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REVIEW

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Cancer to Cataracts: The Mechanistic Impact of Aldo-Keto Reductases in Chronic Diseases

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Aldo-keto reductases (AKRs) are a superfamily of promiscuous enzymes that have been chiseled by evolution to act as catalysts for numerous regulatory pathways in humans. However, they have not lost their promiscuity in the process, essentially making them a double-edged sword. The superfamily is involved in multiple metabolic pathways and are linked to chronic diseases such as cataracts, diabetes, and various cancers. Unlike other detoxifying enzymes such as cytochrome P450s (CYP450s), short-chain dehydrogenases (SDRs), and medium-chain dehydrogenases (MDRs), that participate in essential pathways, AKRs are more widely distributed and have members with interchangeable functions. Moreover, their promiscuity is ubiquitous across all species and participates in the resistance of pathogenic microbes. Moreover, the introduction of synthetic substrates, such as synthetic molecules and processed foods, results in unwanted "toxification" due to enzyme promiscuity, leading to chronic diseases.

INTRODUCTION

The biochemical foundation of life encompasses the ability to evade entropy (death) and trigger its successful duplication. This is guaranteed by the specificity of enzymes that participate in streamlined regulation of metabolites, thereby securing the organism's survival. However, promiscuity also plays an important role in housekeeping, as it involves the detoxification of extraneous molecules that could hamper survival and growth [1,2]. Aldo-keto reductases (AKRs) are one of the promiscuous superfamilies along with other detoxifying enzymes like short-chain (SDR), glutathione-S-transferases (GSTs), medium-chain dehydrogenases (MDRs), cytochrome P450s (CYP450s) etc. AKRs cause multiple chronic diseases like cataracts, diabetes, liver disease, various cancers etc. [3,4]. Despite numerous studies detailing their metabolic functions, detoxification processes, and involvement in chronic diseases, a comprehensive correlation among these areas remains largely unexplored. AKRs play a crucial role in several fundamental metabolic and detoxification pathways, which are vital for the survival of higher eukaryotes [5]. However, the rise in consumption of synthetic molecules (drugs, narcotics, flavoring agents, processed foods) sometimes lead to unfavorable consequences due to the promiscuity of AKRs. While other oxidoreductases like CYP450s, SDRs, and MDRs also participate in core metabolic pathways, AKRs are comparatively more ubiquitous [6-8].

For example, in core biosynthetic pathways like retinoic acid biosynthesis, MDRs, SDRs, and AKRs act as isozymes and oxidize retinol to retinaldehyde, and then to

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Abbreviations: AKRs, Aldo-keto reductases; CYP450s, cytochrome P450s; SDRs, short-chain dehydrogenases; MDRs, medium-chain dehydrogenases.

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retinoic acid. The three superfamilies share comparable and low Km values for retinoids, ranging from 0.12 to 1.1μM. However, they display substantial differences in their kcat values. MDRs are more effective retinol dehydrogenases while RDH11 (an SDR) and AKR1B10 are efficient retinaldehyde reductases *in vitro*. Interestingly, cell culture studies demonstrate RoDH-4 as the main retinol dehydrogenase and AKR1B1 as the main retinaldehyde reductase hinting at differing expression profiles [9]. However, the rest of the pathway is regulated by specific enzymes that are not housekeeping proteins. Interestingly, no diseases are associated with this pathway concerning AKRs or other isozymes due to specificity of other enzymes. On the other hand, AKRs are present in multiple stages during steroid synthesis and are a major cause for breast, prostate, and testicular cancer due to over-production of androgens and estrogens leading to cell proliferation [10].

Additionally, AKRs are major participants of detoxification specifically redox reactions due to their biodistribution. The intestine and liver are responsible for the first-pass metabolism and bioavailability of orally administered drugs. mRNA and protein expression studies of all human AKRs (AKR1A1, AKR1B1, AKR1B10, AKR1B15, AKR1C1, AKR1C2, AKR1C3, AKR1C4, AKR1D1, AKR1E2, AKR7A2, and AKR7A3) and SDRs (CBR1, CBR3, CBR4, DCXR, DHRS4, HSD11B1, and HSD17B12) revealed that AKRs are highly expressed in the liver and the upper regions of the intestine (duodenum and jejunum), with expression declining toward the rectum. Among SDRs, CBR1 and DHRS4 are highly expressed in the upper regions, while the expression levels of the other isoforms are almost uniform across all regions. In the case of AKRs, AKR1B10 exhibits the highest expression, followed by AKR7A3 and CBR1. Each of these contributes to more than 10% of the total AKR and SDR levels in the small intestine. The expression pattern in the human intestine is markedly different from that in the human liver, where the AKR1C isoforms are primarily expressed. It is clear that AKRs have a more uniform distribution and expression throughout the alimentary canal and its associated organs compared to MDRs and SDRs [10,11]. As a result, they are major participants in performing redox reactions against many chemotherapeutic agents resulting in chemo resistant cancers.

Indeed, AKRs play a significant role in essential pathways and detoxification reactions. Their ubiquitous biodistribution makes them a major contributor to many chronic diseases. This is evident even in lower organisms like *Synechocystis* sp. PCC 6803. Here, the native MDR (DMDR) and AKR (DMAKR) detoxify reactive carbonyls like acrolein. This is demonstrated by the inhibition of growth in DMDR and DMAKR double knockouts when exposed to the compound. However, knocking out these genes also inhibits the organism's ability to perform photosynthesis. This illustrates the adverse effects of enzymes that participate in multiple pathways, a direct result of their ability to catalyze various compounds [12]. The current review describes the multifaceted roles of AKRs and their implications in various chronic diseases, as discussed, are largely influenced by their ability to catalyze various compounds, a characteristic that is closely tied to their promiscuous nature.

AKRs AND PROMISCUITY

The promiscuity of AKRs is largely influenced by the enzyme's structure and function. The active site of an AKR possesses a flexible and adaptable structure that allows it to accommodate different substrates and catalyze diverse reactions. Additionally, the enzyme's active site residues and cofactors are strategically positioned to facilitate specific substrate interactions, thus influencing their promiscuity. Overall, the enzyme structure and function play a crucial role in determining the promiscuity of AKR and its ability to catalyze multiple reactions.

Structural Attributes of AKR Promiscuity

Structural elucidation of AKRs is important for understanding their promiscuous nature. All AKRs share a common structural motif known as a (β/α)8-barrel motif. The β-strands and α-helices are connected by three loops (loop A, B, C). They play a crucial role in the function of AKRs and are located at the back of the barrel and determine the enzyme specificity. They utilize pyridine nucleotides (NADH or NADPH) as cofactors and catalyze the reduction of carbonyl groups (aldehydic and ketonic) to their corresponding alcohols (Figure 1A). Each AKR member's specific structure, including its loops' configuration, contributes to its unique function within the body.

A conserved catalytic tetrad (Y55, L84, H117, D50) participates in a "proton-relay", with tyrosine (Y55) as the general acid/base catalyst. Mutations result in different enzymatic activities. For example, H117E mutants display 5β-reductase activity, while Y55F and Y55S mutants retain quinone reductase activity. The mature steroid binding pocket in the ternary complex structure is comprised of ten residues from five loops. Significant loop movement occurs upon ligand binding. Different binding modes for steroid substrates and nonsteroidal inhibitors may reflect ligand-induced loop movement. These findings could lead to selective and broad-based AKR inhibitors [13].

Some closely related AKRs like AKR1B1 and 1B10 can change their function through a single residue mutation. Both enzymes have a unique Trp112 structure. ARKR1B1 involve the role of external loops in inhibitor

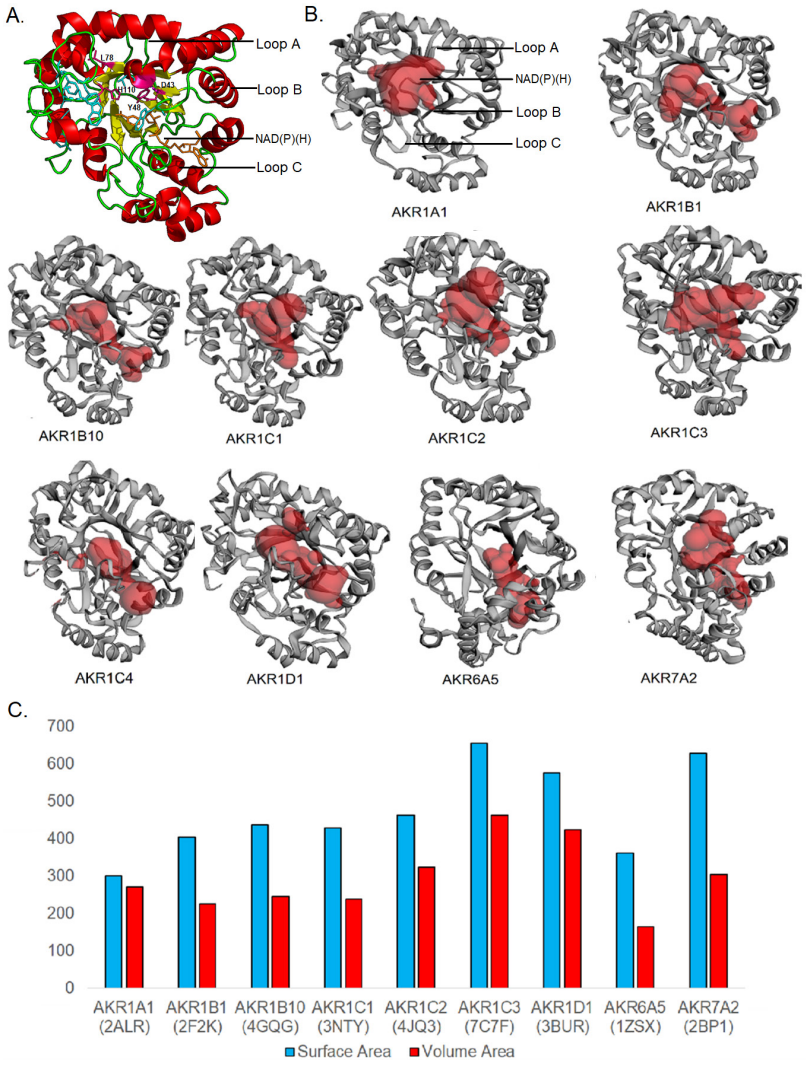

Figure 1. **Structural features of human AKR**. **A**. The general structure of an AKR with (β/α)8-barrel motif where α-helices (Red) and β-sheets (yellow) and loops (green) and active site (magenta) and substrate binding site (cyan). **B**. The substrate space of AKRs shows their diversity (Shown in red). **C**. Chart of the substrate space shows proportional surface area and volume in AKRs.

binding: the alternative conformation of Trp112 (Trp111 in AKR1B1) and loop A mobility, which defines a larger, more loosely packed substrate pocket in AKR1B10. It is stabilized by a G114 hydrogen bond network in AKR1B10. This suggests that AKR1B1 inhibitors could bind to AKR1B10 by flipping Trp112, creating an "AKR1B1-like" active site. The native Trp112 orientation provides a broader active site for selective AKR1B10 inhibitors [14]. The design of inhibitors with unique Markush to uniquely inhibit different AKRs has been a subject of interest and has been addressed in multiple studies [15-18].

