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The Aldo-Keto Reductase Superfamily and its Role in Drug Metabolism and Detoxification

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Abstract

The Aldo-Keto Reductase (AKR) superfamily comprises of several enzymes that catalyze redox transformations involved in biosynthesis, intermediary metabolism and detoxification. Substrates of the family include glucose, steroids, glycosylation end products, lipid peroxidation products, and environmental pollutants. These proteins adopt a $(\beta/\alpha)_8$ barrel structural motif interrupted by a number of extraneous loops and helixes that vary between proteins and bring structural identity to individual families. The human AKR family differs from the rodent families. Due to their broad substrate specificity, AKRs play an important role in the Phase II detoxification of a large number of pharmaceuticals, drugs, and xenobiotics.

Keywords

Aldo-keto reductase; AKR; carbonyl reduction; gene homology; structural motif; α, β-barrel; detoxification; pharmaceutical; xenobiotic

INTRODUCTION

Aldo-Keto Reductases (AKRs) are a group of structurally-related proteins of common ancestry. These proteins adopt a $(\beta\alpha)_8$ or TIM-barrel motif (triosphosphate isomerase), which represents a compact yet adaptable scaffolding with structural variations required for binding a chemically-diverse range of carbonyl substrates (Jez et al., 1997a). The active site of AKRs is located at the C-terminal face of the barrel and it is optimized for high-affinity interaction with pyridine nucleotides in the absence of a canonical Rossman fold. Using pyridine nucleotide as cofactors, most AKRs catalyze simple oxidation-reduction reactions. All plants and animals, ranging from yeast to man, express multiple A*kr* genes. The AKR proteins have also been found in a wide range of microorganisms (Ellis, 2002), however it is difficult to draw direct parallels between genes from lower organisms and mammalian AKRs because AKRs from lower organisms form their own families (AKR familes 2–5 and 8–13). Within each species, several A*kr* genes are expressed in most tissues. The highest aldehyde reductase activity has been found in kidney and liver in mammals, birds, reptiles, amphibians, and fishes. Aldehdye reductase activity is also readily measurable in insects (Drosophila) and yeast, and the enzymes from each species showed molecular weight between 30 and 40 kDa (Davidson et al., 1978). Most studies on AKRs have been performed on mammalian proteins with the exception of xylose reductase from yeast (AKR2B), which has potential biotechnological applications such as xylose fermentation to ethanol and organic synthesis (Kratzer et al., 2008; Nidetzky et al., 1996). The proteins encoded by *Akr* genes catalyze a variety of metabolic oxidation-reduction

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reactions ranging from the reduction of glucose, glucocorticoids and small carbonyl metabolites to glutathione conjugates and phospholipid aldehydes. In this capacity, the AKRs function as independent metabolic units or as inter-linked components of metabolic pathways in which these proteins work in collaboration with other carbonyl-metabolizing enzymes such as aldehyde and alcohol dehydrogenases, cytochrome P450s (CYPs) and glutathione S-transferases (GSTs). Given the diversity of substrates, which includes most biologic aldehydes, it appears that one function common to the AKR superfamily may be transformation and detoxification of aldehydes and ketones generated endogenously during metabolism or encountered in the environment as nutrient, food, drug, or toxin (Bachur, 1976).

A characteristic feature of AKRs is their ability to catalyze aldehyde or ketone reduction. Because these proteins lack metal or flavin cofactors, they are relatively inefficient as alcohol dehydrogenases. Most AKRs prefer NADPH over NADH. In metabolically active cells, $NADP^+$ is mostly in the reduced form (Pollak et al., 2007), therefore, reduction is favored over oxidation. The NADPH/$NADP^+$ ratio is reflective of the synthetic capacity of the cell and is kinetically and thermodynamically dissociated from the NAD^+/NADH ratio, which is mostly regulated by rates of glycolysis and respiration. Hence AKRs can accomplish their tasks of metabolism and detoxification without being affected by fluctuations in the cofactor ratio due to changes in metabolic rate and capacity. The constant supply of NADPH maintained at high levels, therefore, provides a strong driving force for AKRs to catalyze reduction under a wide range of energetic states of the cell, associated with different levels of respiration, growth reproduction or starvation.

Tight binding to NADPH provides some AKRs (e.g. aldose reductase) a thermodynamic advantage for achieving the transition state without placing much energetic demand on the substrate (Grimshaw, 1992). Because most of the energy required for carbonyl reduction is derived from nucleotide, not carbonyl, binding, even substrates that are loosely bound to active site residues are reduced with high efficiency. As a results, aldose reductase reduces a wide range of aldehydes (Grimshaw, 1992). The relaxed structural requirements for carbonyl substrates, i.e. broad substrate specificity of several AKRs and high velocity of chemical interconversion compared with cofactor exchange are the features that favor efficient and rapid detoxification and provide a unique detoxification advantage to some AKR proteins.

The carbonyl group, especially as an aldehyde, has high intrinsic chemical activity and it reacts readily with nucleophilic centers (such as proteins side chains containing sulfhydryl or primary amino substituents). The conversion of aldehydes to alcohols, which results in the reduction of the polar carbonyl group, and decreases the overall chemical (but not necessarily the biological) reactivity of the molecule, therefore, represent one mode of inactivation and detoxification. Prompt reduction by AKRs, though in principle reversible (by alcohol dehydrogenases) primes several detoxification pathways and allows further processing and extrusion of carbonyls, without prolonging the residence time of the toxin within the cell.

Several drugs, pharmaceuticals, foods, and pollutants are reactive carbonyls and aldehydes or are converted to carbonyls during *in vivo* metabolism (e.g. by CYP450 catalyzed conversions). There is increasing recognition of the role of AKRs in preventing carbonyl toxicity and as important components of the Phase II drug metabolism pathways. In the following review, we discuss recent developments in the field and in particular the role of AKRs in drug detoxification and xenobiotic metabolism. For additional historical background and perspective, the reader is referred to several excellent reviews on the structural and biochemical properties of AKR superfamily (Bachur, 1976; Jez et al., 1997b; Jez et al., 1997a; Jin and Penning, 2007; Penning and Drury, 2007) or its individual members (Bhatnagar and Srivastava, 1992; Bhatnagar *et al*., 2004; Kinoshita, 1990; Penning, 1997; Srivastava *et al*., 2005b; Yabe-Nishimura, 1998).

2) Structural and Kinetic Features of AKRs

a) Structural fold

Aldo-keto reductases are ancient proteins that share a common conversed $(\beta/\alpha)_8$ barrel structure and a conserved pyridine nucleotide binding site. To-date more than 100 members of this family have been described. These proteins are found in all phyla ranging from prokaryotes, protozoans, and yeasts to plants, animals, and humans. They are believed to have originated from a now extinct multifunctional ancestor by divergent evolution involving gene duplication and subsequent evolutionary variances in substrate binding and preferences (Davidson *et al.*, 1978; Jez *et al.*, 1997b). Based on the level of sequence homology, the AKR superfamily is divided into 15 families and some families are further divided into subfamilies. Members of each family share more than 40% homology with each other and less than 40% with members of any other family. Mammalian AKRs fall within 3 well-defined families (AKR1, 6 and 7). These proteins are widely distributed in tissues and most cells express several AKRs. In humans, 13 different AKR proteins have been identified that fall within the 3 major families of mammalian AKR (Fig. 1). A web page with current information about AKR proteins is maintained at the University of Pennsylvania by Dr. T. Penning at the URL http://www.med.upenn.edu/akr.

The β-barrel structure of the AKR proteins provides a unique identity to the superfamily. This structural fold exceeds any other known fold in terms of its inclusion in a large number of proteins. It has been estimated that nearly 10 % of all proteins, distributed over 28 families fold into $(\beta/\alpha)_8$ barrels (Vega *et al.*, 2003). Most of these proteins function as enzymes that catalyze five of the six general classes of catalytic activities and 15 different enzymatic functions (Hegyi and Gerstein, 1999) with the exception of ligases. The $(\beta/\alpha)_8$ motif has wide functional utility. It can be utilized to bind redox active cofactors, and metals (Vega *et al.*, 2003), to oligomerize into quanternary arrangements (Wise et al., 2002) that can form active site interfaces or it could be used as a gated barrel for channeling reaction intermediates (Amaro et al., 2003). A distinguishing feature of the $(\beta/\alpha)_8$ barrel fold is the presence of the active site at the C-terminus. Because there is no obvious reason for this preference, this feature is considered to be indicative of a common ancestry. The ready interconversion of the substrate specificity of $(\beta/\alpha)_8$ proteins in a single round to random mutations affecting the C-terminus further supports their common ancestry from progenitor proteins of broader substrate specificity (Jurgens et al., 2000). The $(\beta/\alpha)_8$ motif has been proposed to be the result of gene duplication and fusion of an ancient half-barrel protein. This hypothetical evolutionary pathway could be experimentally reconstructed in the laboratory to assemble $(\beta/\alpha)_8$ barrels from $(\beta/\alpha)_4$ half barrels (Hocker et al., 2004) attesting to the likelihood that this could have occurred during the course of evolution. Another conserved feature of $(\beta/\alpha)_8$ proteins is the presence of a phosphate binding site. Approximately two-thirds of the established $(\beta/\alpha)_8$ barrel enzymes utilize substrates or cofactors that contain phosphate group. In the AKR family this is the pyrophosphate backbone of the pyridine nucleotide.

In the canonical $(\beta/\alpha)_8$ structure, the central inner ring of 8 parallel β-strands in a hyperboloid structure is wrapped by an outer envelop consisting of 8 external α-helices (Fig. 2). This generates a highly symmetrical arrangement of secondary structural elements. The structure of the inner β-strand barrel is constrained, whereas, the arrangement of α-helices is more variable. Various motifs such as loops and extra helixes interrupt the α/β barrel fold and add structural diversity to the family. The $(\beta/\alpha)_8$ motif is particularly well-suited for the evolution of new function and modifications of the loops between the α-helices and β-strands alter the properties of binding and catalysis without affecting the basic structure of the protein. Extensions could be used to construct cavities for binding effector molecules or reinforce and tighten cofactor binding.

Variations of the TIM-barrel motif—In AKRs the $(\beta/\alpha)_8$ motif is preceded by a hairpin of two β-strands (β1 and β2, Fig 3) which form the bottom of the barrel. The $(\beta/\alpha)_8$ barrel itself is interrupted by a number of extraneous loops and helixes that vary between AKRs and bring identity to individual families. The "hot spot" for such variations is the region between the 7^{th} and the 8^{th} β-strands of the barrel (strands β9 and β10 in aldose reductase, Fig 3A). The sequence and structure of these extraneous elements differ between subfamilies and is a "fingerprint" or a signature feature for a particular subfamily of enzymes. For example, aldehyde and aldose reductases (AKRs 1A and 1B) have a long loop between β9 and α7 (residues 209 to 230 in AKR1B1, loop B, Fig. 3A) that opens and closes above the bound nucleotide and holds it firmly in place (Wilson et al., 1992), thus facilitating tight binding to NADPH. The length of this loop is variable among members of the family and in some AKRs, e.g. hydroxysteroid dehydrogenases (AKR1Cs) (Bennett *et al.*, 1996), its small size results in the absence of opening or closing movements. The amino acid sequence of the aldose reductase loop shares 100% identity with other AKR1B members and a 68% identity with AKR1A1 (aldehyde reductase), but not with members of other AKR families. Interestingly, it resembles the Drosophila proteins CG6084 and diaphanous FH3 domain of formin3. Whereas CG6084 possesses the aldehyde reductase activity, the FH3 domain of diaphanous formins interacts with activated Rho-GTPases and regulates their activity. Reasons for this similarity are presently unclear.

The opening of the NADPH binding loop to release the cofactor is a single rate-limiting step in the kinetic mechanism of aldose reductase catalysis (Grimshaw *et al.*, 1995) and contributes to over 60% of the rate-limitation in aldehyde reductase-catalyzed reduction of DL-glyceraldehyde (Barski *et al.*, 1995). In contrast, in 3α-HSD (AKR1C2), where the loop is smaller, the chemical step and the release of the alcohol are more rate-limiting than the rate of cofactor release (Jin and Penning, 2006). Extra helix H1 (aa 243–254) is homologous only to members of the AKR1B and 1E families. Similar H1 helix of the aldehyde reductase also shares homology with AKR1E (70% identity) and *1CL1* (62%), a hypothetical gene member of 1C family. It is homologous to a subset of AKRs from yeast (GRE3, YJR096W, YPR1 and YDR124W) indicating that those must be distant precursors of modern aldehyde reductase. It is interesting that the corresponding helix in aldose reductase does not have any similarity with these proteins or aldehyde reductase. Helixes H1 of AKR1C proteins share 100% homology with each other and a weak 64% homology with AKR1A1. Homology search with Drosophila database revealed the similarity of this helix (QLQR motif at the C-teminal end of the helix) to the AKR CG6084, as well as sif protein, which possesses Rho guanyl nucleotide exchange factor activity. The similarity of both the loop of AKR1B1 and H1 helix of AKR1C proteins to Drosophila AKR and proteins related to Rho-GTPases may be indicative that portions of Rho-GTPases were recruited as AKR structural elements during evolution.

The AKR6 family members (Kvβ) have an extra helix attached to a long loop (245–270) between β9 and α7, which is similar to a seat belt loop in AR (Fig. 3 C). This loop shares 42% identity with arsenite resistance protein, however, the significance of this resemblance is unknown. In addition, a helix H2 is located between α7 and β10 of the barrel similar to other AKRs. Extra helixes form a hammerhead-like structure that leans over the active site in the crystal structure of Kvβ. Helix H2 (303–313 in rat Kvβ2) shares homology only within the AKR6 family, whereas, the whole H1–H2 segment of Kvβ (aa 271–312) is similar to zinc finger protein MYM4, but not other members of the AKR family.

In the AKR7 family, the α7 helix is separated from the strand β8 by a stretch of only 9 amino acids, whereas, the same structure in other AKR families is at least 16 amino acids long. This results in helix H1 in AKR7 proteins being 2-times shorter than other AKRs. This helix of the rat protein, AKR7A1 (and abutting residues 270–279) is only 70% homologous to human AKR7 members, suggesting that these proteins may not be evolutionary orthologs. In addition,

rat AKR7A1 is strongly induced by antioxidants, whereas inducibility of human AKR7 enzymes has not been reported suggesting that these proteins may have different functions. Aflatoxin reductases (AKR7) have a sequence of 4 small α-helixes in place of the long loop between β7 and α7 (Fig 3D). In addition AKR7 proteins do not have a β-hairpin structure at the bottom of the barrel.

An additional variable loop in AKRs is between β6 and α4 (loop A). This loop is long in all AKR1 enzymes and contains several active site residues indispensable for catalytic activity such as His110 (AR numbering). The loop is much shorter in AKR6 and AKR7 proteins making their active site much more shallow and accessible to solvent.

The "hot spot" of variability in the AKR superfamily between the 7^{th} and the 8^{th} β-strands of the barrel contains diverse structural elements such as long loops and singular or numerous helices, which brings identity to individual families. Variability in this region suggests divergent evolution that led to multiplicity in substrate specificity and kinetic properties of AKR members. Homology of these variable sequence elements with lower organisms allows us to infer evolutionary origin of individual mammalian AKRs and to relate them to their yeast or insect precursors. If, as homology analysis suggests, some of these elements were borrowed from non-AKR proteins, an intriguing possibility arises that these may serve as interaction domains through which as yet unknown protein-protein interactions of AKRs with proteins from other pathways occur. Investigation into this possibility may lead to the identification of novel and exciting functions of the AKRs or a better clarification of their previously identified roles.

Quaternary structure of AKRs—Members of the AKR1 family are monomeric, whereas AKR7 proteins are dimers and AKR6 proteins form tetramers. The dimerization domain of AKR7 consists of helices α5-and-α6, loop C and helix H2 (Fig. 3D) (Kozma et al., 2002). These elements are not homologous to any other mammalian AKRs, except within the AKR7 family. The helices α5-and-α6, however, are homologous to yeast aryl-alcohol dehydrogenases - AAD4, AAD10, AAD3, and YPO88. These dehydrogenases are distinct from the yeast AKRs (GRE3, YJR096W, YPR1 and YDR124W) homologous to the helix H1 of aldehyde reductase suggestive that the evolutionary separation of AKR families took place at the level of yeast.

In the AKR6 family, the N-terminal β1-β2 hairpin (Y39-G46) forms a part of the tetramer intersubunit interface: together with the closely located R109-S111 segment (Kvβ2-AKR6A5 numbering) from the α2-β5 loop at the bottom of the barrel it interacts with the loop β5-α3 (consisting of amino acid residues K124-R129) at the top (C-terminal end) of another barrel (Gulbis *et al.*, 1999). Thus, both ends of the β5 strand are involved in the intersubunit interactions resulting in the β5 strand being inflexible in the Kvβ tetramer (see Fig. 3C). Immobility of this part of the protein molecule, which contains important catalytic residues (K118, a part of the catalytic triad, is located on β5) may have profound implications on the catalytic properties of AKR6. Not surprisingly, the regions involved in the intersubunit interaction are >92% conserved within the AKR6 family, but do not share homology with AKRs from other families. In addition, the helices α5 and α6 (residues M193 through H234 of Kvβ2) are also unique to the AKR6 family and are involved in Kvβ docking with the T1 domain of the pore-forming Kv channel (Gulbis *et al.*, 2000).

