with matter much more strongly than X-ray photons, producing less damage per scattering event, allowing crystal volumes a billion times smaller [246,251]), helping to maintain continuous pose confirmation during fragment-to-lead optimization, makes it highly suitable for fragment screening programmes where obtaining large crystals is not feasible [191,192]. For AR, we therefore view MicroED as a complementary option that can preserve the pace of structure-guided iteration, rather than delaying chemistry while crystal size and robustness are optimized [192,217].

4.6. Application Workflow: Using MicroED to Guide Differential Inhibitor Design

In our proposed AR-MicroED workflow, optimized fragments/leads are co-crystallized or soaked into pre-grown AR microcrystals [217,226], transferred to CryoEM grids, and vitrified. Continuous-rotation MicroED data collection yields high-resolution diffraction suitable for placing ligands and evaluating binding interactions [252,253]. The structural readout is then used to confirm whether substitutes engage the intended residues and whether binding extends into inducible subpockets proposed to drive selectivity; this information is fed back into SAR and selectivity profiling to guide further design cycles [189,190].

5. Conclusions and Future Perspectives

Despite decades of effort, the development of clinically effective ARIs remains an unsolved challenge, primarily due to issues of selectivity, bioavailability, and adverse off-target effects. Classical approaches such as HTS and rational drug design have identified promising leads, but these have rarely translated into viable therapies beyond epalrestat, which itself has limited availability. In this review, we listed the current short comes have highlighted how emerging tools such as FBDD and MicroED can be harnessed to overcome these limitations. FBDD offers a structurally efficient and chemically tractable way to identify fragment hits that may exhibit selective binding to distinct sub-pockets within the AR active site. When integrated with MicroED, a technique capable of resolving atomic-level detail from nanocrystals, this pipeline enables high-resolution structural validation of fragment binding even in cases where classical X-ray crystallography is not feasible.

Looking forward, this combined FBDD-MicroED approach opens new avenues for developing differential inhibitors of AR that preserve its physiological detoxification role while selectively suppressing its pathological activity in diabetic complications. Future work should focus on expanding structurally diverse fragment libraries tailored to the unique architecture of AR, validating binding through multi-technique pipelines, and iterating lead optimization using real-time structural feedback.

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