In terms of enzymatic activity, AKR1B15 parallels AKR1B10, exhibiting a similar breadth of activity. Two AKR1B10 mutants resulted in the engineered AKR1B15 mirroring the latter's functional features, including preferences towards retinaldehyde isomers, higher activity with steroids and ketones, and unique behavior with inhibitors. The Phe residues in AKR1B15 contribute to a narrower, more hydrophobic site, accounting for its functional uniqueness. AKR1B15 is also an efficient 17β-ketosteroid reductase, unlike AKR1B10 [19]. It is localized in mitochondria and catalyzes 3-keto acyl CoA conjugates [20]. AKR1B15 exhibits superior catalytic efficiency with 9-cis-retinaldehyde. They show higher activity with ketone and dicarbonyl substrates. Several typical AKR inhibitors do not affect AKR1B15 activity due to large substrate site. Amino acid substitutions clustered in loops A and C result in a smaller, more hydrophobic, and rigid active site with a pocket comparable to AKR1B10, which is consistent with distinct substrate specificity and narrower inhibitor selectivity [21]. This shows that the

promiscuity of AKRs with larger substrate binding site making it harder to design inhibitors. A survey of AKR inhibitors, which are designed to inhibit specific AKRs have overlapping IC50s (see Appendix A: Table S1). This only adds to the conundrum of inhibiting specific AKRs for disease treatment [15-17]. The dimension of the substrate binding pocket is an important facet in determining the enzyme specificity.

This functional diversity (despite similarity) is also observed in other mammalian AKRs. For example, the mouse AKR1C19 is 72% similar to human AKR1C1, a steroid dehydrogenase. It reduces α-dicarbonyl compounds such as camphorquinone, istain, 3-hydroxyhexobarbital, S-indan-1-ol, and cis-benzene dihydrodiol, but is inactive towards steroids, prostaglandins, monosaccharides, and other xenobiotic alcohols. In fact, AKR119 functions as a detoxifying enzyme in the liver and gastrointestinal tract [22]. Similarly, the mouse AKR1C20 is 89% similar to mouse liver 17β -HSD type 5 (AKR1C6) but, it has high Km values for testosterone and 5a-dihydrotestosterone, but low values for 3α-hydroxy- and 3-keto-steroids. Hence, it differs from mouse AKR1C6, 3α-HSD (AKR1C14) making it a novel 3α(17β)-HSD and potential reductase for xenobiotic α-dicarbonyl compounds [23]. Furthermore, unlike polyols, acroleins or phospholipids, the steroids are larger molecules and therefore require broader substrate site. This characteristic along with mutational load enable AKRs to behave as different functional enzymes with varying substrate pockets.

Furthermore, some AKRs have clear orthologs like the murine AKR1B3 and human AKR1B1, but no clear orthologs for human AKR1B10 and AKR1B15 have been identified in rodents (Table S2). This signifies the extent of evolution that has occurred during speciation. On the other hand, there is no ortholog in humans for mouse AKR1B16. It has large substrate substrate-pocket compared to other murine AKRs and reduces aliphatic, aromatic carbonyl compounds and oxidizes 17β-hydroxysteroid. AKR1B16 and its rat ortholog AKR1B17 have a unique activity as retinaldehyde dehydrogenase as well. Hence, the biodistribution and large substrate pocket can lead to multiple functions in AKRs [24].

Similarly, AKR1D1, a human steroid 5β-reductase, catalyzes the reduction of Δ(4)-ketosteroids, forming an A/B cis-ring junction crucial for bile acid biosynthesis. However, it has a large substrate pocket and participates in steroid metabolism as well (Figure 1B,C; Table S3). It reduces all C18, C19, C21, and C27 Δ(4)-ketosteroids at physiological pH and is the sole enzyme needed for all 5β-steroid metabolites in humans. Substrate inhibition occurs with C18 to C21 steroids if the C11 position is unsubstituted, due to an alternative substrate binding pocket in AKR1D1's structure [25]. The enzyme has a similar substrate binding pocket as the promiscuous AKR1C3. Consequently, the ability of the enzyme to catalyze multiple substrates is not unexpected.

To conclude, the promiscuity of AKRs is a result of their flexible and large substrate binding pockets, structural variations, and evolutionary adaptations. This allows them to perform a wide range of functions, making them valuable targets for the development of selective and broad-based AKR inhibitors. However, this also poses challenges for inhibitor design. Future research in this area could provide valuable insights into the design of more effective AKR inhibitors.

Mutations and Promiscuity

Enzyme promiscuity often arises through neutral mutations that are not detrimental to primary enzymatic activity. Despite ordinarily being physiologically irrelevant, under new selective pressures these activities may confer a fitness benefit, prompting the evolution of the formerly promiscuous activity to become the new main activity [26]. However, it is important to note that the native activity of an enzyme is often less robust to mutations than the promiscuous activity. For example, in phosphotriesterase, the deleterious effect of individual mutations on the native phosphotriesterase activity is much larger than their positive effect on the promiscuous arylesterase activity. This suggests that the native activity is more sensitive to mutations, which could potentially lead to decrease in the native activity and an increase in promiscuity [27]. Furthermore, the loss of native activity can lead to metabolic disorders as well. This is further compounded by the presence of variants. Genetic drift leads to changes in the frequencies of different gene variants in a population. This randomness, along with selection, contributes to the occurrence of variants in AKRs as well [28,29].

One such variant is AKR1CL1, located between AKR1C3 and AKR1C4 on chromosome 10. There are two different forms of this gene reported in the NCBI database. One form encodes a 129-amino acid peptide, which includes the complete catalytic tetrad but appears to be too short to be a functional AKR. The other form encodes a complete 326-amino acid protein, which shares 68% sequence identity with AKR1C1-4. It remains unclear whether *AKR1CL1* is a processed pseudogene or a new functional AKR1C member [30].

Another variant, AKR1C2, shares 97% sequence identity with AKR1C1 but exhibits a unique expression pattern and substrate specificity. LOC648517 and LOC100134257 are entries that contain sequence elements matching exons of AKR1C1 and AKR1C2. However, their chromosomal location could not be determined, leaving it unclear whether they might be transcript variants of either AKR1C1 or AKR1C2, or separate

Table 1. Distribution, Role, and Associated Diseases of AKR Superfamily

Protein	Name	Tissue distribution	Physiological role	Chronic diseases	Reference
AKR1A1	Aldehyde reductase	Kidney, Liver, Stomach	Detoxification	Fetal alcohol syndrome, Alcohol dependence	[36]
AKR1B1	Aldose reductase (AR)	Liver, Muscle, Kidney, Eyes	Detoxification, osmotic regulation	Diabetic complications: cataract; retinopathy; neuropathy; nephropathy	[69]
AKR1B10	Aldose reductase-like protein 1 (ARL-1)	Small intestine, colon, adrenal gland, Eyes	Detoxification, lipid synthesis, Retinoate synthesis	NSCLC hepatocarcinogenesis	[104,105]
AKR1C1	20 α-Hydroxysteroid dehydrogenase (HSD); dihydrodiol dehydrogenase (DD1)	Small intestine, Lung, Mammary Gland, Prostate	Elimination of progesterone	Pre-term birth endometriosis, Colorectal cancer, Breast cancer, Endometrial cancer, and Pre-term birth NCSCLC	[106,107]
AKR1C2	Type 3 3α-hydroxysteroid dehydrogenase (HSD); bile acid binding protein	Small intestine, Lung, Mammary gland, Prostate	Elimination of 5α-dihydrotestosterone	Androgen insufficiency; Pre-menstrual syndrome	[108,109]
AKR1C3	Type 2 3α- and Type 5, 17β-hydroxysteroid dehydrogenase (HSD); prostaglandin F synthase	Small intestine, Lung, Mammary gland, Prostate	PGF2 synthesis	Advanced prostate cancer, Breast cancer, Acute myeloid leukemia, Acute myeloid leukemia NSCLC, Bladder cancer	[110-112]
AKR1C4	Type 1 3α-type 1 3α-HSD chlordecone reductase	Liver specific	Hepatic clearance of steroids, bile acid synthesis	Paranoia	[113]
AKR1D1	Steroid 5β-reductase, Δ4-3ketosteroid 5β-reductase	Liver specific	Bile acid synthesis	Bile acid deficiency	[114]
AKR6A3	β-Subunits of the voltage-dependent shaker potassium channels Type 1	Brain and heart	Potassium channel component	Aberrant redox regulation of Kev channels and cardiovascular disease	[115]
AKR6A5	β-Subunits of the voltage-dependent shaker potassium channels Type 2	Brain and heart	Potassium channel component	Epilepsy, Impairment of learning and memory	[4,11]
AKR6A9	β-Subunits of the voltage-dependent shaker potassium channels Type 3	Brain and heart	Potassium channel component	Epilepsy, Impairment of learning and memory	[98]
AKR7A2	Aflatoxin aldehyde reductase; succinic semialdehyde reductase	Liver, Kidney	GABA metabolism	Neuromodulator; succinic semialdehyde dehydrogenase deficiency	[116]
AKR7A3	Aflatoxin aldehyde reductase	Colon, Kidney, and Pancreas	Detoxification	Hepatocarcinogenesis	[107,117]

pseudogenes [30].