Nucleotide binding—AKRs prefer NADPH over NADH as the reducing cofactor. In cases where direct binding constants have been measured, the K_d of AKRs for NADPH is much lower than that for NADH (Ma *et al.*, 2000; Liu *et al.*, 2001). Cofactor dissociation constants of AKRs calculated from fluorescence titration studies are shown in Table 1. In general, NADPH appears to be the preferred cofactor, with K_d in nanomolar range and the K_d for other pyridine nucleotide cofactors is higher than NADPH suggesting that these cofactors bind with

lower affinities. Like other oxidoreductases, the AKRs enzymes seem to be able to differentiate between oxidized and reduced cofactor and bind reduced coenzyme with higher affinity. Some AKRs, however, can use NADH as coenzyme and even exhibit higher k_{cat} with NADH than NADPH (e.g., AKR1C12, MVDP) (Lefrancois-Martinez *et al.*, 1999; Ikeda *et al.*, 1999). Weaker cofactor binding, however, results in higher k_{cat}, but is inevitably accompanied by higher K_m for carbonyl substrate and a decrease in the specificity of reduction versus oxidation. As a result, higher catalytic constant with NADH results in an overall lower catalytic efficiency k_{cat}/K_m. Structural determinants underlying the preference for NADPH over NADH relate to the positively charged lysine and arginine residues that bind the pyrophosphate backbone and the 5' phosphate group of NADPH (Wilson *et al.*, 1992; Liu *et al.*, 2001; Ratnam *et al.*, 1999).

b) Catalytic properties of AKRs

Most AKRs are catalytically active proteins. As enzymes they catalyze oxidation-reduction reactions using a diverse-array of carbonyls and reducing them to primary or secondary alcohols. There are, however, exceptions. Several AKR proteins have been identified in which the AKR motif has been recruited for a purely structural role. For instance, the Rho (AKR1C10) and RhoB crystallins, which are major components of frog (Fujii *et al.*, 1990) and gecko (van Boekel *et al.*, 2001) lens, retain all the major amino acid residues required for catalysis by other AKRs. Although these proteins bind pyridine nucleotides (Fujii *et al.*, 1990), they show little or no catalytic activity with AKR substrates (Fujii *et al.*, 1990; van Boekel *et al.*, 2001). Weak prostaglandin H2 endoperoxide reductase activity has been reported for rho-crystallin (Fujii *et al.*, 1990), nonetheless, the major function of these proteins is to serve as structural components required for maintaining transparency of the ocular lens. Other AKR proteins, e.g., members of the AKR6 (Kvβ) family may have similar structural roles. Although these proteins display weak catalytic activity with model AKR substrates (Weng *et al.*, 2006), the physiological significance of the Kvβ-mediated catalysis remains unclear, and it is possible that these proteins act merely as chaperones which assist in folding and localization of the pore-forming Kvα subunits or impart inactivation to Kv channels. Members of the AKR family that catalytically active, and whose primary function appears to be catalysis display variable preference. The AKR 1A and 1B proteins, for instance, are more efficient in catalyzing reduction, whereas other AKRs e.g., hydroxysteroid dehydrogenases and dihydrodiol reductases are equally effective as reductases or dehydrogenases (Pawlowski and Penning, 1994). Despite this distinction, the chemical mechanism of catalysis appears to be similar for all AKRs.

The catalytic mechanism of AKRs involves stereospecific reduction or oxidation reactions that utilize pyridine nucleotides. The process of reduction by AKRs proceeds in two steps: 1) a hydride ion is transferred from NAD(P)H to the carbonyl substrate, and 2) the proton is added from the solvent to reduce the carbonyl to an alcohol (Fig. 4). These two steps could occur in a concerted or step-wise manner. The specific time-gap between hydride and proton transfer dictates, in a large measure, the extent of charge developed on the carbonyl during the transition state, and may be the source of differences between the catalytic properties and substrate preferences of different AKRs. Oxidation-reduction reactions of AKRs involve general acid-base catalysis, although under some conditions, non-acid-catalyzed reduction mediated entirely by a propinquity effect (as with mutant forms of rat liver AKR1C9 or 3-α hydroxysteroid dehydrogenase (Schlegel et al., 1998b) has been reported. However, it remains unclear whether any of the AKRs catalyze oxidation reduction entirely by a proximity effect and can naturally do so without acid-base catalysis. Structurally, most AKRs retain a conserved catalytic tetrad, which consists of Tyr-48, His-110, Lys-77 and Asp-43 (AKR1B1 numbering). The pK of the group involved in acid-base catalysis has been reported to be 6.5 to 7.0 for AKR1B1 (Liu *et al.*, 1993) and AKR1C9 (Schlegel *et al.*, 1998b). On the basis of the low pK,

His-110 was first proposed to be the acid-base catalytic group for AKR1B1 (Liu *et al.*, 1993). However, later studies showing that Y48F:AKR1B1 (Bohren *et al.*, 1994) and Y55F:AKR1C9 (Schlegel et al., 1998a) were inactive, whereas mutations of His-110 resulted in the generation of a partially active enzyme suggested that the acid-base catalytic group in AKRs is Tyr-48 (AKR1B1). This tyrosine is universally conserved in all AKRs, whereas His-110 is not conserved in some AKRs (e.g., the AKR6 family). In AKRs that do contain the active site histidine (corresponding to His-110 in AKR1B1), this residue was proposed to play an important role in proper orientation of the substrate at the active site. To explain the low pK_a of the acid-base residue, the pK_a of the active site tyrosine (Tyr-48) was suggested to be decreased due to a hydrogen bonding with Asp-43 and Lys-77 (Bohren *et al.*, 1994). A similar mechanism has been proposed for aldehyde reductase – AKR1A1 (Barski *et al.*, 1995). Nevertheless, not all observations support an obligatory role of an active site tyrosine in AKR catalysis. For instance, Y48H and Y48S mutants of AKR1B1 retain catalytic activity (Bohren *et al.*, 1994) and Y55S and Y55F of AKR1C9 are active with 9,10-phenanthrenequinone (Schlegel *et al.*, 1998b), indicating that under some circumstances the reaction proceeds by recruiting residues other than tyrosine or that no acid-base catalysis is required at all. Furthermore, a catalytic role of His-110 could not be entirely excluded. Quantum mechanical calculations of the energetics of reaction pathway (Lee et al., 1998) support a primary role of His-110 in AKR1B catalysis and in recent analysis of AKR5A2 catalysis, Kilunga *et al.* (Kilunga et al., 2005), found that of the 4 residues of the catalytic tetrad, only His-110 and Lys-77 are important for the reduction of PGH_2 to $PGF_{2\alpha}$, and that active site tyrosine and aspartate were not required. Active site tyrosine was, however, required for the reduction of the non-physiological substrate 9,10-phenanthroquinone. Moreover, based on the observation that Y50F:AKR1A1 does not bind to carbonyl substrates, Ye *et al.*, (Ye et al., 2001) suggested that the active site tyrosine plays a critical role in substrate binding, perhaps in stripping water from basally hydrated aldehydes, which must become dehydrated before participating in hydride transfer reactions. Because both His-110 and Tyr-48 form hydrogen bonds with the substrate carbonyl, it is difficult to establish which one of them is the proton donor under a specific set of reaction conditions with a given substrate.

3) Human AKRs

To date 13 AKR proteins have been identified in humans. These include AKR1A1 (aldehyde reductase), AKR1B1 and B10 (aldose reductases), AKR1C1, C2, C3, and C4 (hydroxysteroid dehydrogenases), AKR1D1 (Δ4-3-ketosteroid-5-β-reductase), AKR6A3, A5, and A9 (Kvβ proteins), and AKR7A2 and 7A3 (aflatoxin reductases). Specific features of each of these families are discussed below.

AKR1A1 – ALDEHYDE REDUCTASE

One of the first AKRs to be discovered, ARK1A1 was identified as a key enzyme involved in ascorbic acid biosynthesis (Mano et al., 1961). It is a cytosolic, NADPH-dependent, monomeric oxidoreductase with a compulsory ordered substrate binding and product release. The enzyme is ubiquitously expressed in most tissues with highest levels in the kidney proximal tubules (Barski *et al.*, 2005; Barski *et al.*, 1999). The crystal structure of AKR1A1 reveals a canonical AKR β-barrel with the active site located at the C-terminus (el-Kabbani *et al.*, 1995). Binding of NADPH to the protein is similar to other AKRs (e.g., AKR1B1) in that cofactor binds in the extended conformation across the lip of the barrel, although affinity is slightly lower: the K_d^{NADPH} of AKR1A1 is 13-fold higher than that of AKR1B1, i.e., 130 (Barski *et al.*, 1995) versus 10 (Ehrig *et al.*, 1994) nM. Also, in contrast to AKR1B proteins, AKR1A1 lacks the hyper-reactive active site cysteine and the Nε of the imidazole ring of the active site histidine (His-112) interacts with the amide side chain of the nicotinamide ring of

NADPH (el-Kabbani *et al.*, 1995), underscoring differences between AKRs in the exact positioning of NADPH in the active site.

AKR1A1 displays broad substrate activity. The enzyme prefers carboxyl-group containing negatively charged substrates, although aromatic aldehydes, steroid aldehydes, and small 3-carbon aldehydes are also reduced with high affinity (Wermuth and Monder, 1983; Branlant and Biellmann, 1980; Wermuth et al., 1977). In the reverse direction, AKR1A1 has been shown to catalyze the oxidation of proximate *trans*-dihydrodiols to *o*-quinones (Palackal et al., 2001). In rodents, the physiological role of the enzyme is thought to be the reduction of D-glucuronate to L-glulonate, which is then converted to L-gulonolactone and finally to ascorbic acid (Linster and Van, 2007). An essential role of AKR1A1 in ascorbic acid synthesis is consistent with the observation that inhibition of AKR1A1 in mice increases urinary output of glucuronate and decreases the output of vitamin C (Barski *et al.*, 2005). In contrast to rodents, humans do not synthesize ascorbic acid, and therefore persistence of a functional *AKR1A* gene in humans indicates that the protein may have role that has not been discovered to date. In the kidney aldehyde reductase has been found associated with myo-inositol oxygenase, which converts myo-inositol to D-glucuronate, indicative of the role of aldehyde reductase in the myo-inositol catabolism pathway (Reddy *et al.*, 1981).

AKR1B1 – ALDOSE REDUCTASE

Because of its potential role in mediating hyperglycemic injury and in the development of secondary diabetic complications, (Dvornik *et al.*, 1973; Gabbay *et al.*, 1966) AKR1B1 is by far the most studied AKR. The enzyme was first isolated as a glucose-reducing activity (HERS, 1960). Under basal conditions, it catalyzes the reduction of low amounts of glucose. The increase in glucose reduction by AKR1B1 during hyperglycemia has been linked to the development of tissue injury and inhibition of this enzyme has been shown to prevent, delay, or in some cases even reverse, tissue injury associated with diabetes (Gabbay, 2004; Dvornik *et al.*, 1973; Nicolucci *et al.*, 1996; Bril and Buchanan, 2006). Although widespread clinical use of AKR1B1 inhibitors is limited by off-target effects of these drugs, that AR-mediated catalysis represents a significant biochemical cause of hyperglycemic injury is well established (Kinoshita, 1990). Specific mechanisms by which AKR1B1 contributes to the development of injury have been recently linked to the ability of this enzyme to regulate multiple inflammatory pathways in which inhibition of AKR1B has been shown to interrupt inflammation triggered by high glucose or cytokines such as TNF-α (Ramana et al., 2007; Ramana et al., 2005; Ramana et al., 2003a; Ramana et al., 2006b). Mechanisms by which AKR1B1 regulates these pathways, however, remain unclear.

AKR1B1 is a wide-specificity catalyst. It is known as the low K_m aldehyde reductase; in contrast to AKR1A1, which has higher values of K_m and k_{cat} (HERS, 1960). Aldose reductase has higher catalytic efficiency (k_{cat}/ K_m) for most of the substrates but a lower k_{cat} (Bohren et al., 1991). In the catalytic cycle of the enzyme, nucleotide exchange is rate-limiting. As a result, the k_{cat} is substrate-independent (usually between 30–40 min^{-1}) (Bohren *et al.*, 1994). The enzyme catalyzes reduction of many substrates of physiological significance including AGE precursors (Vander et al., 1992), isocorticosteroids (Wermuth and Monder, 1983), lipid peroxidation products such as HNE and oxidized phospholipids (Srivastava et al., 2004b), glutathione conjugates of unsaturated aldehydes (Srivastava *et al.*, 1995), environmental pollutants, e.g. acrolein and its glutathione conjugate, etc. The enzyme has been shown to play an essential role in vascular smooth muscle cell proliferation during restenosis and atherosclerosis (Ramana *et al.*, 2002) and in mediating mitogenic signaling triggered by growth factors and cytokines (Ramana *et al.*, 2003a). The enzyme has also been shown to be an obligatory mediator of ischemic preconditioning (Shinmura et al., 2002). Genetic deletion of

Akr1b3 in mice leads to the development of nephrogenic diabetes insipidus (Ho *et al.*, 2000), indicating a potentially critical role of the enzyme in concentrating urine.

A unique feature of ARK1B1 is its sensitivity to oxidation. A hyperreactive cysteine residue is located at the active site of the enzyme (Cys-298) and oxidation of this cysteine accelerates catalysis and prevents inhibitor binding (Petrash et al., 1992). This increase in enzyme activity has been suggested to be due to a decrease in the affinity of the enzyme for NADPH (Ehrig *et al.*, 1994). Oxidation of Cys-298 prevents complete closure of the NADPH binding loop and therefore the loop is easier to open during the release of $NADP^+$ (Bhatnagar et al., 1994). Because $NADP^+$ release is rate-limiting in AKR1B1 catalysis (Grimshaw *et al.*, 1995), an increase in the rate of $NADP^+$ release increases k_{cat}. Several studies suggest that oxidation of Cys-298 is a physiological process and that it represents a paradigmatic mode of redox regulation. These studies show that Cys-298 of AKR1B1 could be glutathiolated, nitrosylated or oxidized to a sulfenic acid, and glutathiolated and sulfenic acid forms of AKR1B have been detected *in vivo* (Kaiserova *et al.*, 2006; Srivastava *et al.*, 2005a; Srivastava *et al.*, 2001b; Ramana *et al.*, 2003b). Because oxidation of the enzyme affects catalysis and substrate preference and could be induced by NO and peroxynitrite, it has been suggested that NO regulates the activity of the polyol pathway and the role of AKR1B in cell growth, inflammation and apoptosis (Ramana *et al.*, 2003b; Kaiserova *et al.*, 2008). Further studies are required to fully evaluate this hypothesis and its physiological implications.

AKR1B10 – SMALL INTESTINE ALDOSE REDUCTASE

This enzyme is a recent addition to the AKR family and was identified simultaneously by two groups in 1998 (Cao *et al.*, 1998; Hyndman and Flynn, 1998). In contrast to the ubiquitously expressed aldose reductase, AKR1B10 is expressed mainly in small intestine, colon, liver, thymus (Cao *et al.*, 1998), and adrenal gland (Hyndman and Flynn, 1998). The amino acid sequence of AKR1B10 is 71% identical to that of aldose reductase and the enzyme exhibits substrate-specificity and inhibitor-sensitivity similar to aldose reductase. Kinetic differences include a more basic pH optimum, 3–6 fold higher k_{cat} for some substrates (e.g. methylglyoxal, 2-, 3-, and 4- nitrobenzaldehydes, DL-glyceraldehyde and diacetyl) (Cao *et al.*, 1998), indicating that the nucleotide exchange and associated loop opening-closing are not as rate-limiting in AKR1B10 as they are in aldose reductase. The AKR1B10 exhibits approximately 100-fold higher catalytic efficiency towards all-*trans*-retinal (Gallego et al., 2006) and several ketones including drugs such as daunorubicin and dolasetron (Martin *et al.*, 2006). The AKR1B10 protein is strongly overexpressed in lung and hepatic carcinomas (squamous cell and adenocarcinomas) (Fukumoto *et al.*, 2005; Cao *et al.*, 1998), as well as in colorectal and uterine cancers (Yoshitake et al., 2007). It has been shown that silencing of *AKR1B10* gene using siRNA results in growth inhibition and reduced foci formation rate and colony size of colorectal cancer cells, indicating that AKR1B10 plays a critical role in cancer cell proliferation (Yan *et al.*, 2007). The mitogenic role of AKR1B10 may be related to the depletion of retinoic acid (due to excessive AKR1B10 activity) and subsequent loss of cell differentiation and cancer development (Gallego *et al.*, 2007). The *AKR1B10* gene has been found induced by cigarette smoke condensate in human oral cells (Nagaraj et al., 2006). Because the enzyme shows high catalytic activity with aldehydes in cigarette smoke such as acrolein and crotonaldehyde (Yan *et al.*, 2007), it is likely that, like aldose reductase, AKR1B10 protective against electrophilic injury. In addition, AKR1B10 appears to regulate biosynthesis of fatty acids through association with a rate-limiting enzyme of *de novo* synthetic pathway acetyl-CoA carboxylase-α (Ma et al., 2008), however, the physiological significance of this role of AKR1B10 needs to be explored further.

AKR1C1-C4 – HYDROXYSTEROID DEHYDROGENASES

This is a subfamily of 4 *AKR1C* genes in humans that shares over 86% homology with each other (Jez et al., 1997a). All 4 enzymes are found in liver but they have different extrahepatic distribution. Whereas AKR1C4 is expressed predominantly in liver, AKRs 1C2 and 1C3 are dominantly expressed in prostate and mammary gland (Penning *et al.*, 2004). In contrast to AKR1A and 1B, these enzymes work efficiently with ketones as well aldehydes. Their natural substrates are steroids and prostaglandins. The enzymes of the AKR1C subfamily generally have low k_{cat} values (typically < 30 min^{-1}) and they catalyze reduction and oxidation reactions with comparable efficiency (Penning *et al.*, 2000). The kinetic mechanism of rat AKR1C9 and human AKR1C2 has been studied in detail using steady-state and stop-flow approaches. In contrast to AKR1B and 1A enzymes, where the release of $NADP^+$ is largely rate-limiting, the AKR1C catalyzed reaction is limited by chemistry and the release of the steroid product (Jin and Penning, 2006; Heredia and Penning, 2004). The diversity of residues lining the substrate binding pocket of AKR1C proteins allows for a variable mode of steroid binding and for specific recognition of 3-, 17-, or 20-ketosteroids with variable affinity (Penning et al., 2003). The AKR1C enzymes are also known as dihydrodiol dehydrogenases because of their ability to catalyze $NADP^+$-linked oxidation of *trans*-dihydrodiols of aromatic hydrocarbons to the corresponding catechols (Hara et al., 1986). Directional preferences of these enzymes in intact cells are largely governed by the relative affinities for nucleotide cofactors and existing cofactor gradients. For example, diminishing the NADPH/$NADP^+$ gradient by adding the oxidizing dye methylene blue to the medium or mutating Arg-276, which is responsible for the preference of the enzyme for NADPH over NADH, shifts the preference of the enzyme from dihydrotestosterone reduction to androstenediol oxidation in transfected HEK293 cells (Papari-Zareei et al., 2006; Sherbet et al., 2007), indicating a unique role of the NADH/NADPH ratio in regulating AKR1C catalysis.