Four entries in the database are related to AKR1B10. One of them, AKR1B10L, resides next to AKR1B10 on chromosome 7 and also consists of ten exons. The two proteins share 91% amino acid identity. A single EST from placenta that completely matches residues 23-209 of the predicted 316-amino acid AKR1B10L transcript suggests that AKR1B10L could indeed be a new and thus far uncharacterized human AKR family member [4,31].

Lastly, two additional database entries relate to the AKR7 family. AKR7L clusters together with AKR7A2 and AKR7A3 on chromosome 1p35-1p36.1. Alternative transcription leads to two different transcript variants. Variant 1 is the longer and comprises seven exons that encode a 331-amino acid protein. This form may indeed resemble a new functional AKR that has 92% and 88% identity with AKR7A3 and AKR7A2, respectively. Variant 2 lacks exons 4 and 5 and codes for a probably non-functional alternative peptide234. The AKR7 family pseudogene (*AFARP1*) is a retro-transposed AKR7,

where the complete processed mRNA has reintegrated into the genome. These variants of AKRs highlight the complexity and diversity of this superfamily of enzymes in humans [32].

AKRs have undergone multiple mutations, especially single nucleotide polymorphism (SNPs) leading to unpredictable consequences. For example, mutations like H117E show 5β-reductase activity, while Y55F and Y55S retain quinone reductase activity in AKRs [13]. The most important anti-cancer target ie, AKR1C3, has 14 nonsynonymous single nucleotide polymorphisms (nsSNPs). Five AKR1C3 nsSNPs were screened for their ability to reduce exemestane (an aromatase inhibitor used to treat breast cancer) to 17ß-dihydroexemestane resulting in a 17 to 250-fold reduction in catalytic efficiency of H5Q, E77G, K104D, and R258C variants compared to the wild type. K104D variant showed reduced thermal but NAD(P)+ restored the stability of the variant [33]. On the other hand, SNPs could be beneficial without changing the core function of the enzyme. For example, some SNPs of AKR1C3 (R170C, P180S), AKR1C4 (L311V), and AKR7A2 (A142T) showed reduced participation in metabolism of chemotherapeutic agents like Daunorubicin and doxycycline leading to lower chemoresistance [34].

During the evolution of a new enzymatic function, weak trade-offs between new and ancestral functions are not due to the native activity's inherent mutational robustness. Selected mutations aim for maximum increases in the new function, regardless of their impact on the old function. This leads to a bias towards initial weak trade-offs and the rise of generalist enzymes [26]. This generalist nature of AKRs, combined with their ability to participate in the metabolism of various compounds, sets the stage for a deeper exploration of their functional diversity and wide biodistribution.

FUNCTIONAL ATTRIBUTES AND BIODISTRIBUTION

Despite having sequence similarity, AKRs are functionally diverse. Some AKRs are quite specific and catalyze similar substrates despite originally being a housekeeping enzyme. As aforementioned, another characteristic that makes AKRs lethal is their bioavailability. For example, hepatic CYP450 and AKRs are inhibited by curcumin, ticlopidine, or naproxen and significantly reduces progesterone inactivation. The compounds helped inhibit hydroxylation of 4-nitrophenol to 4-nitrocatechol and glucuronidation of phenol red or 4-nitrocatechol which helped measure the activity of participating enzymes. It showed that CYP2C and 3A contributed 40% and 15% to progesterone inactivation, while AKRs contributed approximately 40% to progesterone inactivation [35]. This shows that they are not only promiscuous but also act as major isozyme for performing the same function as other housekeeping enzymes. Hence, it is important to understand their functional attributes and biodistribution to extrapolate their contribution in chronic diseases. Most of the AKRs are present in human hepatic cytosol, with AKR1B1 and AKR1C1 levels elevated in livers with alcohol-associated injury. AKR7A2 and AKR1B1 are widely distributed in human tissues, while AKR1A1 is primarily in brain, kidney, liver, and small intestine. AKR1C1 and AKR1C4 are less widely expressed (Table 1).

AKRs have ubiquitous presence in the body and perform specific roles in the organs that they are present in. For instance, AKR1A1, a traditional AKR, is recognized for its detoxification function [36]. However, it is primarily expressed in the liver and its malfunction leads to alcohol toxicity. This highlights the importance of having detoxifying enzymes in specific organs like liver. This is further supported by the presence of orthologs in rats and mice (see Figure S1 and Table S2). Similarly, AKR6As, which are not reductases but act as potassium channels, are found exclusively in the brain and heart, aiding in nerve and ion conduction. Diseases associated with these proteins are typically due to enzyme malfunction, indicating their crucial role in maintaining metabolic and housekeeping functions.

However, the distribution of these enzymes in organs that serve other functions, such as the lungs, brain, and kidneys, presents a double-edged sword scenario for AKRs. For example, AKR1Bs and AKR1Cs participate in detoxification (Table 1) and are promiscuous. However, they are widely distributed in organs that metabolize various extraneous molecules such as lungs, liver, kidneys, and urinary bladder. Interestingly, other model organisms like mice and rats have a larger number of members from this family (see Figure S1 and Table S2).

In humans, AKR1A1 reduces a broad spectrum of carbonyl-containing compounds. AKR1B1 also reduces many AKR1A1 substrates but with less activity. AKR1C1 and AKR1C4 do not have high reductase activity towards aliphatic aldehydes, aromatic aldehydes, aldoses, or dicarbonyls but can oxidize 1-acenaphthenol. AKR1C4 can also oxidize di- and tri-hydroxylated bile acids. All these reductases are present in human hepatic cytosol, with AKR1B1 and AKR1C1 levels elevated in livers with alcohol-associated injury. AKR7A2 and AKR1B1 are widely distributed in human tissues, while AKR1A1 is primarily in brain, kidney, liver, and small intestine. AKR1C1 and AKR1C4 are less widely expressed [37].

Functional Specificity

AKR1A1 is an aldehyde reductase participating in aldehyde detoxification, which can be cytotoxic and even

carcinogenic. It reduces reactive intermediates like 3-deoxyglucosone and methylglyoxal, which are involved in glycation or non-enzymatic glycosylation reaction. This suggests that AKR1A may help prevent diabetic complications by suppressing the formation of advanced glycation end products. Additionally, AKR1A and 1B contributes catalysis of d-glucuronate to l-gluconate, a key step in the synthesis of ascorbate (vitamin C) [38]. In fact, its deficiency causes alcohol toxicity in rats [39]. AKR1A also plays a role in reducing S-nitrosylated glutathione and coenzyme A, thereby inhibiting protein S-nitrosylation under conditions where nitric oxide production is stimulated. AKR1A1 is comparatively specific than other members given its small substrate space (Figure 1B,C) [38].

Similarly, human testes AKR (htAKR or AKR1E1), homologous to mouse AKR1B3/B7 is expressed on sperm surface and is concentrated on the mid-piece. It reduces acrolein a by-product of spermine catabolism in the reproductive tract. Acrolein detoxification was observed in human sperm membrane extracts, but not in seminal plasma suggesting sperms are enzymatically protected against reactive aldehyde species in both the male and female reproductive tract [40]. htAKR has multiple isoforms due to alternative splicing of which htakr4 is expressed in both testicle and germ cells. However, it does not have any activity toward steroid and prostaglandins. Interestingly it is active against another xenobiotic ie, 9,10-phenanthrenequinone. The enzyme is downregulated in testicular tumor and is controlled by hormones [41]. Its mouse homolog (AKR1B7) inhibits adipogenesis, as seen in adipose stromal cells and 3T3-L1 preadipocytes. Its expression decreases under a high-fat diet. Similar effects are observed in mouse AKR1B3 and human htAKR [42,43].

This shows that some AKRs have diverted from their promiscuous "past" in microbes and are specific in nature [44]. They metabolize multiple closely related substrates and are expressed locally in a few organs. The divergence from catalysis is evident in the AKR6A family. Humans express AKR6A3, AKR6A5, and AKR6A9 that act as potassium voltage-gated channel beta-subunits and encompass the (β/α)8-barrel motif. Any alterations in these channels could potentially lead to neurological disorders. These enzymes expressed in both the heart and brain and play a crucial role in maintaining the electrical activity of neurons, which is fundamental for processes like memory formation and learning. Both AKR6A3 and AKR6A5 lower expression, have been linked to epilepsy and cognitive impairments, including learning and memory deficits, but the underlying mechanisms are unknown [4].

However, the exact mechanisms by which AKR6A3 and AKR6A5 impair learning and memory are not fully understood. More research is needed to fully understand the specific roles of AKR6A3 and AKR6A5 in learning and memory impairment.

Functional Diversity

AKR1B1 has larger substrate space than specific AKRs and is a rate limiting catalyst for conversion of glucose to sorbitol which is then converted to fructose and redirected to glycolysis (Polyol pathway). Furthermore, AKR1B1 and B10 share similar homology but the former is ubiquitously expressed, and the latter is confined to the gastrointestinal tract and adrenal grands. AKR1B1 reduces glutathione-conjugated carbonyls more effectively than AKR1B10 suggesting its efficiency in eliminating free electrophilic carbonyl compounds [45]. *In vitro* and *in vivo* testing towards carbonyl compounds of dietary origins, including acrolein, crotonaldehyde, 4-hydroxynonenal, trans-2-hexenal, and trans-2,4-hexadienal reveal AKR1B10 as a better catalyst. This is also evidenced by larger substrate space of AKR1B10 (Figure 1B,C). Unfortunately, this promiscuity results in catalysis of anti-cancer agents like doxycycline in gastric cancer cells resulting in their resistance [46]. In fact, it is overexpressed in many cancers and is involved in chemoresistance.

Both enzymes demonstrate overlapping functions *viz* phospholipid oxidation. This results in bioactive aldehydes that remain attached to the glycerol backbone. They have several functions like inducing endothelial cells to produce monocyte chemotactic factors, enhancing monocyte-endothelium adhesion, and serving as ligands of scavenger receptors for the uptake of oxidized lipoproteins or apoptotic cells. The model phospholipid aldehyde POVPC (1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine) was efficiently reduced by members of the AKR1 family, especially AKR1B1. The human AKRs, 1B1 and 1B10, also reduced C-7 and C-9 sn-2 aldehydes as well as POVPE. These proteins catalyzed the reduction of aldehydes generated in oxidized C16:0-20:4 phosphatidylcholine with acyl, plasmenyl or alkyl linkage at the sn-1 position or C16:0-20:4 phosphatidylglycerol or phosphatidic acid. AKR1A and B families are efficient phospholipid aldehyde reductases, with non-overlapping substrate specificity, and may be involved in tissue-specific metabolism of endogenous or dietary phosphatidyl aldehydes. However, the biochemical pathways involved in phospholipid aldehyde metabolism are largely unknown [47].

AKR1C1 and 1C4 reduce aliphatic aldehydes, aromatic aldehydes, aldoses, dicarbonyls and could oxidize 1-acenaphthenol and di- and tri-hydroxylated bile acids. The levels of AKR1B1 and AKR1C1 were markedly elevated in livers with alcohol-associated injury. AKR1B1 was only detectable in livers with evidence of alcoholic liver disease and AKR1A1 is restricted primarily to brain,

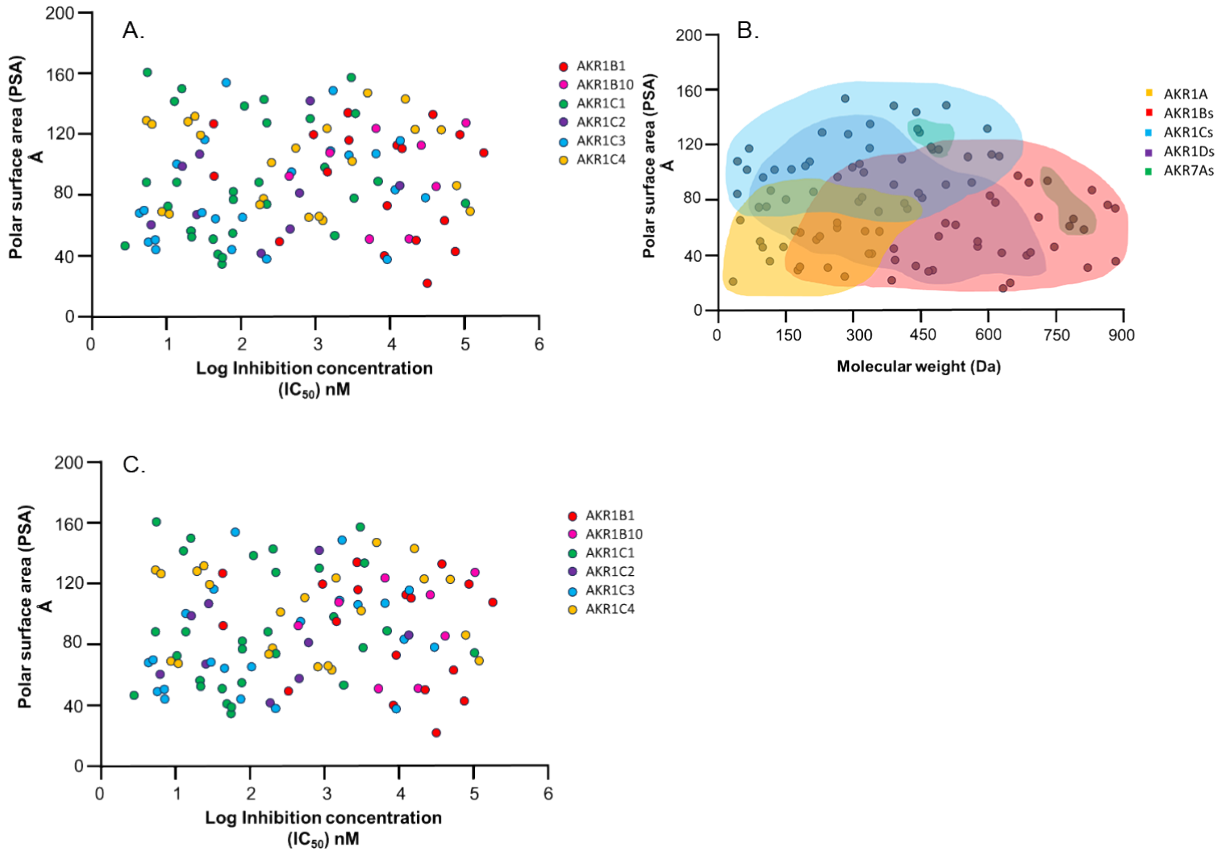

Figure 2. **Functional characteristics and biodistribution of AKRs**. **A**. Distribution of drug metabolizing enzymes in humans across various organs where green shows high, medium, and low protein expression is depicted as green, yellow, and red respectively. **B**. A scatter plot demonstrating the overlapping substrate specificities of different human AKRs. **C**. Scatter plot of the polar surface area (PSA) and AKR1B/AKR1C inhibitors IC50 shows overlapping efficacy of AKR inhibitors with closely related enzymes hence demonstrating the difficulty in designing unique AKR inhibitors.

kidney, liver, and small intestine. AKR1C1 and AKR1C4 are not as widely expressed, with AKR1C1 being observed only in kidney, liver, and testis, and AKR1C4 being found in liver alone [37]. AKR1Cs are predominant players for steroid synthesis. They are known as type 1 3α-HSD (AKR1C4), type 2 3α(17β)-HSD (AKR1C3), type 3 3α-HSD (AKR1C2), and 20α(3α)-HSD (AKR1C1) and share an amino acid sequence identity of 84% with each other. *In vitro* AKR1C4 is the most catalytically efficient, exceeding other isoforms by 10 to 30-fold in kcat/Km values. All isoforms inactivate 5α-dihydrotestosterone (5α-DHT) to yield 3α-androstanediol, but only AKR1C3 reduces Δ4-androstene-3,17-dione to produce significant amounts of testosterone.

All isoforms reduce estrone to 17β-estradiol, and progesterone to 20α-hydroxyprogesterone. Only AKR1C2 converts 3α-androstanediol to the active hormone 5α-DHT. AKR1C3 and AKR1C4 oxidized testosterone to Δ4-androstene-3,17-dione. All isoforms oxidize 17β-estradiol to estrone, and 20α-hydroxyprogesterone to progesterone. AKR1C4 was virtually liver-specific, while AKR1C3 was most prominent in the prostate and mammary glands. AKR1C3 can interconvert testosterone with Δ4-androstene-3,17-dione, but inactivates 5α-DHT, suggesting it eliminates active androgens from the prostate. In the mammary gland, AKR1C3 converts Δ4-androstene-3,17-dione to testosterone, estrone to 17β-estradiol, and progesterone to 20α-hydroxyprogesterone, potentially yielding a pro-estrogenic state. It is dominant form in the uterus and synthesizes 3α-androstanediol, implicated as a parturition hormone. The major isoforms in the brain, capable of synthesizing anxiolytic steroids, are AKR1C1 and AKR1C2. These studies contrast with those in rats where only a single AKR with positional- and stereo-specificity for 3α-hydroxysteroids exists [48].

AKRs are the most ubiquitous reductases in the body compared to other detoxifying enzymes. The next largest bioavailable enzymes are the glutathione S- transferases (GSTs). Other redox enzymes like CYP450s, Aldose oxidases, MDRs, and SDRs are comparatively sparse (Fig-

ure 2A) [6,49–53]. Furthermore, it is quite evident that the promiscuity of these enzymes allows them to perform a wide range of functions in the body, from detoxification to steroid synthesis. Additionally, it is hard to decipher specific functions of AKRs based on sequence alone as closely related enzymes perform different functions. These enzymes have a unique and overlapping set of preferred reactions and substrates, suggesting that they have evolved to fulfil many roles in different tissues (Figure 2B and Table S1). This characteristic makes them difficult targets for further research, particularly in the context of disease states where their activities may be altered.

Additionally, the versatility of AKRs stems from their adaptable and expansive substrate binding pockets, structural diversity, and evolutionary modifications. These characteristics enable them to carry out a diverse array of functions, thereby making them promising candidates for the creation of selective and comprehensive AKR inhibitors. However, this also introduces difficulties in the design of inhibitors. The IC50s of closely related AKRs and the polar surface area of the molecules, as shown in the literature (Figure 2C), clearly illustrate these challenges. Further studies in this field could yield important information for the development of more potent AKR inhibitors.

AKRs, with their adaptable substrate binding pockets and structural diversity, play a crucial role in detoxification by converting harmful aldehydes and ketones into less toxic alcohols. However, this process can generate reactive oxygen species (ROS), leading to potential harmful consequences, particularly in cancer. Understanding this dual nature of AKRs is vital for developing effective AKR inhibitors and therapeutic strategies. It also helps us comprehend the implications of AKR activity in health and disease.

CHRONIC DISEASES DUE TO DETOXIFICATION

The conversion of aldehydes and ketones to their respective primary and secondary alcohols by AKRs, is vital for detoxification. This is generated endogenously and exogenously and contribute significantly to the body's defense mechanism against harmful substances, aiding in maintaining cellular homeostasis. However, during reduction of carbonyl groups, the transfer of electrons from NADPH to the carbonyl group, can lead to the generation of ROS. In the context of cancer, high expression of the AKR1B and AKR1C is associated with worse outcomes in different cancer types. This is partly due to the role of these enzymes in chemoresistance, inflammation, oxidative stress, and epithelial-to-mesenchymal transition [54,55]. Hence, it is important to look at the role of AKRs within aspects where, detoxification is done right and when it may lead to unsafe consequences.

When Scavenging is Done Right

2-tert-Butylhydroquinone (BHQ), a food additive antioxidant, has anticancer effects but is carcinogenic at high doses. It is metabolized into cytotoxic tertbutylquinone (BQ), which is further converted to 6-tert-butyl-2,3-epoxy-4-hydroxy-5-cyclohexen-1-one (TBEH) through 6-tert-butyl-2,3-epoxy-4-benzoquinone (TBE), inducing chromosomal aberration. Reductases for BQ and TBE could protect against their toxicity and AKR1B10 was the most efficient in reducing BQ and TBE into BHQ and TBEH indicating its role in protecting against the toxicity of the two p-quinone metabolites of BHQ [56].

AKR1B10 is found overexpressed in benign and para-carcinoma specimens than in nasopharyngeal carcinoma (NPC) tissues. Lower AKR1B10 levels in NPC was associated with higher T-classification and lymph node metastasis. Stable AKR1B10 expression in nasopharyngeal cancer cells inhibited cell proliferation and migration. Therefore, low AKR1B10 expression could be a prognostic indicator in NPC [57].