AKR1C4 is found almost exclusively in the liver. It is the most efficient member of the family, with k_{cat}/K_m values 10- to 30-fold higher than that of other AKR1C proteins (Penning *et al.*, 2000). The AKR1C3 protein is also known as PGF synthase. It catalyzes the conversion of prostaglandins H_2 and D_2 into PGF2α and 9α,11β-PGF2α respectively (Suzuki-Yamamoto *et al.*, 1999). It also possesses 17β-hydroxysteroid dehydrogenase activity and it catalyzes the interconversion between estrone and estradiol, and androstanedione and testosterone (Deyashiki *et al.*, 1995). The AKR1C2 is also known as the bile acid binding protein because one of its functions may be to transport bile acids from the canalicular to the polar end of hepatocyte (Stolz et al., 1993). AKR1C1 which possesses 20α-HSD activity, has been suggested to play an important role in reductive inactivation of progesterone into 20α-hydroxyprogesterone and deficiency of this enzyme has been shown to cause a delay in parturition (Piekorz *et al.*, 2005). Although much is known about the substrate specificity and tissue distribution of AKR1C enzymes, their physiological roles still remains elusive. Also the extent of redundancy in the function of AKR1C enzymes remains unclear and it is not known whether there are additional yet unknown members of this family expressed in humans. The enzymes of this family share a very high extent of homology and overlapping catalytic properties, such as substrate specificity, hence it may be important to know to which extent and with which substrates these enzymes can substitute one another. Efforts in applying knockout technology to this family of AKRs are hampered by the fact that mice express at least 8 AKR1C proteins compared to 4 in humans. Hence, it is difficult to draw direct parallels between human and murine enzymes. To date, only one knockout model of the enzyme of this family, namely 20α-HSD, has been reported, and this led to a decrease in the survival of pups and an increase in the duration of the estrous cycles and pregnancy (Ishida *et al.*, 2007; Piekorz *et al.*, 2005). Development of specific inhibitors and fluorogenic substrates that can be used to interrogate the role of individual AKR1C isoforms, as well as serve as potential drug candidates is under way (Byrns *et al.*, 2008; Bauman *et al.*, 2005; Yee *et al.*, 2006). Approaches based

both on similarities in activity (such as e.g. 20α− vs 3α−, or 17β− HSD activity) and sequence and expression profile and gene regulation mechanism (promoter) similarities, as well as siRNA based approaches may be useful in answering these important questions.

AKR1D1 – DELTA 4-3-KETOSTEROID-5-BETA-REDUCTASE

has been purified from the liver (Okuda and Okuda, 1984), and it is the only enzyme that catalyzes 5β-stereospecific reduction necessary for bile acid synthesis. The enzyme reduces the Δ4 double bond of Δ4-3 ketosteroids such as 7α-hydroxy-Δ4-cholestene-3-one and Δ4-cholestene-3-one-7α,12α-diol to form the A/B cis-ring structure (Fig. 5). The resulting 5β-dihydrosteroids are further reduced by liver AKR1C4 to ultimately form chenodeoxycholic and cholic acids. Mutations in the gene encoding AKR1D1 (also known as SRD5B1) in human patients lead to neonatal cholestasis, hepatitis, and liver failure (Lemonde et al., 2003; Setchell et al., 1988). AKR1D1 also exhibit enzymatic activity for several steroid hormones including testosterone, progesterone, cortisol and cortisone among others (Okuda and Okuda, 1984) and is present in the brain (Hutchison and Steimer, 1981) and in tissues of the genitourinary tract. Most likely it plays an important role in the degradative metabolism of sex hormones and regulating multiple hormone-dependent processes in concert with 3α-hydroxysteroid dehydrogenases of the AKR1C family. The level of this enzyme in the liver of males and females has been reported to be different (Mode and Rafter, 1985), however, the significance of this difference remains unclear. Metabolomic analysis of a female patient deficient in AKR1D1 activity revealed an almost complete absence of 5β-reduced metabolites of corticosteroids and significantly reduced production of 5β-reduced metabolites of other steroids (Palermo et al., 2008). Despite these changes in steroid profile, there were no clinical symptoms that could be attributed to changes in steroid hormone metabolism, suggesting redundancy and that bile acid biosynthesis may be the major clinically important function of this enzyme.

AKR6 FAMILY – the Kvβ PROTEINS

These proteins associate with voltage-gated potassium (Kv) channels via an N-terminal T1 cytosolic domain of the transmembrane α-subunit of Kv channels (Gulbis *et al*., 2000; Sewing *et al*., 1996). The Kvβ proteins assemble with the α subunit early during channel biogenesis in the ER (Nagaya and Papazian, 1997) and they facilitate subunit assembly and promote protein maturation and cell surface expression (Fink *et al.*, 1996; Manganas and Trimmer, 2000; Shi *et al.*, 1996). In addition, they are also involved in polarized trafficking and axonal localization of Kv proteins (Campomanes et al., 2002; Gu et al., 2003). These proteins have a distinct N-terminus attached to a highly conserved AKR domain. The variable N-terminus, related curiously to *Drosophila* death proteins *grim* and *reaper* (Avdonin et al., 1998) induces fast inactivation in non-inactivating Kv1 channels and accelerates inactivation of Kv1.4 (Rettig *et al.*, 1994). These proteins retain the catalytic and nucleotide binding groups common to other AKRs (Gulbis*et al*., 1999), and despite high affinity NADPH binding (Liu *et al.*, 2001) and weak catalytic activity with aromatic aldehydes, ketones and natural substrates (Weng *et al.*, 2006; Tipparaju *et al*., 2008), the significance of their AKR functions remains obscure. It also remains unclear whether these proteins have been recruited to a structural role due to their sturdy β-barrels for supporting inactivation peptides or whether the oxidoreductase activity of these proteins regulates Kv channel activity by differential effects of reduced and oxidized nucleotides (Tipparaju et al., 2005).

AKR7A2-A3 – AFLATOXIN REDUCTASES

Aflatoxin reductase was first discovered in the rat for its ability to metabolize aflatoxin B1 dialdehyde into a non-toxic alcohol (Ellis *et al.*, 1993). In contrast to the monomeric AKR1 enzymes, AKR7 proteins are functional dimers (Kelly *et al.*, 2000; Kozma *et al.*, 2002). The

2-carboxybenzaldehyde, a compound used to mask charged amino or carboxy groups to improve the absorption of drugs, is a “diagnostic” substrate for the AKR7A family, because it is not reduced by other AKRs (O'Connor et al., 1999). Two AKR7 family members have been characterized in humans: AKR7A3 has tissue distribution limited to stomach, pancreas, kidney and liver (Knight *et al.*, 1999), whereas, the other enzyme (AKR7A2) is widely distributed in extrahepatic tissues (Ireland *et al.*, 1998). Like humans, rats also posses two aflatoxin reductase (*7a1* and *7a4*) genes, whereas, the mouse has only one gene (*Akr7a5*) (Hinshelwood et al., 2002). The ubiquitously expressed AKR7A2 (human) and 7A4 (rat) enzymes have high affinity and catalytic efficiency for succinic semialdehyde (SSA), which is a metabolite of the neurotransmitter GABA. SSA is considered to be a physiological substrate of AKR7A2, which converts it to γ-hydroxybutyrate (GHB) and has been demonstrated to represent the major SSA reductase activity in cell lines of neuronal origin (Lyon *et al.*, 2007). The second enzyme, AKR7A3, and its rat counterpart (AKR7A1) are significantly more efficient in reducing aflatoxin B_1-dialdehyde (Guengerich *et al.*, 2001). Based on its limited tissue distribution and activity with a number of toxic aldehydes such as acrolein, methylglyoxal and aflatoxin dialdehyde (Gardner et al., 2004), this enzyme has been assigned a detoxification role. The *Akr7a1* gene is induced in the rat liver up to 15-fold by antioxidants and electrophiles such as ethoxyquin and butylated hydroxytoluene but the inducibility of *AKR7A3* in humans has not been established (McLellan et al., 1994). Murine Akr7a5 is also not inducible by antioxidants (Hinshelwood *et al.*, 2002).

The AKR7 enzymes were considered cytosolic proteins until an additional sequence of ~40 amino acid residues that serves as a localization signal to the Golgi complex, was discovered at the N-terminus of AKR7A2, A4, and A5 (Kelly *et al.*, 2002). It has been suggested that localization of AKR7A2 and A4 to the Golgi apparatus facilitate synthesis and secretion of γ-hydroxybutyrate (GHB) (Kelly *et al.*, 2002). In addition, AKR7A2 has been found in the mitochondria of SH-SY5Y neuroblastoma cell line, where it was hypothesized to work in concert with SSA dehydrogenase to remove GHB via oxidation (Keenan et al., 2006). It is unclear, however, whether aflatoxin reductase or some other enzyme catalyzes the oxidation of GHB to succinic semialdehyde.

4) Gene Structure

Genomic structure[a] of the AKRs generally conforms to their classification into families (<40% amino acid identity between families, >60% identity defines a subfamily). In general, the *AKR* genes consist of a median of 10 exons and have an average length of ~17 kb. In the human genome, with the exception of Kvβ (AKR6 family), AKR members belonging to the same family form gene clusters on the same chromosome and have similar gene structures. Human subfamilies differ somewhat in the degree of homology between its family members. Whereas the human 7A and 1C subfamilies share at least 82% identity, whereas, the *1B* and *6A* genes are only 70–74% identical.

a) Human Gene Structure

The *AKR1A* family has only one member in humans and its gene is located on chromosome 1p32-33 (Fig. 6). There is also only one *AKR1D1* gene, which is located on chromosome 7q32-33. It is a much larger gene when compared with a majority of other *AKR* genes and it spans 41.85 kb of the genomic sequence (Fig. 7). The *AKR* genes *1B1* and *1B10* are located next to each other on chromosome 7q33-35 and each gene consists of 10 exons. Clustered together with these two genes is a predicted gene locus *tcag7.1260*, which probably results

[a]Discussion of the gene structure of human AKRs and their homologies to rodents are based on the data contained in public accessible databases NCBI http://www.ncbi.nlm.nih.gov/ and Ensembl http://www.ensembl.org/. Murine and rat orthologs of human genes are identified based on the information of Homologenen database at NCBI.

from duplication of the *AKR1B10* gene. This gene locus, which is potentially encodes an unidentified ARK1B protein, also consists of 10 exons and contains an open reading frame that has 91% identity to the *1B10* gene.

The four human *AKR1C* genes consist of 9 coding exons and form a distinct cluster on chromosome 10p14-15. As shown in Fig. 7, *1C1* and *1C3* genes are almost identical in structure and length. The *AKR1C4* gene has the same structure but with longer introns and *1C2* gene is similar but has two additional non-coding exons at the 5'-end. The four known *AKR1C* genes are part of a larger cluster of 9 genes. Out of the remaining 5 genes, one belongs to the *AKR1E* family and encodes a protein named human testis AKR (htAKR) (Azuma *et al.*, 2004). This gene is listed in the Genebank as *AKR1CL2* and should be renamed *AKR1E2*, in accordance with to the nomenclature of the AKR superfamily. Of the remaining 4 predicted genes, two consist of 8 exons according to NCBI database and 9 exons according to Ensembl database and possibly code for yet unknown human AKR1C members (tAKR and AKR1CL1), while two other seem to be pseudogenes (*LOC643789* and *LOC648947*). The properties of these genes as well as human AKR1E member are discussed below in connection with homology with rodent genes.

The two known human aflatoxin reductase genes: *AKR7A2* and *7A3* reside on chromosome 1p35.1-36.23 and consist of 7 exons (Fig. 6, Fig. 7). An additional gene, provisionally labeled as *AFAR3*, reside in the same cluster but codes for a 153 amino acid protein, whereas *AKR7A2* and *7A3* each have 359 and 331 amino acids, respectively. The sequence of AFAR3 protein only partially resembles other AKR7 proteins. Although nucleotide alignment of *AFAR3* and *AKR7A3* cDNAs shows two regions of homology, the 5'-homologous region of *AFAR3* translates with a frame shift relative to *7A3*, thus coding for a completely different amino acid sequence. Perhaps the *AFAR3* arose by duplication of the *AKR7A2/A3* gene, but lost its middle exons in the process. It is presently unknown whether the *AFAR3* gene encodes a functional protein.

Genes of the *AKR6* (Kvβ) family are different from other AKRs in several aspects. First, these genes do not form a cluster on a chromosome as other families do. Three existing human genes are located on 3 separate chromosomes – 1, 3, and 17. Second, despite similarities in intron-exon structure (all 3 Kvβ genes consist of 14–16 exons), the length of these genes differ greatly. Whereas *AKR6A9* (Kvβ3) gene is only 7 kb long, *AKR6A3* (Kvβ1) spans a whopping 416 kb. Third, the *AKR6* genes possess long 3'-untranslated sequences. The *AKR6A3* gene has 2391 nt long 3'-UTR, *AKR6A5* has 1889 nt, and *6A9* has 1270 according to Ensembl database (discrepancies exist in regard to the 3'-UTR of this gene: Enseml lists 2 transcripts with 1270 and 393 nt long UTR; Genbank lists just a 393 nt transcript and original publication by T. Leicher *et al.* (Leicher et al., 1998) lists polyA sites 756 and 760 nt downstream of the stop codon). Also, the 5'-end of this gene, upstream to the open reading frame, has not been characterized. With the exception of *AKR1D1* which has 1642 nt-long 3'-end, all other *AKR* genes have 3'-untranslated regions that are less than 300 nt long. Generally, 3'-UTRs are considered important determinants of mRNA stability and regulation by micro-RNA. Thus, we speculate that long 3'-UTR of Kvβ might have some regulatory elements which control the post- transcriptional expression of these genes. In the Kvβ genes, the AKR domain starts on the second coding exon (marked with arrowhead on Fig. 7) and invariably consists of 13 exons; the 5'-exons encode the "ball and chain" domain (Leicher et al., 1998). From an evolutionary standpoint, it appears that a primordial *AKR* gene was recruited to serve as an auxiliary subunit for Kv channels and combined with various N-termini to form a functional protein with necessary inactivation qualities (Kvβ2 is not capable of inducing channel inactivation). This notion is consistent with large variations in the length of Kvβ genes and with the observation that the 5'- non-AKR exons comprise a large portion of the Kvβ1 and 2 gene.

Gene processing—A majority of the human *AKR* genes have translation start sites at the first exon. Notable exceptions are: aldehyde reductase (1A1), type III 3α-HSD (1C2), and Kvβ2 (6A5), all of which have evolved separate exons at the 5'-end coding for 5'-UTR. As a result, aldehyde reductase has at least 3 transcripts coding for the same protein, but with different 5'-untranslated regions resulting from the use of alternative transcription start sites and alternative splicing (Barski *et al.*, 1999; Barski *et al.*, 2005). The *AKR1C2* also has alternatively spliced transcripts in the 5'-UTR (Lou et al., 1994). The structure of 5'-untranslated regions may contain uORFs and other structures that generally determine translation efficiency. Transcripts with alternative UTR may be a means of fine tuning expression. For instance, in AKR1A1, the two alternative transcripts have different tissue distribution: a long isoform is expressed ubiquitously in tissues, whereas a short isoform is specific for kidney, liver and intestine. It is absent in proliferating cell lines and may be subject to developmental regulation (Barski *et al.*, 2005). Members of the *AKR6* family *6A3* and *6A5* also exhibit alternative splicing/transcription resulting in expression of protein isoforms with alternative N-terminal domains. The *AKR6A3* gene produces 3 alternative isoforms – Kvβ1.1, 1.2 and 1.3 which exhibit distinct tissue distribution (Rettig *et al.*, 1994; England *et al.*, 1995). The *AKR6A5* gene has two isoforms: a longer Kvβ2.1 and shorter 2.2 (Pongs et al., 1999). These isoforms differ in their N-termini, but not in their AKR domains. Specific roles of the alternative sites of Kvβ2 N-terminus remain unknown.

b) Homology between human and rodent *AKR* genes

Although human AKRs are the focus of the present review, extensive use of rats and mice in laboratory research warrants some discussion of their homology to human genes. The AKR families of rodents differ from that of humans and this difference impedes the use of transgenic and knockout animals in elucidating the physiological function of these enzymes. Although some rodent genes are direct correlates of human genes, this is not the case with other rodent AKRs. However, cross-species comparison of known AKR genes across databases developed by large-scale genome sequencing and annotation projects reveals similarities beyond what is obvious from current knowledge of characterized proteins (Table 2). Novel genes clustering within existing families emerge from comparison of human genomic sequence with known rodent AKR genes and *vice versa*. For example, two human (AKR1B1 and 1B10) and three murine (1B3, 1B7, and 1B8) AKR1B proteins are known. The 1B1 protein is a homolog of 1B3, whereas 1B10 is not homologous to either 1B7 or 1B8. Examination of human genome however, reveals a predicted gene locus *tcag7.1260*, which is a homolog of murine *Akr1b8*. Likewise, a *RIKEN cDNA 2310005E10* gene, corresponding to *1b10* has been found in murine genome and its cDNA found in expressed sequence tags (EST) libraries (Table 2). Existence of a fourth gene in the human *Akr1b* locus on chromosome 7 has been predicted but has not yet been confirmed. Whether these newly-predicted genes are expressed as functional proteins remains to be discovered and it is possible that there are other unknown human and rodent AKRs yet to be discovered. Overall, it appears that the rodent and human AKRs are more similar on a genetic level than it appears from the amino acid sequence of these proteins.

The AKR1A, AKR1D, AKR1E, and AKR6 families have clear homology in humans and rodents (Table 2). In the AKR1A family, there is one aldehyde reductase in human (1A1), rat (1A3) and mouse (1A4). The kinetic properties of these enzymes are very similar with the exception of the murine enzyme,which has approximately two-times higher activity (turnover numbers and catalytic efficiencies k_{cat}/K_m) compared with the human enzyme (Barski et al., 2004). There is also one member of the AKR1D family in each of three species, although murine *Akr1d* gene and mRNA (NM_145364 on mouse chromosome 6B1) has been described through a genome sequencing project and has not been characterized. In contrast, less is known about the AKR1E family. Only the mouse protein and gene have been characterized, while the rat sequence has only been identified through large-scale genome sequencing (NM_001008342

on chromosome 17q12.3). Both murine and rat *Akr1es* cluster with *Akr1c* family on chromosomes 13 and 17, respectively, however they possess 10 rather than 9 exons and encode for 301 amino acid proteins (AKRs 1Cs consist of 323 amino acids) (Vergnes *et al.*, 2003). AKR1E1 displays distinct catalytic properties (Bohren et al., 1996), which places it in a separate subfamily altogether. Putative human homolog has been identified recently as human testis AKR (htAKR) and is present in Genbank database under the name of *AKR1CL2* (AKR family member C-like 2). Similar to mouse and rat genes, the human gene also clusters with members of the *AKR1C* family and consists of 10 exons. Its mRNA shares higher homology with AKR1B10 than with AKR1Cs. However, human AKR1E protein possesses 19 extra amino acids (109–127) due to an altered splice acceptor site at the 5'-end of exon 4. The resulting protein consists of 320 amino acids and possesses only weak catalytic activity with phenanthrenequinone (k_{cat}=0.24 min^{-1}) (Azuma *et al.*, 2004). No activity with other typical AKR substrates such as DL-glyceraldehyde, p-nitrobenzaldehyde, steroids and prostaglandins was detected. The protein also bound NADP(H) with K_d in nanomolar range (Azuma *et al.*, 2004). In contrast, murine AKR1E1 protein possesses high reductase activity with DL-glyceraldehyde, xylose, p-nitrobenzaldehyde and 9,10-phenanthrenequinone (Bohren et al., 1996). However, antibodies raised against recombinant protein failed to detect a product in native human tissue (Azuma *et al.*, 2004). Thus, it appears that due to a mutation altering the exon-intron junction during the evolution, the human protein acquired 19 additional amino acids which diminished its catalytic activity, and other changes occurred making expression specific to testis [AKR1E1 is ubiquitously expressed in mouse tissues (Vergnes *et al.*, 2003)]. It has been speculated that human AKR1E regulates spermatogenesis, as it is expressed in germ cells (Azuma *et al.*, 2004).

A high level of conservation of AKR6 (Kvβ) between species attests to the functional importance of these proteins. Genes encoding Kvβ 1, 2, and 3 in humans have similar counterparts in rat and mouse. Alternative splicing observed in Kvβ 1 and Kvβ2 genes, leading to the expression of distinct tissue-specific isoforms with different N-terminal "ball and chain" domains, is also conserved between species. In the mouse, a Kvβ4 transcript has been described in brain and kidney (Fink *et al.*, 1996) resulting from an alternative initiation start site in the intron 7 of Kvβ3 gene. Because this transcript is not derived from a separate gene, but is an alternative splice variant of Kvβ3 gene, its more appropriate annotation is Kvβ3.1. An analogous transcript has not been detected in humans because human gene contains an Alu element inserted in the corresponding position in intron 7 (Leicher et al., 1998).

The AKR1B and 1C families contain different number of known protein members in different species making it difficult to identify homologs (Table 2). The AKRs 1B3 (mouse) and 1B4 (rat) are considered homologs of human aldose reductase (1B1) based on their catalytic activity with glucose as a substrate (Gui et al., 1995;Old et al., 1990). Major differences exist in the aldose reductase activity and expression level between these organisms. The mouse AR is less active than human AR (lower turnover number) (Spite et al., 2007) and is expressed at a lower level (cited as a possible reason why mouse rarely develops diabetic complications such as cataracts), AR is expressed at a much higher level in rat heart and lens (rats develop cataracts readily) (Srivastava et al., 1998a). In the human genome, a predicted gene *tcag7.1260* also known as *LOC441282* clusters together with *AKR1B1* and *1B10* genes. According to sequence similarity this gene is an ortholog of murine *Akr1b8* (FR1) (80.8% identity). Predicted protein has 91% amino acid identity with 1B10 and 67% identity with 1B1 and corresponding EST has been found in large cell lung carcinoma library. However, the ortholog of *Akr1b7* has not yet been found in the human genome.

Murine AKR1B family has 3 well-characterized members - AR, fibroblast dependent growth factor 1 (FR-1) – AKR1B8, and mouse vas deference protein (MVDP, 1B7). Neither 1B7 nor 1B8 is a homolog of the human 1B10 according to their tissue distribution and catalytic

properties (Cao *et al.*, 1998). 1B7 has very low enzymatic activity and limited tissue distribution (it is abundant in the adrenal gland) (Lau *et al.*, 1995; Lefrancois-Martinez *et al.*, 1999). The FR-1 protein (1B8) has very similar catalytic properties to AR, but it has a higher K_m for DL-glyceraldehyde and lacks activity with glucose (Srivastava et al., 1998b). Thus, between mouse and man a total of 4 AKR1B enzymes with distinct tissue distribution and kinetic properties have been found. Accordingly, *Akr1b* cluster on mouse chromosome 6B1 contains four genes: three mentioned previously and *2310005E10Rik*, an ortholog of human AKR1B10 with which it shares 82.9% amino acid identity. Similarly, the rat genome also contains four *akr1b* genes on chromosome 4. The expression of AKR1B8 homolog in rat has been established through proteome analysis (Acc. # CAC80649, 316 aa) (Zeindl-Eberhart et al., 2001). Rat *Akr1b7* mRNA is expressed in liver and adrenal cortex, but accumulation in vas deferens is seen only in the mouse (Val et al., 2002). The expression of this protein in the liver is regulated by sexually dimorphic expression pattern of growth hormone in rats resulting in differential expression in females, but not in males (Kotokorpi et al., 2004).

The *AKR1C* family forms a cluster of 10 genes on rat chromosome 17, 8 genes on mouse chromosome 13, and 8 genes on human chromosome 10 according to the current information on the NCBI web site. In all three species an *AKR1E* gene is found together with the *AKR1C* cluster. Among 8 human genes 4 genes encode known hydroxysteroid dehydrogenases AKR1C1-C4. The gene *AKR1CL1* consists of 8 or 9 exons (NCBI and Ensembl databases, respectively) and conceptual translation predicts two transcripts: one encoding a 129 amino acid peptide (NCBI) and one encoding a 302 amino acid protein (Ensembl). The transcript of this gene has been found in several tissues; however its function remains unknown. Truncated AKR, or *tAKR* gene also consists of 8 (NCBI) or 9 (Ensembl) exons, encode a 305 amino acid protein 75% homologous to AKRs 1C3 and 1C4, and has associated ESTs. Two other genes of the cluster, *LOC648947* and *LOC643789* appear to be pseudogenes (Table 2).

In mice, a cluster of 8 *Akr1c* genes and *Akr1e1* gene is located on chromosome 13A1 (Vergnes *et al*., 2003). AKR1C proteins all consist of 323 amino acids (same as human) and share more than 75% similarity in their amino acid sequence, making it difficult to assign specific parallels between them and human HSDs. Based on substrate specificity, 20α-HSD activity is assigned to murine AKR1C18 (human 1C1) (Ishida et al., 2003), 17β-HSD to AKR1C6 (Deyashiki *et al.*, 1995) (human 1C3, prostaglandin F synthase, similar to bovine PGFS). Murine AKR1C14 possesses the highest 3α-HSD enzymatic activity and thus may be compared to rat 1C9 and human 1C4. However, according to the degree of sequence identity, human AKRs 1C1 and 1C2 are most similar to murine AKR1C21 (73.4%). The AKR1C3 is homologous to 1C18 (73.4% identity), and 1C4 corresponds to murine 1C6 (75.5%). Parallels between the other 2 human genes: *tAKR* and *AKR1CL1* and murine genes have not yet been assigned. Murine AKRs 1C12 and 1C13 cluster together in phylogenetic analysis and possess very low catalytic activity (Endo *et al.*, 2006; Ikeda *et al.*, 1999). The AKR1C12 was reported to be induced by IL-3 in EML myeloid cell line (Du et al., 2000). Murine AKR1C21 is the only enzyme in the AKR family that possesses 17α- (in contrast to 17β-) HSD activity, and therefore is able to sterospecifically reduce androstenedione (Δ4) into epi-testosterone (Ishikura *et al.*, 2004). Elegant crystallographic studies were performed on this enzyme revealing a unique mode of steroid binding consistent with 17α-HSD activity (Faucher et al., 2007; Dhagat et al., 2007).

Among the rat enzymes, AKR1C8 has well-documented 20α-HSD activity and it catalyzes the conversion of progesterone into inactive 20α-hydroxyprogesterone. The AKR1C9 is a prototypical 3α-HSD studied in great detail before human enzymes were cloned (Jez *et al.*, 1996; Bennett *et al.*, 1996; Bennett *et al.*, 1997). The catalytic properties of a number of murine and rat AKR1C enzymes have been described recently (Endo *et al.*, 2007; Sanai *et al.*, 2007; Matsumoto *et al.*, 2006; Ishikura *et al.*, 2004; Endo *et al.*, 2006).

Non-uniformity in the AKR presence in different species imposes limitation on the use of knockout technology for studying the physiological function of the AKRs. Even in cases where the homologous protein has been established and clearly identified in mice (as with aldose reductase) the presence of different family members that can partly compensate for the loss of function, limits our ability to fully understand their corresponding function in humans. This explains why to-date only four knockout models of the AKRs have been described. Aldose reductase knockout mouse exhibits diabetes insipidus (Ho *et al.*, 2000), whereas Kvβ1 knockout mice have learning and memory impairments (Giese et al., 1998; Need et al., 2003) and alterations in cardiac currents I(to) and I(K_{slow}) (Aimond et al., 2005). The Kvβ2 knockout has reduced life span, seizures, and exaggerated cold swim induced tremors (Connor et al., 2005; McCormack et al., 2002). The 20α-HSD knockout mice has decreased survival of pups and prolonged duration of the estrous cycle and pregnancy indicating this enzyme's importance for the survival of newborn mice (Ishida *et al.*, 2007). However, as mentioned above, homology between the species on the gene level runs much deeper than on the level of known enzymes. Thus, analysis of the expression patterns and properties of the corresponding proteins may bring order into the field and help identify similarities with human AKRs.

5) Natural Substrates of AKRs

a) Lipid peroxidation products

Oxidative stress has emerged as a key metabolic disturbance associated with the development of several disease states such as diabetes mellitus, Alzheimer's disease, atherosclerosis and heart failure (Brownlee, 2001; Glass and Witztum, 2001; Thomas et al., 1996; Srivastava et al., 2002). Although excessive generation of reactive oxygen species and free radical, unquenched by cellular antioxidants in the main initiating feature of oxidative stress, the generation of metastable products of lipid peroxidation, particularly aldehydes and related carbonyls, leads to the expansion and amplification of oxidative injury (Negre-Salvayre et al., 2008). Oxidizing lipids generate an array of bioactive molecules including alkoxyl and peroxyl radicals, peroxides, isoprostanes, and epoxides. Of these, aldehydes, which are generated from the scission of bis-allyic double bonds in unsaturated fatty acids, are the major end products (Fig. 8). Because several aldehydes derived from lipid peroxidation are toxic; and because they represent the most abundant products of lipid peroxidation, it appears that aldehyde generation may be one of the significant biochemical causes of atherogenesis. Protein-aldehyde adducts are present in arterial plaques of humans and animals, and atherosclerosis is associated with the development of auto-antibodies against these adducts (Jurgens et al., 1993; Horkko et al., 1999). Among the several free and esterified aldehydes generated, the C9 unsaturated aldehyde HNE and the C5 esterified aldehyde POVPC are the most abundant (Fig. 8) (Esterbauer *et al.*, 1991; Leitinger *et al.*, 1999). HNE is generated from the peroxidation of ω-6 polyunsaturated fatty acids, and under some conditions, accounts for >95 % of the alkenals produced. The esterified aldehyde, POVPC, is derived from the oxidation of 1-palmitoyl-2-arachidonyl-glycerol-3-phosphocholine (PAPC), which is one of the most abundant phospholipids in low-density lipoprotein (LDL). POVPC is generated in high concentration in miminally modified LDL (mmLDL) and in fatty streak lesions (Berliner et al., 1990; Watson et al., 1997). Protein adducts of POVPC have been detected in human atherosclerotic lesions and positive reactivity of the plasma with anti-POVPC antibodies correlates with lesion formation in apoE-null mice and with angiographically documented coronary artery disease in humans. POVPC has been shown to be responsible, in part, for the ability of mmLDL to activate endothelium to bind monocytes (Leitinger *et al.*, 1999).

AKR1B1 catalyzes the reduction of a wide range of saturated aldehydes, including HNE and POVPC with 10^3 to 10^4-fold higher efficiency than glucose, suggesting that under euglycemic conditions aldehyde detoxification rather than glucose reduction is the primary role of the enzyme (Srivastava et al., 2004a; Vander et al., 1995). In contrast to other pathways of

metabolism, reduction of aldehydes by AKR1B1 appears to represent true detoxification (Srivastava et al., 2001a; Srivastava et al., 2004c). Although HNE readily forms glutathione conjugates, the formation of GS-HNE may not, by itself be sufficient for detoxification. The glutathione conjugates of unsaturated aldehydes are markedly toxic and can induce DNA damage or stimulate the production of reactive oxygen species. Therefore, reduction of the glutathione-aldehyde conjugate by AR may be necessary to substantially annul the reactivity of the conjugate (Ruef et al., 2000). Recent studies, however, suggest that reduction of GS-HNE by AKR1B1 transforms the conjugate into a bioactive alcohol (GS-DHN), indicating that AKR1B1-catalyzed reduction could generate mediators of intracellular signaling that may be key regulators of inflammation and cytokine production (Ramana et al., 2006a). Reduction by AKR1B may also be important for inactivating POVPC. Chemical reduction of POVPC by sodium borohydride abolishes its ability to activate endothelial cells to bind monocytes, suggesting that its reductive product PHVPC is inactive (Subbanagounder et al., 2000). In comparison, hydrolysis by phospholipase A2 could generate more reactive metabolites such as lysoPC, which impairs arterial relaxation, induces growth factor gene expression, superoxide production and arachidonic acid release (Kita et al., 2000; Tselepis and John, 2002).

b) Advanced glycosylation end-products (AGEs)

AGEs arise from modification of proteins with carbohydrates or products of their metabolism containing reactive carbonyl group. These reactive carbonyl compounds initially form Schiff bases with amino groups of proteins followed by formation of Amadori products and further oxidative and nonoxidative reactions that eventually form advanced glycation end products (AGE) (Thornalley et al., 1999) (Fig. 9). AGEs are characterized by cross-links, brown color and fluorescence and are recognized by specific AGE receptors (Baynes and Thorpe, 1999). AGEs accumulate with normal aging as well as in diseases such as diabetes, atherosclerosis, renal failure, hemodialysis-associated amiloidosis and Alzheimer's disease (Horiuchi, 2002). Proteins modified include long-lived extracellular proteins such as collagens (Brownlee, 1992; Verzijl et al., 2002; Verzijl et al., 2001), plasma proteins, and cellular proteins, such as the well-known marker of glycemic control - hemoglobin A_{1C} (Horiuchi, 2002). Several studies implicate AGEs in the etiology of conditions characterized by high carbonyl load such as the secondary complications of diabetes, especially diabetic nephropathy and uremia.

Several studies report that AGEs accumulate in characteristic diabetic glomerular lesions, such as the expanded mesangial matrix and nodular lesions (Suzuki and Miyata, 1999; Vlassara and Palace, 2002; Mason and Wahab, 2003). These observations have led to the hypothesis that AGEs might contribute, at least in part, to the pathogenesis of diabetic nephropathy. AGEs accumulate in the extracellular matrix and basement membrane proteins which are among the longest lived proteins in the body (Mason and Wahab, 2003). There is evidence that accumulation of AGE accompanies the development of glomerulosclerosis and renal disease. Blockade this process by several experimental strategies such as quenching of AGEs by aminoguanidine (Thornalley, 2003), genetic deletion of the receptor for AGE (RAGE) (Wendt et al., 2003) or up-regulation of enzymes that metabolize AGE precursors (methylglyoxal) e.g. glyoxalase (Shinohara et al., 1998) delays disease progression. Thus, detoxification strategies aimed at reducing carbonyl stress and prevention of AGE formation and accumulation may be useful in preventing tissue injury associated with diabetic nephropathy and related syndromes.

In 1998 Miyata introduced the term "carbonyl stress" which refers to accumulation of reactive carbonyl compounds derived from carbohydrates and lipids and the subsequent modification of proteins (Miyata et al., 1998). The major reactive carbonyl compounds derived from carbohydrates are: glyoxal, methylglyoxal, D-arabinose, 3-deoxyglucosone (Miyata et al., 1999). In addition to carbohydrates, AGEs could also be derived from polyunsaturated fatty acids, amino acids, and ascorbate (Fig. 8, Fig. 9). The concentration of 3-deoxyglucosone is

increased in both diabetic and uremic plasma, and 3-DG-arginine adduct (imidazolone) is increased in blood and tissue proteins in diabetes in association with nephropathy (Niwa et al., 1996). Blood levels of methylglyoxal are also increased in diabetes in association with methylglyoxal-derived dilysine imidazolium cross-links (MOLD) (Nagaraj et al., 1996). It has been proposed that the increase in carbonyl stress in diabetes is caused by deficiencies in or overload on, pathways for the detoxification of carbonyl compounds leading to an imbalance between the rates of production and detoxification of reactive carbonyls (Baynes and Thorpe, 1999).

Detoxification of reactive carbonyl compounds proceeds through reduction, conjugation or oxidation. Methylglyoxal is detoxified to lactate by the glutathione-dependent glyoxalase system or through reduction by AKR1B1 and AKR1A1 (Vander et al., 1992). Other AKRs also display catalytic activity with methylglyoxal but AKRs 1A1 and 1B1 display the most favorable kinetic parameters. Like other AGE precursors, 3-deoxyglucosone (3-DG) is also detoxified by reduction. It is converted to 3-deoxyfructose, which is the major urinary metabolite of 3-DG (Niwa and Tsukushi, 2001). Kidney is the major site of 3-DG reduction and renal reduction of 3-DG has been ascribed to AKR1A, AKR1B, and AKR1C (Sato et al., 1993). In support of the cellular role of AKR1A, it has been shown that overexpression of AKR1A1 in PC-12 cells protects against methylglyoxal and 3-DG toxicity (Suzuki et al., 1998). Kinetic parameters of porcine aldose and aldehyde reductases and human AKR1B1 for the reduction of osones have been reported. Both enzymes display high catalytic efficiencies for methylglyoxal, and 3-deoxyxylosone indicating that they may be capable of reducing AGE precursors *in vivo*. (Feather et al., 1995). Collectively, these data suggest that reduction by AKRs may be an important pathway of AGE metabolism, however, the *in vivo* role of these enzymes has not been directly studied and their quantitative contribution to AGE metabolism viz-a-biz the glyoxalase pathway remains unclear. Because AKR-mediated reduction is independent of glutathione it is likely that under conditions of oxidative stress such as diabetes, AKR-mediated metabolism may be more prominent than that due to glyoxalase. Further studies are, however, required to test this intriguing hypothesis.

c) Prostaglandins

The first step in the synthesis of prostaglandins is the conversion of arachidonic acid into PGH_2 by cyclooxygenase. PGH_2 is then converted to PGD_2, PGE_2, PGI_2 or thromboxane A_4. The convertion of PGH_2 to $PGF_{2\alpha}$, is catalyzed by PGF synthase which belongs to the AKR superfamily and is designated AKR1C3 in humans (Suzuki-Yamamoto *et al.*, 1999; Matsuura *et al.*, 1998). The enzyme also converts PGD_2 to 9α, 11β-PGF_2 but PGE_2 is not a substrate (Watanabe et al., 1985; Watanabe et al., 1986) (Fig. 10). The 9α,11β-PGF_2 is a biologically active stereoisomer of $PGF_{2\alpha}$ (9α,11α-PGF_2). The $PGF_{2\alpha}$ has been shown to induce uterine constriction, contraction of coronary arteries and raise blood pressure (Makino et al., 2007; Camu et al., 1992). The 9α,11β isomer of PGF_2 produces similar biological effects but it inhibits ADP-induced platelet aggregation whereas $PGF_{2\alpha}$ is devoid of such activity (Liston and Roberts, 1985). AKR1C3 (PGF synthase) has an active site which is capable of reducing both the endoperoxide group of PGH_2 and the aldehyde group of PGD_2. The PGF synthase protein is abundant in the lung and the spleen (Urade et al., 1990) although transcripts of the gene have also been detected in kidney, skeletal muscle, leukocytes, uterus, and liver by RT-PCR (Nishizawa et al., 2000). Other reductases with PGD_2 11-ketoreductase activity have been localized to the liver, heart and other organs, however, the identity of those enzymes remains unclear and they could be other members of the AKR1C family. The $PGF_{2\alpha}$ can also be formed from PGE_2 due to reduction of its 9-keto group. The enzyme responsible for this activity has been purified from human brain (Hayashi et al., 1990) and it belongs to the carbonyl reductase family (Fig. 10). A recent report, however, raises a possibility that AKRs 1C1 and 1C2 may also be able to catalyze the conversion of PGE_2 to $PGF_{2\alpha}$ (Dozier et al., 2008).