AKR1C1-C4 also act as dehydrogenase for dihydrodiols of polycyclic aromatic hydrocarbons (PAH) like benzo[a]pyrene 7,8-diol which leads to formation of carcinogenic PAHs. They are formed via two pathways: one forming anti-BPDE (catalyzed by CYPA1/1B1), and the other forming BP-7,8-dione (catalyzed by AKR1A1 and AKR1C1-AKR1C4) [58]. Both anti-BPDE and B[a]P-7,8-trans-dihydrodiol induced p53 expression, indicating DNA damage. However, B[a]P-7,8-dione generated reactive oxygen species but did not induce p53. In fact, the overexpression of AKR1A1 in the cell lines protected them from the toxic effects of B[a]P-7,8-trans-dihydrodiol, suggesting that AKRs overexpression may protect lung cancer cells from PAH toxicity [59]. However, as most PAH are xenobiotics, there are unexpected consequences of detoxification. The catalyzed product can sometimes lead to unfavorable consequences.

Scavengers to Predators

PAH procarcinogens like 7,12-dimethylbenz[a]anthracene-3,4-diol, and benzo[g]chrysene-11,12-diol are catalyzed by AKR1C1-C4 into highly reactive carcinogenic ketones in A549 lung cancer cell lines [60]. AKR1C3 regulates myelopoiesis by metabolizing prostaglandin D2 and participating in oxidative activation of polycyclic aromatic hydrocarbon (PAH) procarcinogens. Exposure of AKR1C3 expressing cell Ines to 7,12-dimethylbenz(a)anthracene-3,4-dihydrodiol, a PAH causes DNA damage. However, when knocked down oxidative DNA damage was reduced but single strand breaks were prevalent. However, it also triggered of acute myeloid

leukemia cells to differentiate, thereby suggesting the enzyme's role in chronic myeloid leukemia as well [61].

Both CYP1A1/1B1 and AKR1A1,C1-C4 play competing roles and the dominant pathway depends on the redox state of the cells. Further studies with another PAH, ie, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) reflect this as well where, CYP450s have greater kcat/Km values than AKRs *in vitro* and compete for the same substrate at a specific NADPH/NADP+ ratio. However, *in vivo* experiments with lung cell lines (HBEC-KT) and lung cancer cell lines (A549) show mRNA levels of AKR1C1–1C3 exceed basal TCDD induced CYP1A1/1B1. Functional assays of both cell lysates demonstrated a lack of TCDD-inducible P450 1A1/1B1 activity, but robust basal expression of AKR1A1 and AKR1C1-4 activities [62]. The studies also show a presence of oxidative state and rise in ROS levels. Moderate ROS amounts can cause oxidative damage aiding in cancer pathogenesis. However, excessive ROS can initiate programmed cell death in cancer cells. Thus, ROS levels have a paradoxical effect on cancer.

AKR-dependent ROS generation and stress response is reflected even in unicellular microbes like *Saccharomyces cerevisiae* where three AKRs with functional similarity to human aldose reductase (AKR1B1) were identified and functionally validated. The knockout strain of these genes showed enhanced sensitivity to stress. Ongoing studies show that the triple null strain has an altered transcription profile consistent with an enhanced stress response compared to the parental strain [63].

Sometimes it is not the direct actions of AKRs, rather their ubiquitous presence that causes resistance of anti-cancer agents. For example, AKR1C inhibitor meclofenamic acid increased cis-platin sensitivity. The total NAD and NADH/NAD+ ratio was higher in resistant cells, with NADH acting as an antioxidant against cis-platin-induced radicals. NADH, produced by NAD(P)H-dependent oxidoreductases, plays a key role in cis-platin resistance in liver cancer cells and as AKR is quite prominent it acts as a major player in promoting cancer resistance [64].

Chemoresistance is significant issue in cancer treatment, with metabolic inactivation by carbonyl reduction being a primary cause. Oracin, an anticancer drug currently in phase II clinical trials, undergoes metabolic inactivation by carbonyl reduction. AKR1C3, part of the AKR superfamily, is identified as the enzyme catalyzing oracin's inactivation. AKR1C3 also mediates the carbonyl reduction of doxorubicin to its inactive metabolite, doxorubicinol, which is linked to cardiomyopathy in doxorubicin chemotherapy. As AKR1C3 is overexpressed in hormone-dependent cancers like prostate and breast cancer, co-administering AKR1C3 inhibitors could potentially enhance the efficacy of oracin and doxorubicin chemotherapy, while also reducing the risk of doxorubicin-induced cardiomyopathy [65]. Other members, *viz* AKR1C1, 1C2, and 1C4, were also identified to mediate oracin carbonyl reduction in humans. AKR1C2 and 1C4 are exclusively (100%) stereospecific for (+)-DHO formation, while AKR1C1 showed some 3% of (-)-DHO formation. However, AKR1C1 significantly reduces oracin [66].

The broad substrate space containing (more promiscuous) AKR1B10 plays a role in acquisition of resistance to anticancer drug mitomycin-c. It is mediated by its ability to detoxify cytotoxic aldehydes like 4-hydroxy-2-nonenal (HNE) [67]. AKR1B15 shares 92% identity with AKR1B10 and acts as retinaldehyde reductase. It is also involved in the development of several cancers.

While AKRs' role in cancer and drug resistance is significant, their metabolic functions also have profound implications. For instance, AKR1B1's role in the polyol pathway is crucial in diabetes-related complications, highlighting the diverse impacts of AKRs in health and disease.

CHRONIC DISEASES DUE TO METABOLIC FUNCTIONS

Cataracts

AKR1B1 converts glucose to sorbitol in the polyol pathway. In individuals with diabetes, the blood glucose levels are high. This signals AKR1B1 overexpression consequently, increasing sorbitol accumulation. This causes osmotic stress and oxidative damage, contributing to the development of diabetic complications [4]. AKR1B1, is the rate-limiting enzyme of the polyol pathway and furthermore, it is ubiquitous as it is expressed in skeletal muscles. Hence, accelerated glucose metabolism through this pathway is associated with diabetic cataract and retinopathy [68]. AKR1B1-mediated polyol accumulation causes osmotic imbalances leading to fiber cell swelling, liquefaction, and eventually cataract [69].

However, AKR1B1's function in non-diabetic conditions is unclear. It shares 65% protein identity with other AKR1Bs and reduces many substrates, leading to their classification as detoxifying enzymes. Recent mouse model studies on AKR1B homologs revealed their role in regulating glucido-lipidic metabolism and adipose tissue homeostasis. Contrary to the idea of being mere detoxification enzymes, they are found to modify or generate signal molecules, shifting their classification from scavengers to messengers [70].

Diabetic Retinopathy

AKR1B1 plays a crucial role in causing diabetic retinopathy (DR) eventually causing blindness [68].

Vascular Endothelial Growth Factor (VEGF) promotes retinal blood vessel growth, a key DR development factor. Animal studies show that AKR1B1 null mutation reduces VEGF elevation in the diabetic retina, preventing blood-retinal barrier (BRB) breakdown and retinal apoptosis [71]. This deletion also protects mice from streptozotocin-induced DR by inhibiting retinal capillary degeneration and superoxide generation. AR activity reduction using inhibitors normalizes VEGF levels, suppresses VEGF-induced tube formation in retinal endothelial cells, and alleviates hyperglycemia-induced retinal pigment epithelial cell damage [72].

Besides VEGF regulation, AR is involved in diabetic eye inflammatory responses. AKR1B1 downregulation reduces retinal microglia (RMG) inflammatory responses, suggesting AR inhibition prevents DR by suppressing diabetic eye inflammation. Advanced glycation end-products (AGEs) induce VEGF production and matrix metalloproteinases (MMPs) in the diabetic retina, altering the BRB and initiating retinal endothelial cell morphological changes. AKR1B1 inhibition or genetic deficiency suppresses the NF-κB and MAPK pathways, raising questions about AKR1B1 mediation of AGE or AGA-induced diabetic retina inflammatory responses [73].

Other inflammatory diseases such as Uveitis (contributes to 10-20% of legal blindness annually) is speculated to be caused by AKR1B1. The RMG cells in the eye, become activated in uveitis, leading to morphological transformation and migration into the outer nuclear layer where they secrete cytokines and peroxynitrites. RMG activation can be suppressed by an AKR1B1 inhibitor in mice. However, the exact role of AKR1B1 in causing the disease is not deciphered [69].

Diabetic Neuropathy

AKR1B1 is the first and rate-limiting enzyme in the polyol pathway, where it catalyzes the NADPH-dependent reduction of glucose to sorbitol. This pathway becomes more active when blood glucose levels are high, such as in diabetes. The accumulation of sorbitol can lead to osmotic stress and nerve damage, contributing to the development of diabetic neuropathy [74].

Moreover, polymorphisms (variations) in the *AKR1B1* gene have been associated with the development and progression of diabetic neuropathy [74-76]. For instance, a 7-year longitudinal study found that certain *AKR1B1* polymorphisms were strongly associated with the rate of decline of nerve function in an adolescent diabetic cohort [74]. Another study suggested that painful diabetic neuropathy is a condition with enhanced genomic instability characterized by increased chromosomal aberrations and possible mutations in the *AKR1B1* gene.

Therefore, understanding the role of the *AKR1B1* gene and its variants can help in the prediction, prevention, and treatment of diabetic neuropathy. However, the exact mechanisms are complex and likely involve other genetic and environmental factors [75].

Chronic Liver Disease

The AKR7A enzymes are expressed in liver, brain, colon, and kidney. They are responsible for biosynthesis of gamma-hydroxybutyrate (GHB). However, in liver they detoxify, 4-Hydroxynonenal (HNE), a byproduct of lipid peroxidation that is involved in chronic liver diseases. It reacts with DNA, lipids, and proteins, and its effects vary based on concentration. High levels of HNE causes cell death, while low levels stimulate an adaptive response. The study found that sub-lethal HNE levels induce the expression of certain enzymes, heme oxygenase-1 (HO-1) (2.5-fold), NADPH:quinone oxidoreductase (NQO1) (4.5-fold), AKR1C3 (2-fold) and AKR7A2 (3-fold) enzymes. Individuals who have mutated AKR7A2 suffer from alcohol poisoning and chronic liver disease. AKR7A2 has been shown to protect against HNE toxicity in HepG2 cells (Liver cell lines). Furthermore, 7-hydroxycoumain a natural compound, protects the liver from HNE through AKR7A2 induction, suggesting the enzyme as a potential therapeutic target for chronic liver disease [77].