PGD_2 and PGE_2 each have important biological functions such as constriction of smooth muscles and vasoconstriction, body temperature regulation, sleep-wake regulation in the brain (Oka, 2004; Huang et al., 2007; Davidge, 2001), thus their reduction to PGF_2 may play a role in regulating their level in addition to the synthesis of biologically active PGF_2, indicating that AKR1C3 can be an important regulator of these physiological processes.

d) Steroids

Members of the AKR1C family participate in the synthesis and the metabolism of steroid hormones. They are 3α-hydroxysteroid dehygrogenases also called dihydrodiol dehydrogenases (Jez et al., 1997a). Differential substrate specificity and tissue distribution characteristic of these enzymes may reflect the diverse roles they play in the formation and the inactivation of sex hormones (Penning *et al.*, 2000) (Fig. 11). In the liver, the 3α-HSDs work in concert with 5α- and 5β-reductases to convert 5α/5β-dihydrosteroids into 5α/5β-tetrahydrosteroids. They inactivate circulating steroid hormones and thereby limit their biological activity and prevent overload (Penning *et al.*, 2000). The liver-specific AKR1C4, which has the highest catalytic activity among 3α-HSDs, is well suited to perform this function (Penning *et al.*, 2004). In the steroid target tissues, the differential distribution of AKR1C isoforms contributes to the maintenance of a pro-oestrogenic or a pro-androgenic state. In the prostate, the most potent ligand for the androgen receptor, 5α-DHT, is formed from circulating testosterone of adrenal derived steroids. The AKR1C2 and AKR1C3, both present in the prostate, reduce DHT to a weakly androgenic metabolite 3α-diol (5α-androstane-3α, 17β diol) and therefore regulate the occupancy of the androgen receptor. Excessive levels of 5α-DHT have been linked to prostate disease, and a decrease in AKR1C2 levels during prostate cancer possibly contributes to disease progression (Ji et al., 2003). In the mammary gland, the predominant isoform is AKR1C3 which converts Δ4-androstane-3,17-dione to testosterone which can then be aromatized to 17β-estradiol, estrone to 17β-estradiol, and progesterone to 20α-hydroxyprogesterone, thus contributing to the pro-oestrogenic state of this tissue (Penning *et al.*, 2004; Penning *et al.*, 2000). AKR1C1 (20α-HSD) catalyzes the reduction of progesterone to inactive metabolite 20α-hydroxyprogesterone (in ovaries and uterus) (Fig. 11) (Vergnes *et al.*, 2003). Ensuing reduction in the progesterone level initiates parturition and termination of pregnancy. Synthesis of $PGF_{2\alpha}$ by AKR1C3 (vide supra) also contributes to this process. Levels of AKR1C1 and C2, but not that of C3, have been found to be significantly decreased in ovarian cancer tissue and are associated with a decrease in the ability to catabolize progesterone (Ji et al., 2005).

Members of the AKR1A and 1B family (aldehyde and aldose reductases) also contribute to the catabolism of steroid hormones by reducing isocorticosteroids, which are among the best substrates of these enzymes described so far (Wermuth and Monder, 1983). Isocorticosterone and isocortisol are intermediates in the metabolism of steroid hormones and have an aldehyde group at the 21 position and a hydroxyl group linked to C-20. The AKR1A1 displays a marked preference for isocorticosterone over isocortisone, whereas AKR1B1 does not differentiate between these two substrates (Wermuth and Monder, 1983). In the liver, AKR1A1 competes with aldehyde dehydrogenase for the reduction of isocorticosterone to 20,21-diol (Wermuth and Monder, 1983). Of the total excreted metabolites of cortisol, 18–33% are recovered as diols, possibly due to the action of AKR1B1 in kidney and other extrahepatic tissues (Wermuth and Monder, 1983), indicating that this enzyme plays a quantitatively important role in regulating steroid function in a variety of tissues.

e) Succinic Semialdehyde

Metabolism of the neurotransmitter GABA by monoamineoxidase (MAO, GABA-T) results in the generation of succinic semialdehyde as an intermediate. Succinic semialdehyde is oxidized to succinic acid by succinic semialdehyde dehydrogenase (SSADH), or is converted

to γ-hydroxybutyrate (GHB) by aflatoxin and aldehyde reductases (Fig. 12). Although both transformations can be considered to be a form of elimination, GHB is physiologically active and is recognized by a specific receptor or could bind to the GABA receptor at high concentrations (Buzzi et al., 2006). SSADH deficiency in humans is associated with seizures and mental retardation (Gibson et al., 1998). Recently, GHB has attracted attention as a date-rape drug. The enzymes capable of converting succinic semialdehyde to GHB, AKR1A1 and AKR7 isozymes 7A2 and 7A3 are members of the AKR superfamily. In contrast to the high K_m nonspecific aldehyde reductase (AKR1A1) (K_m 170 μM) and low affinity AKR7A3, AKR7A2 has K_m for SSA of 10–20 μM. The AKR7A2 and 1A1 enzymes are expressed in the brain and neuronal cells and thus could participate in SSA metabolism (Hoffman *et al.*, 1980;Ireland *et al.*, 1998). Recently, using siRNA, Lyon *et al.* have demonstrated that in 2 human neuronal cell lines: neuroblastoma derived SH-SY5Y and astrocytoma 1321N1, AKR7A2 is responsible for over 80% of the SSA reductase activity (Lyon *et al.*, 2007). Golgi localization of this enzyme has been speculated to facilitate the secretion of GHB (Kelly *et al.*, 2002). The reverse reaction involving the oxidation of GHB to succinic semialdehyde is not inhibited by AKR7A2 siRNA and was attributed to alcohol dehydrogenase and aldehyde reductase (AKR1A1). The GHB dehydrogenase activity is mainly localized to the mitochondria, where succinic semialdehyde is deemed to enter tricarboxylic acid cycle after oxidation to succinate (Lyon *et al.*, 2007).

f) Glucuronate-xylulose pathway and Vitamin C biosynthesis

AKR1A catalyzes the conversion of D-glucuronate to L-gulonate, which is an essential step in the biosynthesis of vitamin C in most animals (Bosron and Prairie, 1972) (Fig. 13). An important role of AKR1A is indicated by the observation that its inhibition leads to a marked reduction in urine vitamin C content in mice (Barski *et al.*, 2005). An alternate and probably more important fate of L-gulonate is to enter the pentose phosphate pathway after conversion to D-xylulose -5 phosphate. In humans and guinea pigs, species which have lost the ability to synthesize vitamin C, all of the L-gulonate produced by the AKR1A enters the pentose phosphate pathway. This pathway is very active in renal cortex which is also the tissue most abundant in AKR1A localized to the proximal tubules (Barski *et al.*, 2005). The major source of glucuronate in the renal cortex is *myo*-inositol, which is catabolized to D-glucuronate by inositol oxygenase – an enzyme also specific to the renal cortex (Reddy *et al.*, 1981). Free glucuronate can also be generated from the hydrolysis of glucuronides or UDP-glucuronate in the liver and other organs, but quantitative contribution of this pathway to glucuronate formation remains unclear (Linster and Van Schaftingen, 2003; Linster and Van, 2007).

g) Glucose

Reduction of excess glucose to sorbitol by AKR1B1 during diabetes with subsequent accumulation of sorbitol in cells has long been considered a major reason for the development of diabetic complications (Gabbay, 1975; Dvornik *et al.*, 1973). Sorbitol has been shown to accumulate in cataractous lens, and in Schwann cells. Mice overexpressing AKR1B1 in the lens or Schwann cells show greater susceptibility to diabetic cataractogenesis or neuropathy, supporting the idea that AKR1B1 is involved in the development of some diabetic complications (Lee et al., 1995; Song et al., 2003). Nevertheless, the role of AKR1B1 in diabetes remains controversial and the reader is directed to several extensive reviews on this topic (Demaine, 2003; Chung and Chung, 2003; Srivastava *et al.*, 2005b; Oates, 2002). Numerous clinical studies have been conducted to test the effect of AKR1B1 inhibition on diabetic complications. The results of these clinical trials are mixed and marred by unintended off-target effects of inappropriate dosing (Gabbay, 2004). However, the use of newer, more specific inhibitors with better pharmacokinetic profiles shows considerable improvement in nerve conductance velocity in patients with diabetes (Bril and Buchanan, 2006). The major controversy in the field that remains to be resolved is the dual role of AKR1B1, which on one

hand functions as a detoxifying enzyme involved in the removal of toxic lipid peroxidation products (*vide supra*), and on the other hand, mediating hyperglycemic injury by converting excessive glucose to sorbitol. An understanding of the role of AKR1B1 is further confounded by the recent demonstration of an obligatory role of this enzyme in mediating the effects of growth factors and mitogens (Srivastava et al., 2005b). Clearly, much remains to be learned in regard to the myriad physiological roles of AKR1B1 and whether they relate to the reduction of a singular aldehyde or multiple aldehydes and their glutathione conjugates.

6) Exogenous Substrates of AKRs

Xenobiotics are generally metabolized by phase I and phase II detoxification enzymes. Phase I metabolism introduces a polar group in xenobiotics to enhance detoxification. Phase I transformations typically involve oxidation by the cytochrome P450 monooxygenases (CYPs) which introduce a hydroxy- or an epoxy- group into the compound. Resulting metabolites are subjected to hydrolysis, conjugation, or reactions involving oxidation/reduction. The AKRs are involved in redox transformations of carbonyls introduced by metabolic transformations by CYPs or other enzyme systems, or present on the parent xenobiotic. The preferred transformation catalyzed by AKRs is reduction, although AKR-catalyzed oxidation has also been reported. In general, it is believed that reduction represents aldehyde and ketone detoxification because it leads to the formation of chemically less reactive products (alcohols). However, it is important to point out that chemical and biological activities do not always correspond, therefore these reactions could often lead to the bio-activation of xenobiotics.

AKRs and short chain dehydrogenases/reductases (SDRs) are the main enzymes that catalyze oxidation-reduction reactions involving a xenobiotic carbonyl. The SDR family consists of 63 enzymes in humans. Most SDR enzymes are dimers and tetramers (Jornvall et al., 1995). These proteins typically contain 250 amino acid residues, and they utilize both NADH and NADPH, which bind to a Rossman fold. The SDR enzymes utilize catalytic tetrad consisting of Asn, Ser, Tyr and Lys residues (Oppermann et al., 2003; Kallberg et al., 2002a). The family is divided in “classical” and “extended” SDRs; “classical” enzymes prefer NADPH as a cofactor, whereas “extended” SDRs prefer NADH (Kallberg et al., 2002b). They display wide substrate specificity, and their substrates range from steroids, alcohols, and sugars to aromatic xenobiotics.

Several strategies have been devised to identify the enzyme systems involved in xenobiotic metabolism, and to specifically delineate the role of AKRs in xenobiotic transformations (Rosemond and Walsh, 2004). The first step in elucidating the detoxification enzymes is the identification of metabolites in the urine or plasma. If the reduced metabolite, which is a secondary alcohol, or its conjugation product (sulfate, glucuronide), is found, the next step is to identify the enzyme(s) generating this metabolite. Search strategies include identification of the subcellular localization of activity, i.e. cytosolic, microsomal, mitochondrial, etc.; determination whether the activity is NADPH or NADH-dependent; purification of the activity from tissue; and finally, identification of the protein responsible for the enzyme activity. NADH-dependence usually indicates alcohol dehdrogenases or NADH quinone oxidoreductases (NQO), whereas a dependence on NADPH would suggest two classes of reductases: either short chain dehydrogenases (SDR) or AKRs. Most AKR enzymes are cytosolic, although isoforms of AKR7 have been detected in the Golgi apparatus and mitochondria (*vide supra*), and phosphorylated AKR1B1 has been found to associate with mitochondria (Varma et al., 2003). In the SDR family, carbonyl reductase is cytosolic enzyme, whereas others, such as 11β-HSD are microsomal proteins. Further, the use of specific inhibitors or molecular biological techniques such as siRNA suppression or overexpression of a particular gene, allow precise determination of the contribution of individual enzyme to the metabolic fate of a specific xenobiotic. The following discussion focuses on the xenobiotic

compounds for which significant contribution of reductive metabolism has been demonstrated. Specific xenobiotics have been grouped into 2 broad categories – (1) naturally occurring toxins found in the environment (bio-toxins), and (2) drugs and related pharmaceuticals.

I) Bio-Toxins

a) Aflatoxin B_1—Aflatoxin B_1 (AFB_1) is synthesized by the fungus *Aspergillus flavus* and is present in foods such as grain, milk, meat and fish contaminated with this fungus due to poor storage conditions. The AFB_1 is a potent hepatotoxin and carcinogen and it represents a significant cancer threat, especially in developing countries. It requires metabolic activation by the CYPs to form the ultimate carcinogen aflatoxin B_1 epoxide, which is capable of forming a covalent adduct with N^7 of guanine in DNA. (Fig. 14). If these DNA adducts are not repaired, they can undergo spontaneous depurination leading ultimately to mutagenic events. The epoxide is removed by conjugation to glutathione, catalyzed by glutathione S-transferases (GSTs). The conjugate is converted mercapturic acid and excreted in urine (Hayes et al., 1991). Additionally AFB_1 epoxide could undergo enzymatic and nonenzymatic hydrolysis to form AFB_1 8,9-dihydrodiol and subjected to base-catalyzed conversion to a ring-opened metabolite AFB_1-dialdehyde. The dialdehyde forms Schiff bases with lysine residues of proteins contributing to the cytotoxicity of AFB_1. The dialdehyde is detoxified by reduction catalyzed by aflatoxin reductases, which belong to the AKR7A family (Guengerich *et al.*, 2001). One or both of the aldehyde groups can be reduced generating a C6 or a C8 monoalcohol, or a C6,C8 dialcohol. As pointed out above, rats and humans express two AKR7 proteins, whereas mice have only one (Kelly *et al.*, 2000). Among the human enzymes, AKR7A3 possesses higher catalytic activity with aflatoxin B_1 dialdehyde than does AKR7A2 (Knight *et al.*, 1999). Its rat homolog, AKR7A1, the first aflatoxin reductase to be discovered, is expressed in liver, kidney, testes and pancreas and is strongly inducible (up to 15-fold) by antioxidants such as ethoxyquin, butylated hydroxyanisole, oltipraz (Ellis *et al.*, 1993). Using isotope dilution tandem mass spectroscopy, it was estimated that the reduction products AFB_1-C6 monoalcohol and C6,C8-dialcohol (Fig. 14) account for 8.4% of the administered AFB_1 dose that was found in urine of rats pretreated with aflatoxin reductase inducer 3H-1,2-dithiole-3-thione (D3T) (Johnson et al., 2008). Thus, reduction by AKR7 family proteins represents an important metabolic step in the detoxification of AFB_1. Induction of AKR7A1 and GSTs by ethoxyquin in Fisher rats is associated with increased resistance to aflatoxin-induced hepatocelluar carcinoma (Hayes et al., 1993). The other rat enzyme AKR7A4 is not inducible. Similarly, the murine AKR7A5 is not induced by ethoxyquin (Hinshelwood et al., 2002). The inducibility of human enzymes has not been reported but it cannot be excluded. Clinical trials with antioxidant oltipraz in populations exposed to dietary aflatoxins show a significant increase in the excretion of the phase II product - aflatoxin-mercapturic acid, highlighting the general feasibility of inducing phase II detoxification enzymes in humans (Kensler et al., 2000).

b) NNK—4-Methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK) is one of the strongest nitrosamine carcinogens in tobacco. It is generated by nitrosation of nicotine during tobacco curing and smoking and is a very potent pulmonary carcinogen in both humans and laboratory animals. The main detoxification pathway for NNK in humans involves reduction of its ketone group to the corresponding alcohol - NNAL (butanone to butanol), followed by glucuronidation and subsequent removal in urine (Fig. 15). Reduction competes with metabolic activation via α-hydroxylation by CYPs however, NNAL-glucuronide is the major NNK metabolite and it represents 39–100% of the total NNK dose excreted in the urine of smokers (Atalla and Maser, 2001). Reduction of NNK carbonyl may be an important transformation because it initiates detoxification by generating the hydroxyl group required for glucuronidation.

In the human lung, microsomal enzymes are responsible for three quarters of NNK reduction, whereas in the liver the activity is evenly distributed between the cytosolic and the microsomal fractions (Maser et al., 2000). Among the cytosolic enzymes in the liver, carbonyl reductase shows higher K_m values (7 mM), but also a higher catalytic efficiency, and has been found to contribute 60% to the total NNK detoxification capacity of the cytosol. The AKRs 1C1, 1C2, 1C4 and 1B10 have K_m in the submillimolar range (0.2–0.8 mM) and contribute to the remaining 40% activity. The AKR 1C1 and 1C2 each contribute 20% of the overall reduction, whereas AKR1B10 and 1C4 make only minor contribution (Atalla *et al.*, 2000; Martin *et al.*, 2006). The contribution of individual enzymes is assigned based on inhibition of carbonyl reductase activity by menadione and quercetin, whereas medroxyprogesterone acetate, phenolphthalein, and flufenamic acid were used as inhibitors of AKR1Cs. In the microsomal fraction, the11β-hydroxysteroid dehydrogenase 1, a member of SDR family is responsible for the NNK reductase activity. It has recently been reported that glycyrrhetinic acid, the main constituent of licorice, which is used as an additive in cigarettes and aromatizer in a number of foods, is a potent inhibitor of 11β-HSD-1 and AKR1C enzymes. In addition, it induces CYPs, thereby shifting NNK metabolism from detoxification to activation and, hence, potentially contributing to the carcinogenicity of tobacco smoke (Maser, 2004).

NNK has been demonstrated to cross the placental barrier; consequently NNAL and its glucuronide have been found in the urine of newborns whose mothers smoked cigarettes during pregnancy in the amounts equal to 5–10% of the level found in smokers. In the placenta the NNK-reducing activity is distributed between the microsomes and the cytosol. The K_m of the microsomal enzyme is 1.6 mM, whereas that of the cytosolic enzyme is 5.5 mM. NADPH is a strongly preferred cofactor for both activities. The catalytic efficiency of microsomes and cytosol is comparable; however, due to greater protein content of the cytosol its contribution to the NNAL formation is 20-fold higher than that of the microsomes. Inhibition of NNK-reductase activity by menadione and ethacrynic acid, but not barbital and pyrazole, is consistent with a major role of carbonyl reductase in the cytosolic metabolism of NNK (Atalla and Maser, 2001). Although reduction to alcohol decreases the carcinogenicity of NNK, the alcohol metabolite is not innocuous and is carcinogenic by itself; the S-stereoisomer being more tumorigenic in the mouse than the R-isoform (Upadhyaya et al., 1999). Cytosolic enzymes, including AKRs, produce more than 90% of S-stereoisomer, whereas microsomal enzymes produce more of the R-isomer, especially in lung and placenta. The content of these enzymes in human lung (and other tissues) is subject to large interindividual variation, which may alter the risk of lung cancer in smokers (Breyer-Pfaff et al., 2004).

II) Pharmaceuticals

A number of pharmacological compounds have a carbonyl (mainly ketone) moiety and are thus they are potential substrates (or regulators) of AKRs. Potential list of AKR substrates includes a wide range of pharmaceuticals, which includes anticancer, antipsychotic, antidepressants, opiate antagonists, antiasthmatic, antidiabetic and antiemetic drugs (Table 3). For some, like the anticancer drug - daunorubicicn, reduction represents deactivation step, which diminishes its anti-tumor efficacy. For others, such as the antiemetic dolasteron, modification by AKRs leads to the formation of the active compound and is therefore necessary for the drug to be active. Levels and activities of metabolizing enzymes vary among individuals and, therefore, it becomes a primary consideration in prescribing drugs and in monitoring their efficacy. Hence, it is important to understand the role of metabolism as key determinant of drug action and toxicity. Below we discuss the enzymology of the carbonyl-containing drugs, which are reduced by AKRs.

a) Acetohexamide—Acetohexamide is an antidiabetic drug. Its major metabolite is a reduced product – hydroxyhexamide (Kishimoto et al., 1994). Like its parent,

hydroxyhexamide is also pharmacologically active (Imamura et al., 2001). In human liver, AKR1A1 (aldehyde reductase) has been identified to be the major acetohexamide reductase with K_m of 0.22 mM and k_{cat}/K_m of 17 $min^{-1}mM^{-1}$ (Ohara *et al.*, 1995) (Table 4). Three hydroxysteroid dehydrogenases, AKR1C1, C2, and C4 (referred to as dihydrodiol dehydrogenases 1, 2, and 4 in the original report), but not carbonyl reductase, possess such activity, but they have a higher K_m and lower catalytic efficiency than AKR1A1 (Ohara *et al.*, 1995). In rats, but not humans, androgen-dependent microsomal carbonyl reductase has also been suggested to be involved in acetohexamide metabolism and this enzyme has been linked to interspecies differences in the pharmacokinetics of acetohexamide (Imamura and Shimada, 2005).

b) Anticancer drugs—Anticancer drugs **daunorubicin, doxorubicin, oracin** are substrates for several AKRs including AKR1A, 1B, and 1C as well as carbonyl reductase. Reduction of the carbonyl group of these cytostatic compounds renders them inactive and thus contributes to drug resistance. This is particularly important in light of findings that some of these enzymes are overexpressed in tumors (e.g. AKR1B10, (Martin *et al.*, 2006)), thus they could contribute to the resistance of these tumors to chemotherapy. In addition, clinical use of anthracycline-type anticancer drugs such as doxorubicin and daunorubicin is limited by development of chronic cardiomyopathy and congestive heart failure upon completion of cumulative anthracycline regimens (Weiss, 1992). Several lines of evidence suggest that reduced metabolites of these drugs, 13-hydroxy-anthracyclines may be mediators of chronic cardiomyopathy (Mordente *et al.*, 2001; Minotti *et al.*, 2000). Disregulation of iron metabolism in the myocardium through irreversible inactivation of aconitase/iron regulatory protein-1 was suggested as likely mechanism of cardiotoxicity (Mordente *et al.*, 2001). The higher toxicity of reduced alcohol metabolites may be attributed to their reactivity with the fourth iron atom of the [4Fe-4S] cluster; they can reduce Fe(III) to Fe(II) and separate it from the cluster while oxidizing back to the parent carbonyl anthracycline (Minotti *et al.*, 2000). This hypothesis is supported by the observation that in the human myocardium, epirubicin and MEN 10175, which exhibit reduced cardiotoxicity form fewer alcohol metabolites than doxorubicin (Minotti *et al.*, 2000).

Members of the AKR1 family (A1, B10, and C2) as well as carbonyl reductase display enzymatic activity with daunorubicin (Table 4). The specific activity of carbonyl reductase by far exceeds that of AKRs by approximately 7-fold (Ohara *et al.*, 1995). On the basis of the observation that doxorubicin reductase activity is inhibited by AL1576, but not by sorbinil, AKR1A1 has been assigned a major role in reducing doxorubicin in human cardiac cytosol, whereas carbonyl reductase was found responsible for reduction of daunorubicin (Mordente *et al.*, 2003). Inhibitors of AKR1C enzymes have been found to be ineffective in inhibiting reduction of either daunorubicin or doxorubicin, suggesting that AKR1C proteins do not play a major role in their metabolism. In contrast to the human myocardium, in the rabbit heart carbonyl reductases have been found to play a major role in the reduction of both of these drugs, underscoring the danger of extrapolating preclinical results obtained in animal models to human patients (Propper and Maser, 1997;Kaiserova and Kvasnickova, 2005;Mordente *et al.*, 2003).

The anticancer drug **oracin** belongs to isoquinoline rather than anthracycline class of compounds. Pharmacokinetic studies show that oracin carbonyl undergoes reduction to form dihydrooracin, which abolishes the therapeutic efficacy of this drug. The absence of cardiotoxicity is one of the key advantages of using this drug (Gersl et al., 1996). In human liver microsomes 11β-hydroxysteroid dehydrogenase type 1 (an SDR member) have been found to participate in oracin reduction, whereas in the cytosol three members of AKR1C family do this. The AKR1C1 possesses the highest activity in terms of both turnover number and catalytic efficiency, followed by AKR1C2 and AKR1C4 (Wsol *et al.*, 2007; Wsol *et al.*,

2004). Thus, as the reduction of the carbonyl group of anticancer agents by AKRs emerges as a major factor contributing to chemotherapy resistance and cardiotoxicity of these drugs, it may be prudent to entertain the possibility of including AKR inhibitors in cancer therapy regimens as a means to reduce negative side-effects or to increase drug efficacy (Wsol *et al.*, 2007; Mordente *et al.*, 2001).

c) Befunolol—The antihypertensive drug **befunolol** is a good substrate for AKR1C1 and C2, but not carbonyl or aldehyde reductases (Table 4). Reduction is a major metabolic pathway for befunolol in humans and its reduced metabolite is also pharmacologically active (Ohara *et al.*, 1995).

d) Ethacrynic acid—The diuretic drug *ethacrynic acid* acts as an inducer, an inhibitor or a substrate of AKRs. Ethacrynic acid is a AKR1C1 and 1C4 substrate, albeit these enzymes display modest catalytic efficiency, and their K_m for ethacrynic acid is in the millimolar range (Ohara *et al.*, 1995). This drug is an inhibitor of carbonyl reductase (Wermuth, 1981) and it weakly inhibits aflatoxin reductase (Hinshelwood *et al.*, 2003). As a Michael acceptor it is a potent inducer of AKR1C1. The induction of AKR1C1 by ethacrynic acid may be of consequence to the metabolism of other xenobiotic compounds as well as steroids, particularly progesterone (Burczynski et al., 1999).

e) Neurological drugs—Naloxone, naltrexone, haloperidol, timiperone: Opiate antagonist naloxone is reduced in the human liver sterospecifically to 6β-naloxol. The AKR1C4 is the most efficient catalyst; however AKR1C1 and 1C2 also contribute to a significant extent (Ohara *et al.*, 1995). Neither carbonyl reductase nor AKR1A1 is able to reduce naloxone, making AKR1C the exclusive naloxone reductases in human liver cytosol. Direct purification of activity led to the identification of peaks corresponding only to AKR1Cs, adding strength to the conclusion of their exclusive role (Ohara *et al.*, 1995). Studies in rats and rabbits (Yamano et al., 1999) also point to the major role of AKR1C enzymes in naloxone reduction.

Naltrexone, a drug closely related to naloxone, is also reduced stereospecifically to 6β-naltrexol in humans (Gonzalez and Brogden, 1988). The reduced product has a longer half-life than the parent compound and it is also a potent μ-opioid receptor antagonist. It has been reported that the AUC concentration of naltrexol in plasma exceeds that of naltrexone; however, both were subject of large interindividual variations (Breyer-Pfaff and Nill, 2004). Such variations, however, are unlikely to significantly influence clinical efficacy because both ketone and alcohol compound display pharmacological activity. Two separate reports (Ohara *et al.*, 1995; Breyer-Pfaff and Nill, 2004) indicate that AKR1C proteins are responsible for reducing naltrexone; however, these reports differ in kinetic parameters and relative efficacies assigned to the individual AKR1C isozymes. In the report by Ohara *et al.* (Ohara *et al.*, 1995) AKR1C1 (DD1) was suggested to be the predominant enzyme involved in naltrexone reduction and AKR1C4 was found to display roughly 2-times lower catalytic efficiency, although 2 enzymes had similar K_m values ~0.2 mM. In contrast, Breyer-Plaff and Nill (Breyer-Pfaff and Nill, 2004) report a significantly higher catalytic efficiency for AKR1C4 with a low K_m of 0.029 mM. In their determination AKR1C2 was found to exhibit high affinity (K_m 0.13 mM), but low turnover, whereas AK1C1 was much less active. Breyer-Plaff and Nill conclude that metabolism of naltrexone is governed by AKR1C4 with a 20-fold higher efficiency than 1C2. Both reports agree; however, that naltrexone is not reduced by carbonyl reductase.

In contrast to naloxone and naltrexone, the antipsychotic haloperidol is reduced mainly by carbonyl reductase to dihydrohaloperidol, which possesses 20–50% activity of the parent drug (Chang, 1992). Comparable haloperidol and dihydrohaloperidol concentrations are attained in human plasma during treatment, though large interindividual variations have been noted. It has

been suggested that oxidation of the dihydrohaloperidol by CYP3A4 in liver microsomes back to the monodiol may be responsible for low alcohol/ketone ratios in some patients. The CYP3A4, carbonyl reductase and uridine diphosphoglucose glucuronosyltransferase are the three enzymes responsible for the biotransformation of haloperidol in man (Kudo and Ishizaki, 1999). Enzymes of the AKR1C family may also contribute to haloperidol reduction; however, the reported catalytic efficiencies of these enzymes differ. Ohara *et al.* report a K_m of 0.19 mM for AKR1C1, a much lower value than that of catbonyl reductase (1.2 mM) (Ohara *et al.*, 1995), whereas, Breyer-Plaff and Nill (Breyer-Pfaff and Nill, 2000) find much lower catalytic efficiencies and higher K_m values for haloperidol reduction by AKR1Cs (Table 4).

Timiperone, a potent butyrophenone neuroleptic, is reduced in the cytosol of human liver similarly to haloperidol with slightly higher efficiency, but with lower K_m and lower maximal velocity (Shimoda et al., 1998a). Reduced product is a major timiperone metabolite *in vivo* (Shimoda et al., 1998b). Inhibition by menadione and ethacrynic acid, but not phenobarbital and methylpyrazole, strongly suggests that carbonyl reductase may be the major enzyme involved in timiperone metabolism.

f) Tricyclic aromatic system compounds—Like haloperidol, **ketotifen, E-10-oxonortriptyline, and Z-10-oxonortiptyline** have a carbonyl group attached to the aromatic ring of the tricyclic system and are potential AKR substrates. Ketotifen is used for asthma, and nortriptylines belong to the group of antidepressants, but their structural similarity dictates similar metabolic fates. The ketone of ketotifen has been demonstrated to undergo reduction *in vivo*, and its alcohol product appears to be the major metabolite in humans (Breyer-Pfaff and Nill, 2000). No reduction was observed with human liver microsomes, hence cytosolic enzymes were assumed to be responsible. Similarly, secondary alcohols E- and Z-10-hydroxynortriptyline are the major metabolites of the antidepressants amitriptyline and nortriptyline (Breyer-Pfaff and Nill, 2000). E-10-oxonortriptyline is present as a minor amitriptyline metabolite in human urine. Reduction occurs in humans and rabbits but not in rats or guinea pigs, underscoring again the danger of extrapolating metabolic findings from one species to another.

The highest catalytic efficiency for Z-10-oxonortriptyline reduction was observed with AKR1C1. The AKR1C2 has a two-fold lower catalytic efficiency with a similar K_m value in the low micromolar range. Ketotifen is preferentially metabolized by AKR1C2, which has a K_m value of 3.6 μM and catalytic efficiency somewhat lower than that with Z-10-oxonortriptyline (Table 4). Reduction of all compounds occurs in a stereospecific manner although some differences between catalytic efficiencies with substrate enantiomers have been reported. The AKR1C4 and carbonyl reductase are, however, unable to catalyze the reduction of these drugs (Breyer-Pfaff and Nill, 2000). Thus, the high affinity of AKR1C1 and 1C2 for the above ketonic drugs in conjunction with high NADPH/$NADP^+$ ratio in cytosol may be the underlying reasons why the corresponding alcohols are the predominant metabolites of these drugs detected in human urine.

g) Dolasetron—Dolasetron is a serotonin (5-HT_3) receptor antagonist which is used as antiemetic drug during chemotherapy and other conditions. The chiral alcohol resulting from dolasetron reduction is ~ 40 times more potent as a receptor antagonist as dolasetron itself, threefore dolasetron may be regarded a prodrug (Breyer-Pfaff and Nill, 2000). Dolasetron becomes undetectable in human plasma 2 h after intravenous administration, whereas half-life of hydrodolasetron is 7–8 h. Thus, reduction of this compound *in vivo* is imperative for its clinical efficiency. It has been reported that AKR1B10, 1C1, and 1C4 are the most effective enzymes in dolasetron reduction with catalytic efficiencies of ~100 $mM^{-1}min^{-1}$ (Table 4) (Martin *et al.*, 2006). The AKR1C2 and carbonyl reductases may also be involved since their catalytic efficiencies are only about 3-times lower than that of AKR1B10, 1C1 or 1C4. The

widespread tissue distribution of carbonyl reductase could, however, make up for its slightly lower catalytic efficiency. The participation of AKR1B10 in the reduction (i.e., activation) of orally administered dolasetron must be of primary importance since this enzyme is highly expressed in small intestine. Most likely, AKR1B10 is responsible for very fast elimination of orally administered parent compound which is faster than even i.v. infusion. Reoxidation of the alcohol apparently does not take place which would rationalize the exclusive presence of the reduced metabolite in the plasma (Breyer-Pfaff and Nill, 2004).

h) NSAIDs—Ketoprofen and loxoprofen are similar NSAID drugs, which contain a ketone group. Ohara *et al.* place ketoprofen within a group of drugs that are reduced exclusively by AKRs, namely, AKR1C2 and 1C4 with comparable kinetics (Table 4). In contrast, loxoprofen is a better substrate for carbonyl reductase in terms of catalytic efficiency, albeit the K_m of carbonyl reductase for loxoprofen is rather high (38 mM) (Ohara *et al.*, 1995). In accord with these data, reduction has been found to be the major metabolic pathway in the transformation of loxoprofen, but not ketoprofen probably due to participation of carbonyl reductase in the metabolism of loxoprofen.

i) Nafimidone—Nafimidone is an anticonvulsant drug. In humans it is rapidly reduced to pharmacologically active alcohol nafimidol (Rush et al., 1990). The enzymes responsible for this conversion have not yet been identified.

j) S-1360—The compound **S-1360** is the first HIV integrase inhibitor to enter clinical trials. It is a 1,3-diketone compound and reduction of one of its keto-groups constitutes the major pathway for its clearance. Unchanged S-1360, its reduced metabolite (designated HP1) and their glucuronides have been detected in the plasma and the urine of treated humans (Rosemond *et al.*, 2004). Examination of microsomal, mitochondrial and cytosolic fractions of pooled human livers suggests that the cytosol possesses the majority of S-1360 reductase activity, which requires NADPH as a cofactor. Further dissection with inhibitors has led to implication of AKRs in catalyzing the reduction of over 60% of drug with carbonyl reductase contributing to approximately 30% of overall reduction. Specific enzymes involved in the reduction have not yet been identified; however inhibition by flufenamic acid and phenolphthalein suggests the enzymes belong to the AKR1C family. This possibility is consistent with a higher preference for ketone substrates, which is a characteristic feature of this family of AKRs. Curiously, the activity exhibited strong positive cooperativity in regard to substrate concentration with a Hill coefficient of 2 (Rosemond *et al.*, 2004). Such a behavior has not been reported for AKRs. Positive cooperativity has been described for 11β-HSD (Maser et al., 2003), however, this enzyme is found in the microsomal, and not the cytosolic fraction.

In summary, reduction appears a significant pathway in the metabolism of several pharmaceuticals containing ketone groups. Whether reduction is an activating or a deactivating transformation is determined by the nature of the drug and the relative abundance of the reduced (alcohol) and the parent (aldehyde or ketone) metabolite is determined by the efficiency of reduction and oxidation of the alcohol back to the carbonyl, which is often catalyzed by CYPs. Among enzymes carrying out reduction, microsomal hydroxysteroid dehydrogenases, cytosolic carbonyl reductase, and AKRs play a major role. The AKR1C family appears to be involved in the reduction of the widest variety of pharmacological compounds; and a group of drugs has been found to be exclusively reduced by the AKR1C enzymes. Carbonyl reductase closely follows AKR1C enzymes in plasticity and plays very essential role in the metabolism of a variety of drugs due to its abundance, nonselective substrate specificity, and wide tissue distribution. The AKR1A and 1B enzymes have been found to be involved in the biotransformation of only a selective group of drugs, most likely due to the fact that these enzymes are aldehyde rather than ketone reductases, whereas majority of drugs possess ketone group.