Other AKR7 homologs like the mouse AKR7A1 are shown mildly protect cells against acrolein that is found in cigarette smoke resulting in less DNA damage and had a significantly lower mutation rate [78]. Similarly, another mouse homolog, AKR7A5, detoxifies aldehydes from lipid peroxidation and protects against 4-hydroxynonenal-induced apoptosis and oxidative stress [79,80]. Therefore, it is plausible that mutations in the human homolog AKR7A2 could potentially lead to other diseases, necessitating further investigation.

Keloid Scars

Keloids, expanding scars more common in individuals of African descent, have been linked to increased mast cell activity. The main compound secreted by these cells, prostaglandin D2 (PGD2), can convert to 15-deoxy-(Δ2,14)-prostaglandin or 15Δ-PGJ2 or be metabolized to 9α,11β-PGF2 by AKR1C3. *In vivo* studies showed relation between increase of keloids in mast cells and AKR1C3 overexpression. 15Δ-PGJ2 inhibits keloid fibroblast (KF) proliferation, reduces collagen gel contraction, and increases caspase-3 activation. It also activates P38-MAPK, induces ROS, and upregulates superoxide dismutase-1 (SOD-1). Inhibiting P38-MAPK enhances 15Δ-PGJ2 -induced caspase-3 cleavage and reduces its effect on SOD-1 transcription. Therefore, dual inhibition of AKR1C3 and P38-MAPK can inhibit keloid progression by creating an oxidative environment and

sensitizing keloid cells to ROS-induced apoptosis [81].

Lipedema

Lipedema, a chronic disorder causing disproportionate fat accumulation in women, is often underdiagnosed. It is linked with anxiety, depression, and pain, suggesting a role for steroid metabolism and neurohormone signaling. Whole exome sequencing revealed a missense mutant (L213Q) in AKR1C1, an enzyme that reduces progesterone to 20α-hydroxyprogesterone. Comparative molecular dynamics simulations and bioinformatic analysis suggest this variant results in partial loss-of-function, leading to slower reduction of progesterone, increased subcutaneous fat deposition, and potentially lipedema. Thus, AKR1C1 is suggested as the first candidate gene associated with nonsyndromic lipedema [82].

Cancers

Squamous cell carcinoma: AKR1C3 is overexpressed in squamous cell carcinoma (SCC) and converts prostaglandin 2 (PGD2) to 9α11β-PGF2, diverting the spontaneous conversion of PGD2 to the PPARγ agonist *viz* 15-Deoxy-Δ12, 14-prostaglandin J2 (15Δ-PGJ2). Although reducing AKR1C3 does not affect growth, PGD2 and its metabolite 15Δ-PGJ2 decrease SCC proliferation via peroxisome proliferator-activated receptor gamma (PPARγ). The PPARγ agonist pioglitazone significantly inhibits SCC proliferation. Therefore, PPARγ agonists (like pioglitazone) and AKR1C3 antagonists can benefit those at high risk of SCC [83].

Renal Cell carcinoma: As mentioned previously, AKRs have overlapping functions. Despite having a smaller substrate space compared to the promiscuous AKR1B1, the AKR7A2 has narrower substrate specificity. Both enzymes are overexpressed in renal cell carcinoma suggesting "yet to be discovered" pathways that cause its malignancy. However, it is quite evident that despite lesser activity an overexpressed enzyme can invariably convert the same substrates at a competitive rate than its promiscuous counterpart (AKR1B1) [37].

Prostate, ovarian, and breast cancers are hormone-dependent malignancies of the aging male and female, they require the local production of androgens and estrogens to stimulate cell proliferation and AKRs play key roles in this process. In the prostate and ovaries, AKR1C3 (type 5 17β-HSD) reduces Δ4-androstene-3,17-dione to yield testosterone while AKR1C2 (type 3 3α-HSD) eliminates 5α-dihydrotestosterone (5a-DHT), and AKR1C1 forms 3β-androstanediol (a ligand for ERβ). In the breast, AKR1C3 forms testosterone, which is converted to 17β-estradiol by aromatase or reduces estrone to 17β-estradiol directly. AKR1C3 also acts as a prostaglandin (PG) F synthase and forms PGF2a and 11β-PGF2a, which stimulate the FP receptor and prevent the activation of PPARγ by PGJ2 ligands. This proliferative signaling may stimulate the growth of hormone-dependent and -independent prostate and breast cancer [84].

The role of AKRs extends beyond human physiology and into the realm of microbiology. This is evidenced by the influence of the human microbiome on health and disease [85]. This includes both beneficial and harmful microbes. The diverse families of AKRs present in these microbes interact with human AKRs in complex ways, influencing processes from drug interactions to disease progression and warrant further inspection.

MICROBIAL AKRs AND CHRONIC DISEASES

The human microbiome not only encompasses the actively residing microbes in the body of a healthy human being rather, it also contains bacteria that reside in various disease states as well. As described before, the human genome expresses only three families of AKRs *viz* AKR1, AKR6 and AKR7 however, microbes contain the rest of the known families. The participation of external AKRs is not well studied as multiple enzymes, ie, human and microbial AKRs can perform same reactions due to promiscuity. For example, human AKRs reduce the bioavailability of noncompetitive inhibitors like tolrestat and epalrestat, resulting in their limited clinical use. Interestingly, these compounds also bind to AKRs from distantly related microbes like Tm1743 from *Themotoga maritima* [86].

Another interesting example, albeit partially related to AKRs, is the dysfunction of human UDP Glucuronosyltransferase Family 2 Member B17 (UGT2B17). The enzyme plays a crucial role in the glucuronidation process, an intermediate step in the metabolism of steroids. In individuals with *UGT2B17* gene deletion, the gut bacterial β-glucuronidases significantly impact testosterone disposition. Normally, the gene deletion results in the upregulation of AKR1D1 and AKR1C4, which convert testosterone to 5β-dihydrotestosterone and 3α, 5β-tetrahydrotestosterone. However, gut bacterial enzymes can reactivate testosterone glucuronide into testosterone resulting in higher amount of the hormone and metabolic dysregulation [87].

Hence, the overall effect of gut or pathogenic microbiome is quite hard to decipher. They could be essential for pathogenicity due to their participation in metabolic pathways or they can enhance pathogenicity by acting as detoxifying enzymes for drugs. Additionally, the promiscuity of AKRs makes this even more difficult to pinpoint significant functions. For example, babesiosis is a tick-borne disease caused by the *Babesia microti*.

Symptoms range from none to severe, including fever, fatigue, and anemia. It is most common in the Northeastern and Midwestern US and parts of Europe. Its native AKR (BmAKR) is upregulated on day 8 post-infection and downregulated later. It is found in the cytoplasm of *B. microti* merozoites in mouse models. Its expression increased under oxidant stress and in response to anti-babesiosis (Atovaquone) and anti-coccidiosis drugs (Robenidine), suggesting a role in anti-parasite drug response. However, the relation of the AKR corresponding to the pathogenesis of the protozoan needs further studies [88].

On the other hand, the *Staphylococcus aureus* AKR IolS is the target of thymol, a natural compound used to treat *S. aureus* infections. Its binding increases the AKR activity of IolS and depletes NADPH within *S. aureus* cells, leading to a bactericidal effect making it a promising target for developing new antimicrobials [89]. Interestingly, recent studies have discovered AKRs that play a role in chronic diseases, but no ensemble study has been reported in any review yet.

Stomach Ulcers

The bacteria *Helicobacteria pylori* infects the stomach, weakening its protective mucus lining. They produce urease, which neutralizes stomach acid, creating a more hospitable environment. This allows the bacteria to multiply and damage stomach tissue. The resulting irritation, combined with the stomach's own acid, can lead to ulcers. Long-term infection may even lead to stomach cancer. Interestingly, its native AKR13C1 (HpAKR) functions over a broad pH range (4-9), with an optimum at pH 5.5. It performs cinnamyl alcohol dehydrogenase activity in *H. pylori*, enabling the organism to reduce a wide range of aldehydes. An isogenic HpAKR helps the organism to survive and grow under acidic conditions, suggesting its crucial role in adapting to the gastric mucosa [90].

Chagas Disease

AKRs are also involved in pathogenesis like Chagas disease caused by *Trypanosoma cruzi*. Chagas disease becomes chronic when the parasites inhabit heart and digestive muscles. This phase can last for years, with 30-40% of patients developing organ dysfunction. The disease often remains asymptomatic during this phase, making diagnosis and treatment challenging. It is treated using O-napthoquinone derivatives and Benzonidazole which are activated by a parasitic NADH-dependent type I nitroreductase (NTR I) but are rendered ineffective by native AKR (TcAKR) leading to drug-resistant *T. cruzi* and chronicity of the disease [91,92].

DISCUSSION AND CONCLUSION

The complexity of human metabolic processes is such that even a single malfunction can lead to significant disruptions. The AKR superfamily, known for its functional diversity and involvement in numerous essential pathways, is particularly susceptible to mutagenesis. Consequently, it is often implicated in various chronic diseases (Table 1). There is a clear correlation between their bioavailability and diseases caused by the enzymes.

As aforementioned, AKR1A1, a conventional AKR, is known for its detoxification function. It is primarily located in the liver, and its malfunction leads only to alcohol toxicity. This underscores the importance of having detoxifying enzymes in specific organs, such as the liver. This is further supported by the presence of orthologs in rats and mice (see Figure S1 and Table S2). Additionally, AKR1A1 participates only in comparatively lesser number of metabolic reactions. It enables modification of glucoronate and retinaldehyde which encompass aliphatic ketone chains (Figure 3). This is reflective of the enzyme's comparatively narrow substrate site. Interestingly, AKR1A1 reduces a broad spectrum of carbonyl-containing compounds [36]. AKR1B1 also reduces many AKR1A1 substrates but with less activity. However, this is untrue as ketoreductases with larger substrate sites are better at catalyzing aromatic ketones compared to aliphatic ketones [93,94]. However, this notion is hypothetical at best as AKRs have not been extensively compared against the same substrates and warrants further exploration. Furthermore, AKR1A1's role in detoxification and its presence in detoxifying organs (liver and kidney) portrays its distribution in the "right" place and does not cause any diseases in adults [3]. However, their presence in the fetal developmental stage is less, thereby resulting in fetal alcohol syndrome [4].