III) Inhibitors

The major impetus driving research on AKR1B has been the potential involvement of this enzyme in hyperglycemic injury and promise of specific AKR1B1 inhibitors for the treatment of diabetic complications. With the rationale that excessive flux of glucose via this enzyme contributes to diabetic injury, several AKR1B-specific inhibitors have been synthesized. Initial small-scale clinical trials showed that inhibition of this enzyme could ameliorate symptomatic somatic and autonomic neuropathies (Jaspan et al., 1983), improve joint mobility (Eaton et al., 1985) and could prevent or delay fiber degeneration in neuropathy (Dyck et al., 1988) and partially correct nerve conduction defects in diabetics (Sima et al., 1988). Later, large-scale clinical trials, however, failed to demonstrate clear benefit and were marred with non-specific sensitivity reactions or off-target effects. It is currently unclear whether the poor clinical efficacy of these drugs is due to inappropriate pharmacokinetics (poor tissue penetration), insufficient duration of the trials or inappropriate end points (Pfeifer et al., 1997). Nonetheless, recent trials with some AKR1B inhibitors such as ranirestat (Bril and Buchanan, 2006) continue to show promise. Inhibition of AKR1B does offer a mechanism-based rationale for treating diabetic complications and extensive animal and biochemical studies continue to show protections against several end points including inhibition of hyperglycemia--induced inflammation (Ramana et al., 2004; Shaw et al., 2003; Campbell and Trimble, 2005), cytokine production (Ramana *et al.*, 2002), sepsis (Ramana et al., 2006c) and high glucose-induced Ang-II generation (Lavrentyev et al., 2007). Perhaps longer clinical trials with other endpoints (e.g., microvascular complications) and better statistical power may be able to fully assess the efficacy of this class of agents.

A distinguishing feature of AKR1B1 inhibitors is that these inhibitors display uncompetitive or noncompetitive inhibition pattern in the reduction direction and competitive pattern in the direction of alcohol oxidation (Bhatnagar *et al.*, 1990; Liu *et al.*, 1992; Kador and Sharpless, 1983; Sato and Kador, 1990; Ehrig *et al.*, 1994; Matsumoto *et al.*, 2008). The absence of competitive inhibition in the carbonyl reduction direction has been interpreted by various investigators as an indication that these inhibitors bind to an allosteric site distinct from the active site (Kador and Sharpless, 1983; Sato and Kador, 1990). Further investigations, however, unequivocally established that inhibitors do bind into the active site and that the observed inhibition pattern results from inhibitors binding preferentially to the E:$NADP^+$ form of the enzyme (Bhatnagar *et al.*, 1990; Liu *et al.*, 1992; Harrison *et al.*, 1994; Ehrig *et al.*, 1994; Wilson *et al.*, 1993). The preference for the $NADP^+$ form is brought about by the presence of an anion-binding site which is formed due to the positive charge on the nicotinamide ring of $NADP^+$ (Harrison *et al.*, 1994) and absent in the NADPH-bound form of the enzyme. Electrostatic potential surface calculations support the presence of a positively charged residues lining at the bottom of the active site cavity of the E:$NADP^+$ binary complex (Urzhumtsev *et al.*, 1997).

Most effective AKR1B inhibitors are either carboxylic acids (examples are tolrestat, zopolrestat) or spyrohydantoins (e.g. sorbinil, AL1576, ranirestat) (for structures see Table 5). The active site of the enzyme possesses two contact domains involved in inhibitor binding: 1) a hydrophilic region which contains a recognition sequence for hydrogen bond acceptors near the coenzyme and 2) a hydrophobic domain lining of the active site cleft, which includes the “specificity pocket” (Urzhumtsev *et al.*, 1997). Inhibitors bind to the active site with their polar heads toward the coenzyme and the bulk of the molecule in the hydrophobic pocket. The hydrophilic region located at the bottom of the active site cavity includes three centers composed of possible hydrogen bond acceptors His 110, Tyr 48 and Trp 111. Center 2 (Urzhumtsev *et al.*, 1997) delineated by His 110, Tyr 48 and C4 of $NADP^+$ forms the anion-binding site first described by Harrison et.al (Harrison *et al.*, 1994) and is the most occupied among the three centers. As inhibitors of both classes possess a negative charge at physiological

pH, their negatively-charged head binds into the anion-binding site, thus explaining their preference for the $NADP^+$ form of the protein. Recent subatomic resolution AKR1B1-inhibitor structures suggest the possibility that the spirohydantoin inhibitors bind in a neutral state and then become charged inside the active site pocket (Podjarny et al., 2004).

ARK1B inhibitors make multiple contacts with mostly hydrophobic active site residues of the protein active site via a significant number of van der Waals contacts (Wilson *et al.*, 1995; Wilson *et al.*, 1993; Steuber *et al.*, 2008). The active site of the enzyme is capable of large conformational changes to accommodate the inhibitor and thus offer a high-efficiency template for binding to inhibitors complementary to the active site. For example, binding of zopolrestat to AKR1B1 induces a hinge-flap motion of two peptide segments. As a result of these movements new interactions that were not present in the holoenzyme structure are formed. These interactions create a hydrophobic bridge over the bound inhibitor and effectively close the active site pocket thus ensuring high affinity binding of zopolrestat (Wilson *et al.*, 1993).

Quest for greater specificity of binding has stimulated extensive crystallographic and kinetic studies of AKR1B1 itself as well as other members of the AKR superfamily in order to pinpoint differences in the kinetic mechanism and structure of the active site that could serve as a specificity determinants. This has been addressed mainly through comparisons of the AKR1B1 with the AKR1A1 kinetics and crystal structures. The two active sites differ in the “specificity sub pocket”, which is composed of Trp 111 (1B1 numbering) and the C-terminal loop region of the enzymes. In case of AKR1B1 the C-terminal loop exhibits the higher level of plasticity throughout the known crystal structures. AKR1A1 possesses an insertion of 10 amino acids between Met 301 and Arg 311, which, although does not directly contribute to the shape of the binding pocket, is likely to be involved in the modulation of its dynamic properties (Steuber *et al.*, 2008; Barski *et al.*, 1996). Several AKR1B1 inhibitors have been shown to both penetrate and consequently open the specificity pocket (e.g. tolrestat) or leave the specificity pocket closed (e.g. sorbinil) (Urzhumtsev *et al.*, 1997). Establishment of a similar specificity pocket in AKR1A1 appears rather unfavorable as it formation would involve the breakage of a salt link between Arg 311 and Asp 312 (Steuber et al., 2008). Thus, inhibitors that penetrate the specificity pocket display the highest selectivity towards 1B1 (e.g. tolrestat, 72-fold, zopolrestat, 450-fold) whereas those that do not are relatively nonspecific (e.g. sorbinil) (Barski *et al.*, 1995).

An alternative approach to AKR1B inhibition is based on the discovery that glutathione conjugates of unsaturated aldehydes are good substrates for this enzyme (Srivastava *et al.*, 1995; Srivastava *et al.*, 1999) but has not been reported as substrates of any other AKR. Crystal structure of AKR1B1 with a substrate analog S-(1,2-dicarboxyethyl) glutathione (DCEG) reveals that the glutathione-containing compound fills the active site and binds also in the “specificity pocket” of the enzyme (Singh et al., 2006). Further development of inhibitors that bind to the AKR1B glutathione binding site of AR may provide more selective drugs to inhibit this enzyme and not its close relatives (e.g. AKR1A1).

At the time of intensive development of aldose reductase inhibitors, AKR1B1 was the only AKR1B member known in humans. Therefore, all attempts were made to develop inhibitors that could differentiate between AKR1A1 and 1B1 enzymes. It is surprising that 10 years after the discovery of the second human AKR1B enzyme, AKR1B10, remarkably few studies have examined the cross-reactivity of AKR1B1 inhibitors with AKR1B10, whereas, similar studies aimed at the development of inhibitors specific for AKR1C isoforms are well under way (*vide infra*). Tolrestat has been shown to display similar IC_{50} values with both AKR1B1 and AKR1B10 (Crosas *et al.*, 2003; Gallego *et al.*, 2007). The K_i values reported for zopolrestat and sorbinil with the 1B10 enzyme exceed significantly that of 1B1; however direct comparison of these inhibitors with both enzymes has not been attempted (Verma *et al.*, 2008). In summary,

some of the *in vivo* effects of AR inhibitors as well as their off-target effects observed in clinical trials may be due to inhibition of 1B10. Because AKR1B10 is a poor catalyst for glucose reduction and, therefore, not expected to play a significant role in the sorbitol production during diabetes, development of inhibitors that can distinguish between the two AKR1B isoforms may provide more specific drugs for combating diabetic complications with fewer side effects.

To date, AL1576 is the only high affinity inhibitor that displays preference for AKR1A1 over AKR1B1 (13-fold) (Barski *et al.*, 1995). AKR1A1 is also known to be inhibited by anticonvulsant drugs such as barbiturates and hydantoins, however, these inhibitors are nonspecific and inhibit AKR1B1 as well. Flavonoids such as quercetin and rutin have long been used to inhibit AKRs 1A and 1B, however, these are also nonspecific inhibitors (Wermuth, 1985). Valproic acid is relatively specific for AKR1A1 (Whittle and Turner, 1981) and so are dicarboxylic acids, such as tetramethyleneglutaric acid, which was the first compound tried for its ability to inhibit aldose reductase, however it appeared to possess higher affinity for AKR1A1 than 1B1 (Branlant, 1982; Wermuth, 1991). Valproate has also been shown to inhibit AKR7 (Hinshelwood *et al.*, 2003). Although selected aldose reductase inhibitors inhibit AKR1C enzymes also, their affinity for AKR1C is several orders of magnitude lower than that for AKR1B1 (Jez *et al.*, 1996; Dhagat *et al.*, 2008a). Selected inhibitors displaying specificity for different AKR isoforms are listed in Table 5.

In addition to AKR1B1, enzymes of the AKR1C subfamily have also emerged as potential drug targets. Regulation of the prereceptor levels of steroid hormones and prostaglandins by AKR1C enzymes may contribute to the development of human cancer (Byrns *et al.*, 2008; Bauman *et al.*, 2005). AKR1C3 is particularly relevant for the following reasons: 1) its activity in testosterone production in prostate enhances 5α-DHT formation and increases androgen receptor activity. Likewise, in the breast, AKR1C3 helps create pro-estrogenic state by enhancing the synthesis of 17β-estradiol and inactivating progesterone (Byrns *et al.*, 2008); 2) by converting PGD_2 to 9α,11β-PGF_2, AKR1C3 diverts the metabolism of the former from forming 15-deoxy-$\Delta^{12,14}$-PGJ_2, thus depriving PPARγ receptor of its ligand and preventing terminal differentiation of myeloid leukemia cells (Desmond et al., 2003). PGF_2 and its isomers stimulate proliferation through F-prostanoid receptor (Chen et al., 1998; Sales et al., 2004); 3) AKR1C3 is overexpressed in several types of cancer (Stanbrough et al., 2006; Li et al., 2004).

NSAIDs indomethacin and mefenamic acid has long been known to inhibit AKR1C enzymes in addition to cyclooxygenase (Penning and Talalay, 1983). In addition, compounds of other chemical classes such as phenolphthalein and bile acids have been demonstrated to inhibit enzymes of the AKR1C family (Steckelbroeck *et al.*, 2006; Higaki *et al.*, 2003). Based on this knowledge N-phenylanthranilic acid derivatives and steroid carboxylates have been developed as specific inhibitors of AKR1C isoforms which do not inhibit COX-1 and COX-2, and possess various degrees of selectivity among AKR1C isoforms (Bauman *et al.*, 2005). Most steroid carboxylates display marked preference for AKR1C2, whereas an indomethacin analogue (N-(4-chlorobenzoyl)-melatonin) is selective for AKR1C3. The inhibition pattern depends on the substrate and the reaction studied (oxidation or reduction) and it was concluded that these inhibitors bind to E:$NADP^+$ as well as the E:NADPH form of the enzyme with a higher affinity for the E:$NADP^+$ form (Bauman *et al.*, 2005; Byrns *et al.*, 2008), a situation similar to AKR1B inhibition. These inhibitors will be useful to examine the involvement of AKR1C3 in cell proliferation and cancer development. A potent inhibitor of another AKR1C enzyme, AKR1C1, displays over 12-fold selectivity over its closest analog (AKR1C2) (Dhagat *et al.*, 2008b). Specific inhibition of this enzyme may be useful for treatment of several neurological disorders, where it may be involved in the metabolism of neuroactive steroids, as well as cancer; however, as with AKR1C3 inhibitors, this concept requires validation.

In addition to their value as potential drug candidates inhibitor studies contributed greatly to our understanding of the mechanism of AKR catalysis. Recent ultrahigh resolution structure of AKR1B1 complexed with its inhibitor IDD552 show that the proton of Tyr 48 is shared among the Tyr and the carboxy-group of IDD552 (Ruiz et al., 2004), confirming the assignment of Tyr 48 as proton donor made a decade ago based on the mutagenesis studies. An exciting new field for the AKR inhibitors may be their application to the AKR6 class. Such drugs, if they are carbonyl substrate-like molecules and increase the E:$NADP^+$ form of the protein would decrease inactivation, and thus may be useful in treating pulmonary hypertension. Conversely, dead-end inhibitors that increase the E:NADPH form of the protein could be used to decrease excitability (increase inactivation) in hyperexcitable states such as attention-deficiency or epilepsy. No AKR6-specific inhibitors have been developed as yet, however.

CONCLUDING REMARKS

The aldo-keto reductase family is a collection of diverse proteins with a multitude of functions. The AKRs display broad substrate specificity in *in vitro* assays and as a result their in vivo substrates are in most cases difficult to identify. Potential endogenous substrates have been identified for many of the AKR members. Nonetheless, despite many years of research and great strides in understanding enzymatic mechanism, structure, and pharmacological modulation and gene regulation, the physiological roles of AKRs are unclear and the specific metabolic pathways that require these enzymes remain a mystery. It is likely that application of modern technologies such as knockout and transgenic animals, *in vivo* siRNA, fluorescent substrates and inhibitors, combined with metabolomic analysis will shed more light on the normal physiological functions of these proteins. Studies in simpler organisms such as *Drosophila, C. elegans* or yeast are advantageous since these models are relatively easier to manipulate and their use might help in assessing the metabolic roles of AKRs and their evolutionary significance. We are confident that future developments in this area will significantly enrich our basic understanding of AKRs and provide new avenues for using this knowledge to improve human health and technology.

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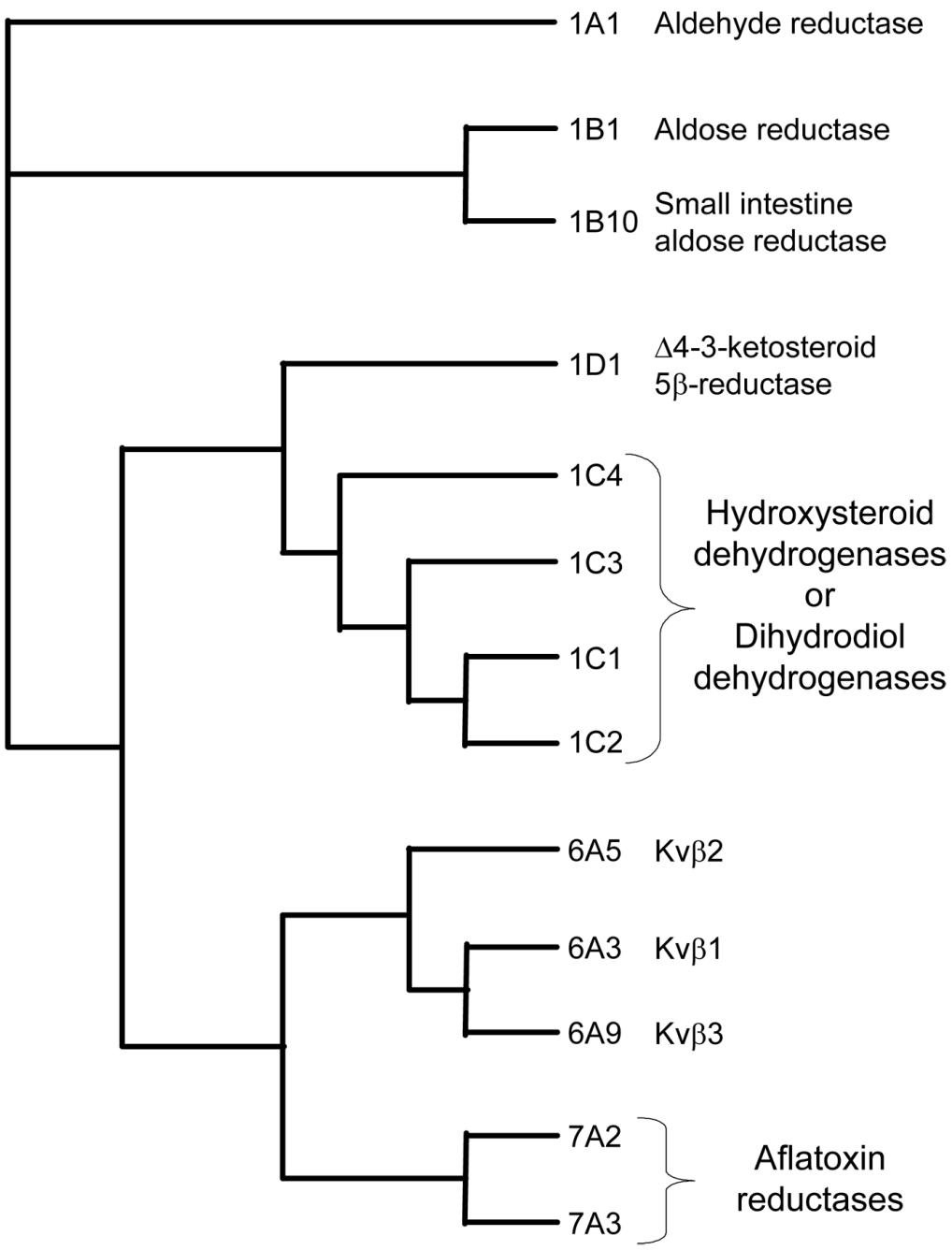


Fig. 1. Phylogenetic tree of human AKRs

Thirteen well-known and characterized human proteins belong to 3 AKR families.