Although AKR1A1 is a known catalyst for producing L-gluconate, it is not able to convert the product further. This is not surprising as the forward reaction km is much lower than the reverse reaction. However, microbial AKRs are known to synthesize vitamin C in large amounts. For example, *Corynebacterium sp.* AKR is used to convert L-gluconate to Ascorbate (vitamin C) in industries [44]. Furthermore, gut bacteria are known source for vitamin C in humans [95]. Therefore, it would not be overly optimistic to speculate the contribution of gut bacteria AKRs for synthesizing vitamin C.

Another AKR which has no reported promiscuity is the htAKR, which is confined to testes and is not known to cause any diseases. Comparatively AKR1Bs are more distributed and participate in central carbon metabolism thereby resulting in metabolic "ubiquity" causing cataracts and diabetic complications like retinopathy and neuropathy. Comparatively the AKR1Ds and 7As are sparse

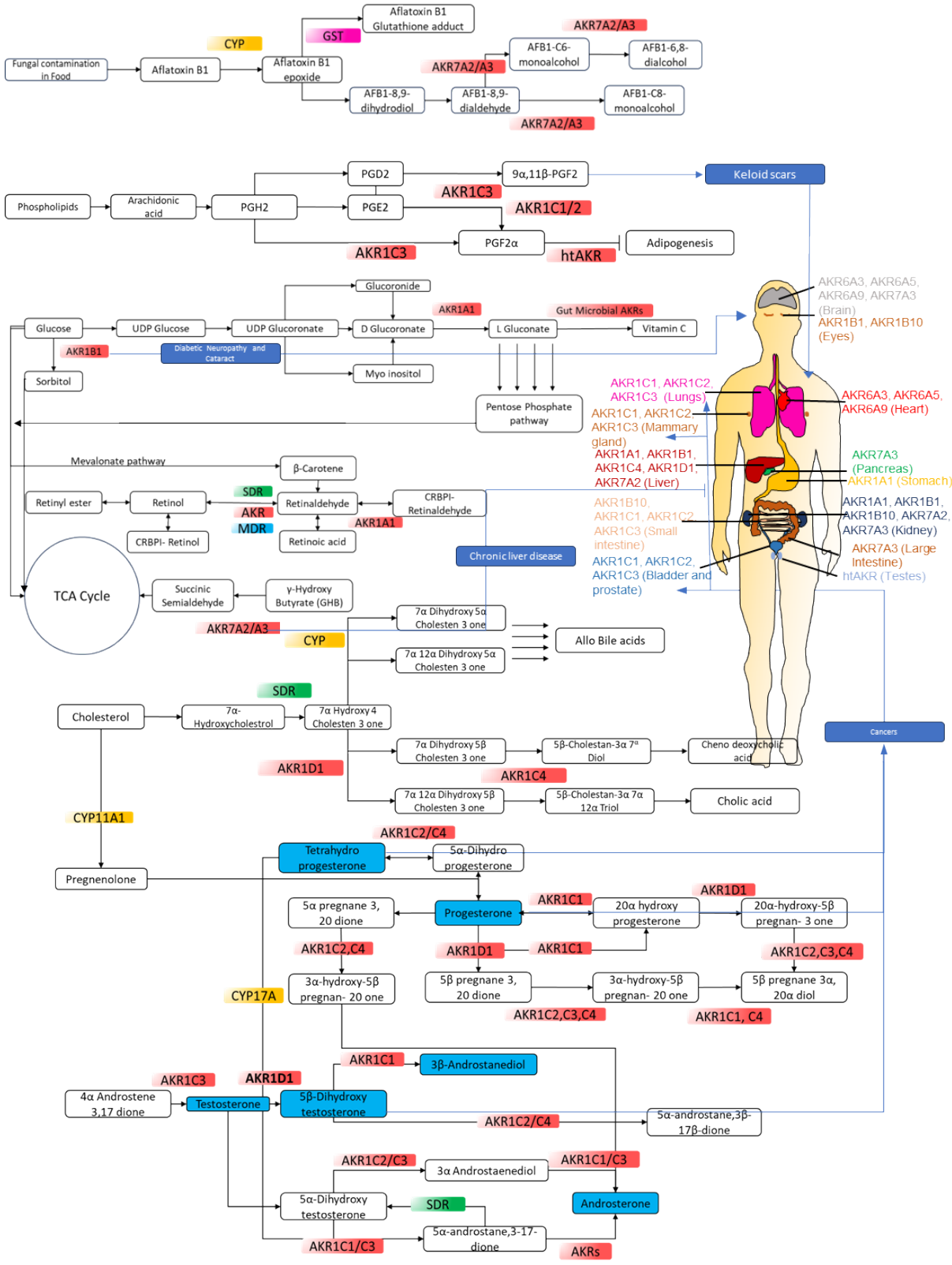

Figure 3. Metabolic Pathways AKRs and the diseases caused by their biodistribution.

but are involved in specific metabolic diseases which are more treatable (Figure 3) [96,97].

Similarly, AKR6As, which are not reductases but act as potassium channels, are found exclusively in the brain and heart, aiding in nerve and ion conduction (Figure 3) [98]. Diseases associated with these proteins are typically due to enzyme malfunction, indicating their crucial role in maintaining metabolic and housekeeping functions. Although AR6As are known biomarkers for gastric cancer, their specific function in this context remains elusive [99]. In contrast, AKR1Bs are more widely distributed and participate in central carbon metabolism, resulting in metabolic "ubiquity" causing cataract and diabetic complications like retinopathy and neuropathy. The AKR1Ds and 7As are sparse but are involved in specific metabolic diseases which are more treatable, for example, diabetic neuropathy, retinopathy, obesity, etc. [42,68,75].

Lastly, the metabolic map of AKRs shows that AKR1Cs are the most widely dispersed of all AKRs and are involved in multiple pathways (Figure 3). Due to their promiscuity they either take role of chemo resistant enzymes (liver, gut, and lung) or direct (breast, prostate) perpetrators of cancer (steroid metabolism) [41]. Furthermore, their overexpression often leads to higher production of steroids. Although there are isozymes that synthesize specific steps, noticeably, AKR1Cs and AKR1Ds are present throughout the synthesis of steroids such as testosterone, progesterone and androsterone [8]. The former is crucial for the development of male reproductive tissues and the manifestation of secondary sexual characteristics. Some studies suggest that testosterone therapy may elevate the risk of prostate cancer. Furthermore, an association between elevated testosterone levels and an increased risk of melanoma, a type of skin cancer, has been reported [100]. Similarly, androsterone an androgen pheromone, can influence the development of breast cancer. Androgens bind to androgen receptors, which are expressed in many breast cancers, potentially promoting tumor growth.

Similarly, progesterone, a hormone vital for the menstrual cycle and pregnancy in women, has also been linked to cancer and is synthesized from AKR1C1 through 20α hydroxy progesterone. However, as seen in Figure 3 AKR1Cs participate not only in synthesizing the hormone but participate in the multiple steps in the pathway. High levels of progesterone are known to increase the risk of breast cancer. Conversely, some studies propose that naturally occurring progesterone may confer protection against breast cancer. The relationship between progesterone and cancer is multifaceted, influenced by factors such as the type of progesterone (synthetic or natural), its interaction with estrogen during HRT, and individual genetic variables [101].

Notably, AKR1Cs do not act as rate limiting steps for hormone synthesis, rather they are involved in "essential" function throughout the pathway thereby are major contributors of biochemical flux towards the production of steroids and their diseases. Hence, unsurprisingly they are known biomarkers for multiple cancers [102]. AKR1C1 and AKR1C4 do not have high reductase activity towards aliphatic aldehydes, aromatic aldehydes, aldoses, or dicarbonyls but can oxidize 1-acenaphthenol. AKR1C4 can also oxidize di- and tri-hydroxylated bile acids [37].

In conclusion, designing inhibitors for AKR1Cs that are closely related but have distinct functions presents a significant challenge for medicinal chemists, particularly when the goal is to design novel small molecules for inhibition. Future research is needed for developing methods to differentiate substrates and inhibitors that can bind to different conformers of AKR, a critical step towards resolving this issue [103]. Potential strategies could include structural characterization or molecular docking studies with a range of current inhibitors, the use of artificial intelligence to differentiate molecules, and the creation of a physiological atlas of AKRs in different subsets of cell populations within a tissue. Such an atlas could aid in the design of antibodies carrying specific small molecules.

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Appendix A

Supplementary methods and materials:

1. Modeling and measurement of substrate binding pocket.

Protein structures were derived from the Protein Data Bank (PDB) (https://www.rcsb.org/). The specific PDB IDs used in this study include: ARK1A1 (2F2K), ARK1B1 (4GQG), ARK1B10 (3NTY), AKR1C1 (4JQ3), AKR1C2 (7C7F), AKR1C3 (3BUR), AKR1D1 (1ZSX), AKR6A5 (2BP1). These identifiers correspond to various enzymes that were examined in this study.

The active site dimensions were determined and analyzed using PyMOL [1]. This allowed for precise measurements within the active sites of each enzyme model. Subsequently, surface area and volume calculations were performed based on the derived dimensional parameters utilizing the Computational Analysis of Surface Topography of Proteins (CASTp) tool [2]. This provided detailed insights into the spatial characteristics of each active site, which is crucial for understanding enzyme-substrate interactions and potential drug-binding efficacy (Supplementary Figure S2).

2. Parametric analysis of AKR inhibitors.

As, AKRs have overlapping activities, this characteristic also makes them challenging targets for research, especially in disease states where their activities may be altered. E.g., AKR1C1 to AKR1C4, show different ratios of 3-, 17-, and 20-ketosteroid reductase activity, demonstrating their promiscuity in terms of regiospecificity, positional specificity, and stereospecificity. This is reflected in the properties of inhibitors.

We analyzed this by plotting the Polar Surface Area (PSA) of the molecules against their molecular weight using the Data Warrior software. PSA is a frequently used measure in medicinal chemistry to optimize a drug's cell permeability. Molecules with a PSA greater than 140 angstroms squared ($Å^2$) are generally poor at permeating cell membranes. For molecules to penetrate the blood-brain barrier (and thus act on receptors in the central nervous system), a PSA less than 90 $Å^2$ is usually required. This helps predict the bioavailability of the molecule within the body and offers a holistic perspective of the chemical and activity spaces that a set of compounds occupy.

In a PSA plot, the x-axis, was used to plot molecular weight of the compounds. On the other hand, the polar surface was plotted in the y-axis. Each point on the plot corresponds to a specific compound and its associated PSA value. It allows for the observation of patterns, trends, and clusters. These observations can indicate the impact of structural changes or variations on the activity of the compounds. This information is valuable in identifying areas of the chemical space that are associated with desirable activities or properties. Furthermore, it assists in choosing compounds for further optimization or lead discovery in the process of drug development.

3. Pathway analysis.

Pathways for microbial AKRs and Human AKRs were derived using BioCyc and HumanCyc databases respectively [3,4].

Supplementary Table S1: IC50s (μM) of various AKR inhibitors that demonstrate inhibition against multiple members

Compound name	Target enzyme and IC50s				Reference
	AKR1C2	AKR1C1 and C4			[5]
Ursodeoxycholic acid	0.34	0.22			
1,10-phenanthroline	0.13	0.65			
	AKR7A1	AKR7A5			
Succinate	0.4	0.3			
Citrate	0.2	0.4			
	AKR1B1	AKR1B10			[6]
Xanthohumol	9.11	6.56			
Isoxanthohumol	0.57	1.09			
8-prenylnaringenin	0.81	0.99			
Dinaciclib		0.23			
	AKR1B10				[7]
phlorofucofuroeckol-A	6.22				
	AKR1C3				[8]
49a	0.07				
49g	0.07				
	AKR1B10	AKR1B1			[9]
(Z)-2-(4-methoxyphenylimino)-7-hydroxy-N pyridin-2-yl)-2H-chromene-3-carboxamide	0.0047	0.024			
7-hydroxy-2-(4-methoxyphenylimino)-2H-chromene-3-carboxylic acid benzylamide	0.006	0.011			
	AKR1C2	AKR1C3			[10]
4-chloro-N-phenylanthranilic acid	1	3			
	AKR1C2	AKR1C3			[11]
Indomethacin	8.97	7.35			
Derivative 1		0.3			
Derivative 2		0.94			
	AKR1C2	AKR1C3			[12]
4-chloro-N-phenylanthranilic acid	1	3			
	AKR1B1	AKR1B10			[13]
Sulindac	0.36	2.69			
Sulindac sulfone	0.46	1.27			
K-80003	0.29	0.37			
Tolrestat		0.012			
Fidarestat		33			
Sorbinil		9.6			

Caffeic acid Phenethyl ester		0.08			
Flufenamic acid		0.76			
Zopolrestat		0.62			
Epalrestat		0.33			
JF0064		1			
	AKR1C2	AKR1C3			[14]
Flufenamic acid	0.37	0.05			
mCF3 derivative	0.22	0.06			
mNO2 derivative	0.19	0.08			
pNO2		0.03			
	AKR1C2	AKR1C3			[15]
S-naproxen	1.26	0.18			
R-naproxen	2.75	0.05			
Fatty acids	AKR1C1	AKR1C2	AKR1C3	AKR1C4	[16]
Palmitoleic acid	2.1	0.91	1	0.92	
Oleic acid	1.8	1	0.72	0.84	
Linoleic acid	1.4	1.1	0.69	0.8	
a-Linolenic acid	12	16	8.3	4.2	
g-Linolenic acid	4.5	5.6	2.3	3.4	
Arachidonic acid	1.9	4.4	0.69	0.81	
EPA	3	4.4	0.81	0.99	
DHA	1.7	3.7	0.76	1.2	
	AKR1B10	AKR1B1			[17]
Oleanolic acid	0.09	124			
Maslinic acid	0.63	72			
Betulinic acid	2	11			
Ursolic acid	4	41			
Asiatic acid	5.9	34			
Glycyrrhetic acid	4.9	280			
Erythrodiol	30	84			
	AKR1B10	AKR1B1			
RARb/c agonist (UVI2008)	6.1	70			[18]
RARb/c agonist (UVI2007)	8.3	39			
	AKR1B10	AKR1B1			
Fidarestat	33	0.026			[19]
	AKR1B1	AKR1B10			[20]
Caffeic acid Phenyl Ester	0.57	0.08			
3,5-Dicaffeoylquinic acid 0.13	0.088	0.013			
3,4-Dicaffeoylquinic acid 0.24	0.078	0.024			

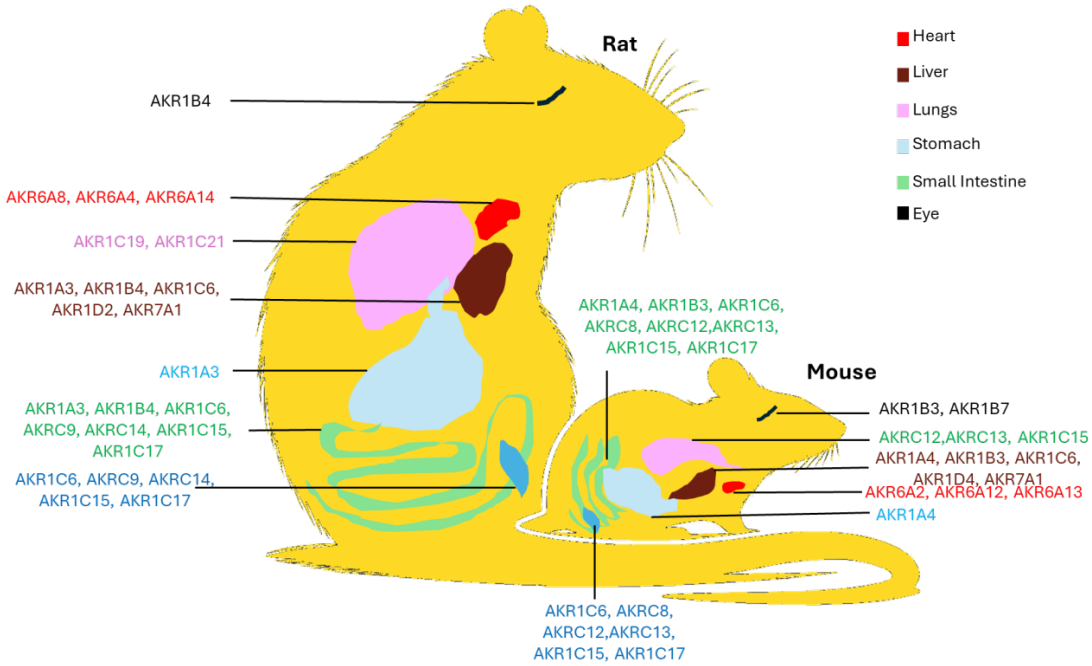
3-Caffeoylquinic acid	0.3	7.9			
Drupanin	38	49			
Artepillin C	23	53			
p-Coumaric acid	76	63			
Caffeic acid	32	90			

Supplementary Table S2: Biodistribution of aldo-keto reductases (AKRs) in mouse (*Mus musculus*) and rat (*Rattus norvegicus*)

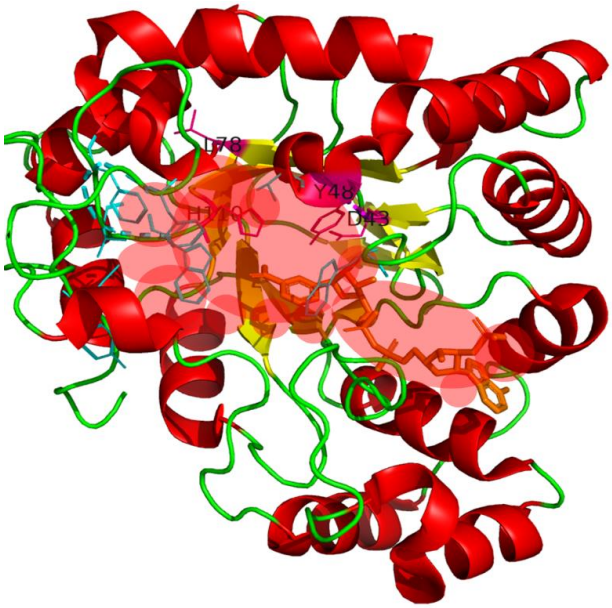
Human	Rat	Mouse		Reference
AKR1A1	AKR1A3	AKR1A4		[21]
AKR1B1	AKR1B4	AKR1B3	AKR1B7	[21]
AKR1B10	AKRB10	AKRB7		[22]
AKR1B15	AKR1B14 and 17	AKR1B16		[22]
AKR1C1	AKR1C9	AKR1C21	AKR1C19	[23]
AKR1C2	AKR1C13 and 14	AKR1C15		[24]
AKR1C3	AKR1C17	AKR1C8	AKR1C18	[25]
AKR1C3	AKR1C6	AKR1C6		[26]
AKR1C3	AKR1C15	AKR1C12 and 13		[27]
AKR1C4	AKR1C6	AKR1C6		[28]
AKR1D1	AKR1D2	AKR1D1 and D4		[21]
AKR6A3	AKR6A13	AKR6A8		[24]
AKR6A5	AKR6A2	AKR6A4		[24]
AKR6A9	AKR6A12	AKR6A14		[24]
AKR7A2	AKR7A1	AKR7A1		[29]
AKR7A3	AKR7A4	AKR7A5		[30]

Supplementary Table S3: The dimension of substrate pocket in Human AKRs

Protein (PDB ID)	Surface Area	Volume Area	Ratio
AKR1A1 (2ALR)	301	271	1.11
AKR1B1 (2F2K)	405	226	1.79
AKR1B10 (4GQG)	436	246	1.78
AKR1C1 (3NTY)	429	238	1.8
AKR1C2 (4JQ3)	463	323	1.43
AKR1C3 (7C7F)	655	463	1.41
AKR1D1 (3BUR)	577	423	1.36
AKR6A5 (1ZSX)	362	165	2.2
AKR7A2 (2BP1)	628	303.9	2.07



Supplementary Figure S1: Biodistribution of aldo-keto reductases (AKRs) in mouse (*Mus musculus*) and rat (*Rattus norvegicus*)



Supplementary Figure S2: Computation and active site volume of AKR1B10 (3NTY) using CastP and PyMOL.

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