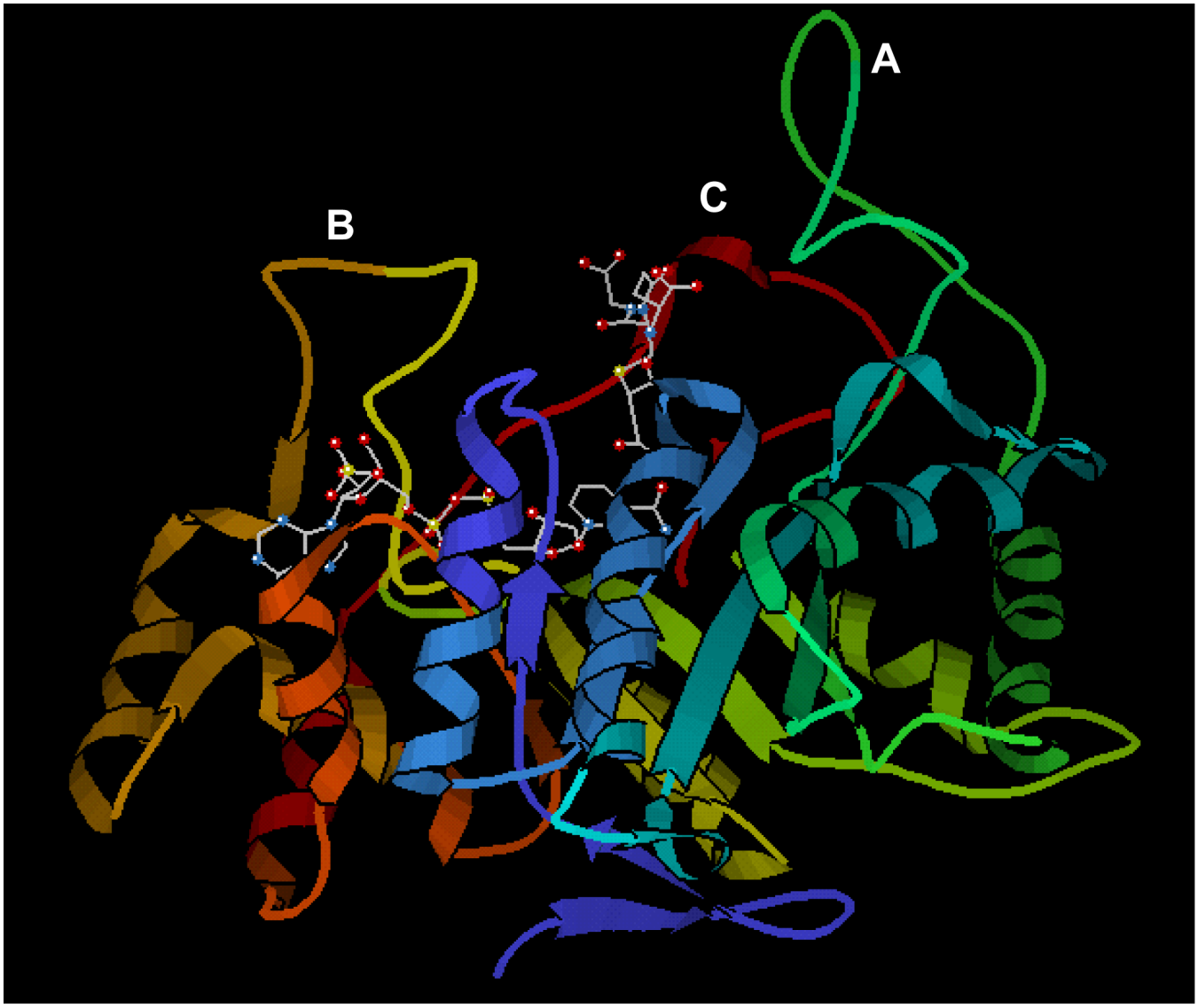


Fig. 2. Crystal structure of aldose reductase

The structure was downloaded from RCSB Protein Data Bank (ID # 2f2k). The ribbon drawing is a side-view representation of the protein with NADPH bound to the active site. The active-site loops are marked - A, B and C. The ball and stick structure at loop B is the NADPH molecule and the structure at loop C is the glutathione analog 1,2-dicarboxyethyl glutathione, which binds to the substrate binding site of the enzyme.

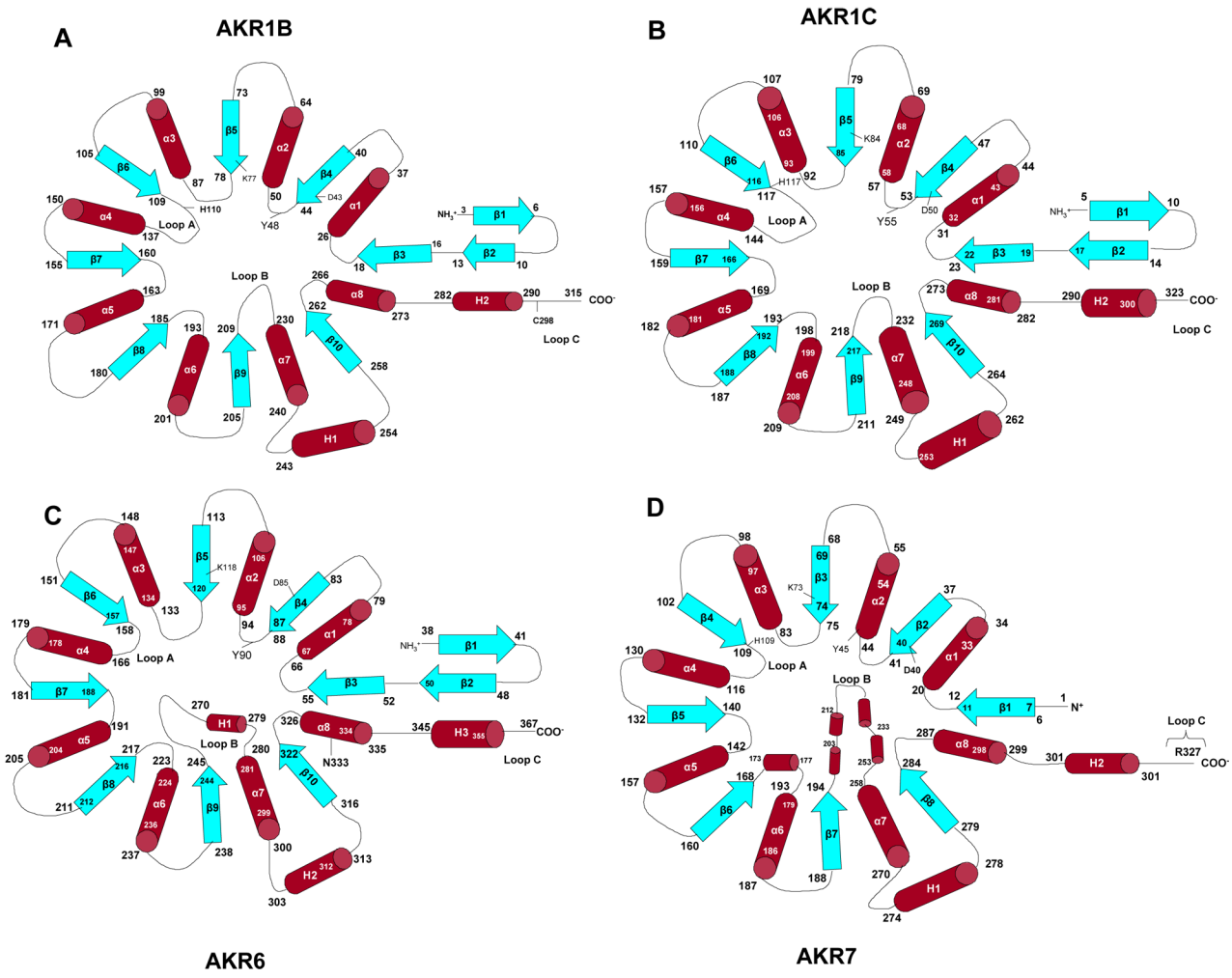


Fig. 3. Topology and structural features of AKRs

Topological representations of the amino acid sequence of AKR1B (A), AKR1C (B), AKR6 (C), and AKR7 (D). The β-strands (blue) are represented as arrows and the α-helices (red) as cylinders. Each major strand and loop is number. Connecting random coils are shown as extended loops. The position of the active site residues in sequence is indicated. Note the shorter loop A and the extra helices in AKR6 and 7 (C and D).

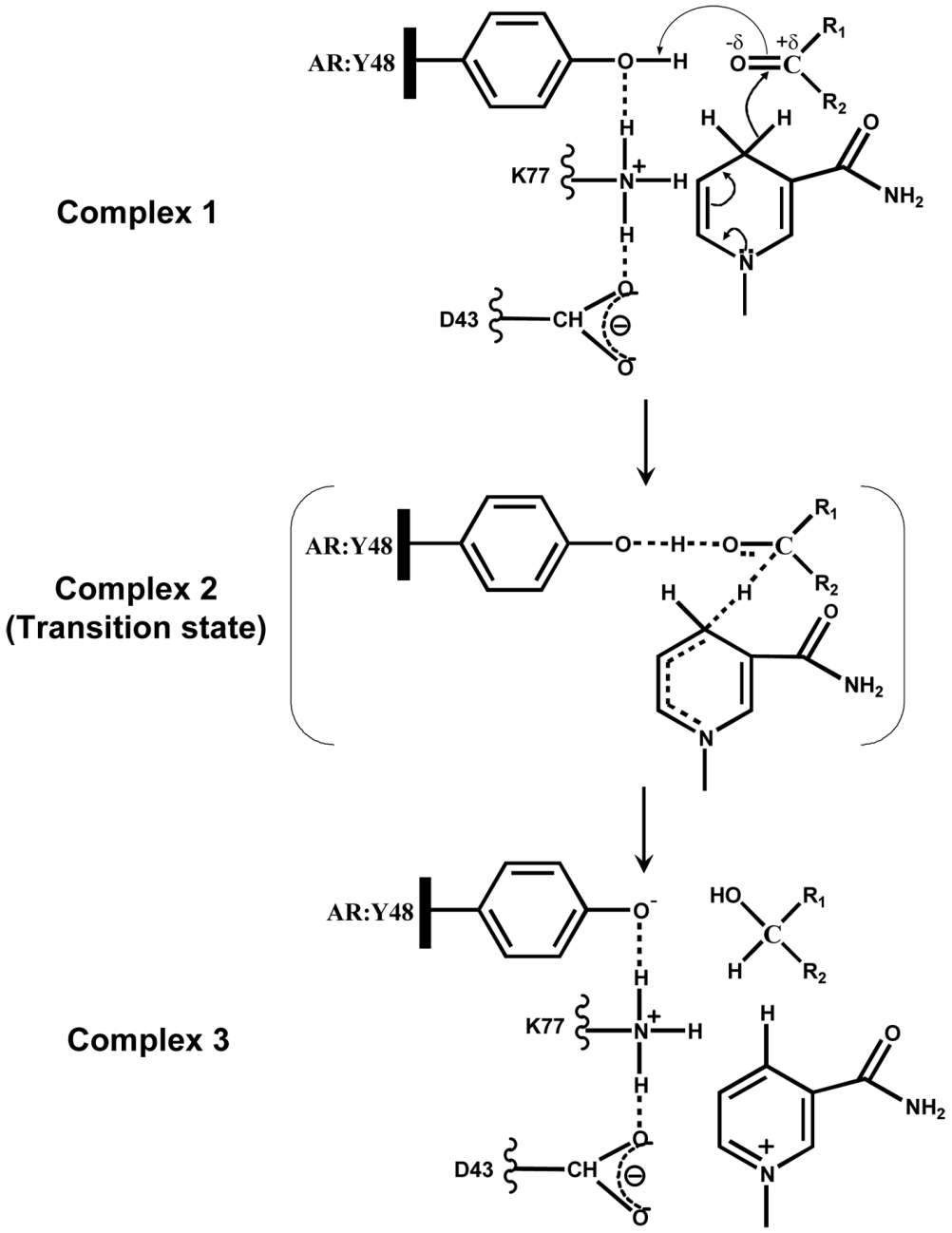


Fig. 4. Catalytic mechanism of AKRs

In complex 1, active site Tyr-48 (AKR1B1 numbering) is shown to form a hydrogen bond with the substrate carbonyl resulting in the carbonyl polarization and accelerating the hydride transfer of the pro-R hydrogen from the nicotinamide ring of NADPH to the carbonyl carbon of the substrate. The hydrogen bond network provided by Lys-77 and the Asp-43 serves to lower the pK_a of tyrosine making the proton transfer easier. Complex 2 shows a transition state in which the polarization at the carbonyl is quenched by the proton transferred from the protein tyrosine and a concerted hydride transfer to the carbonyl carbon. The reduced carbonyl then dissociates from the acid-base catalyst and a net charge on the tyrosinate anion is stabilized by the hydrogen-bonding network (complex 3).

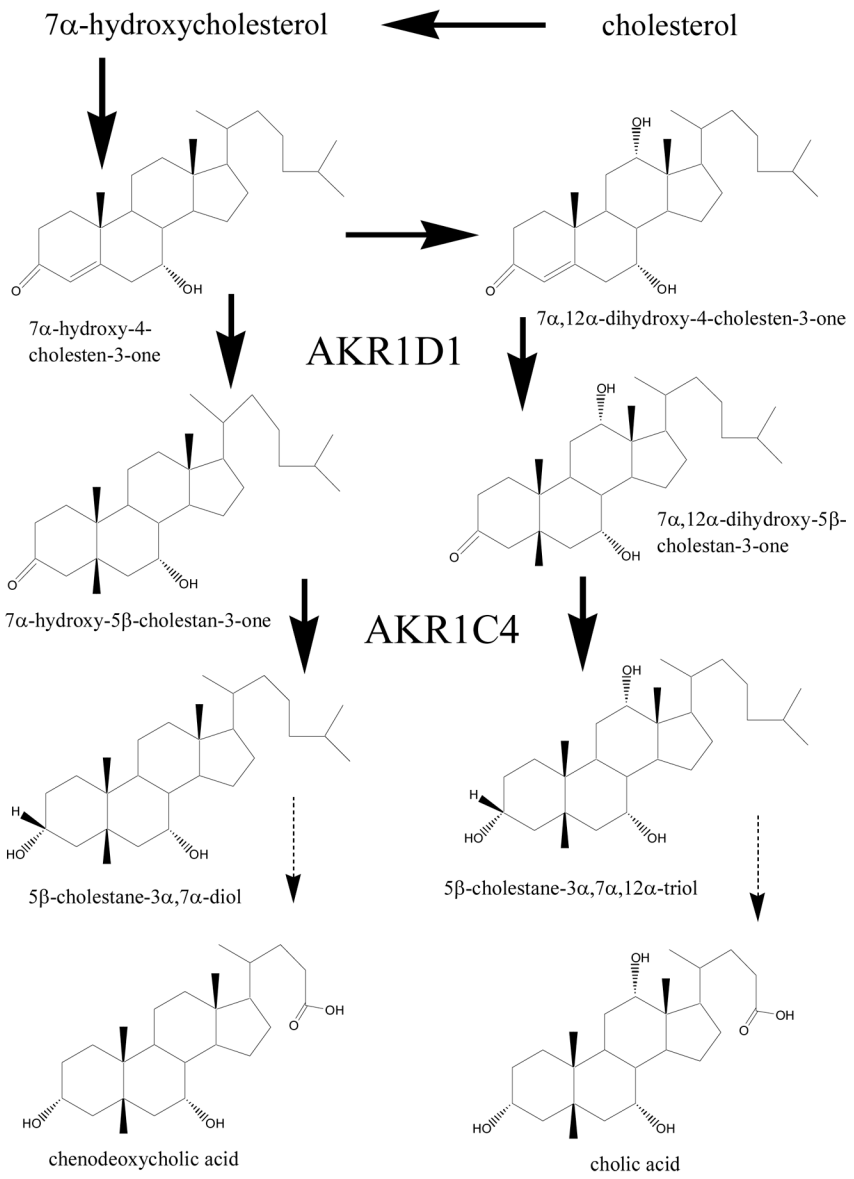


Fig. 5. Biosynthesis of bile acids

The AKR 1D1 and 1C4 catalyze two consecutive steps in the synthesis of cholic and deoxycholic acids.

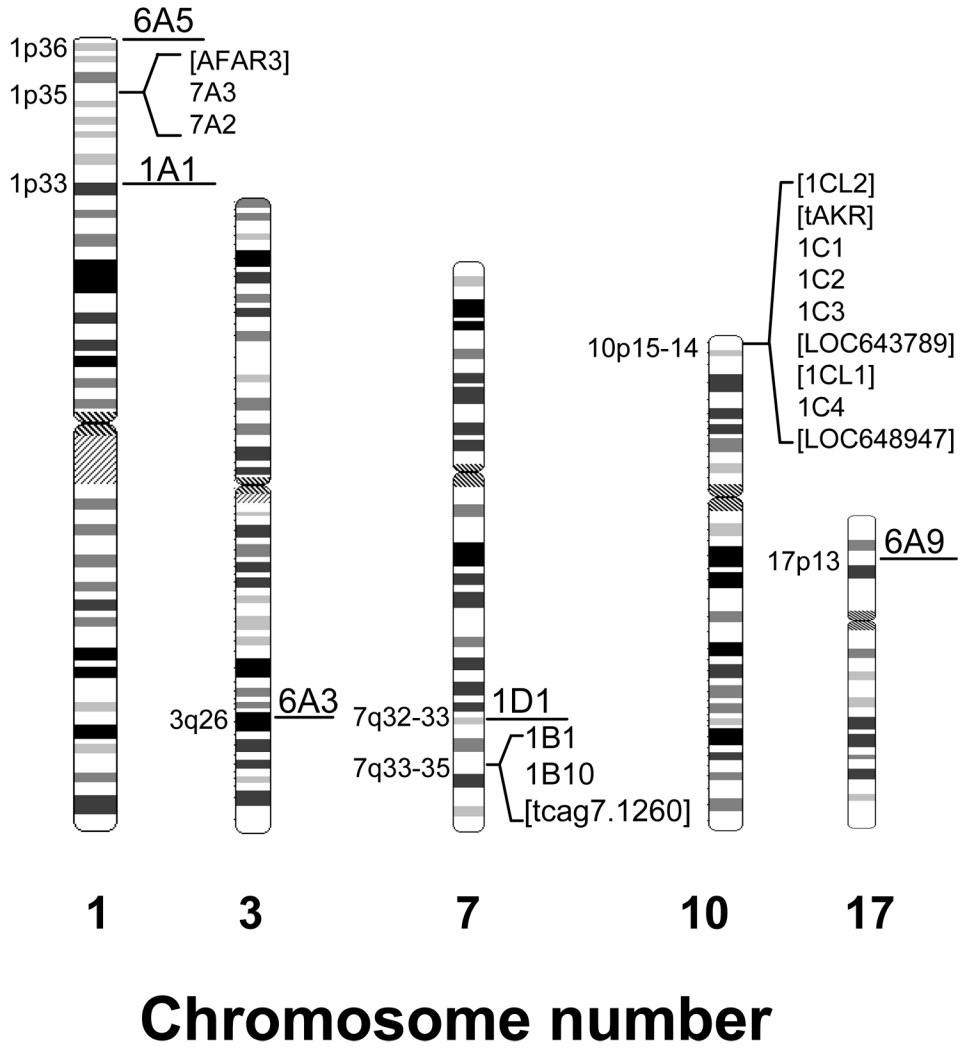


Fig. 6. Chromosomal localization of human AKR genes

With the exception of AKR6, the AKR families form clusters on the same chromosome. Genes for which protein product has not been demonstrated are shown in square brackets.

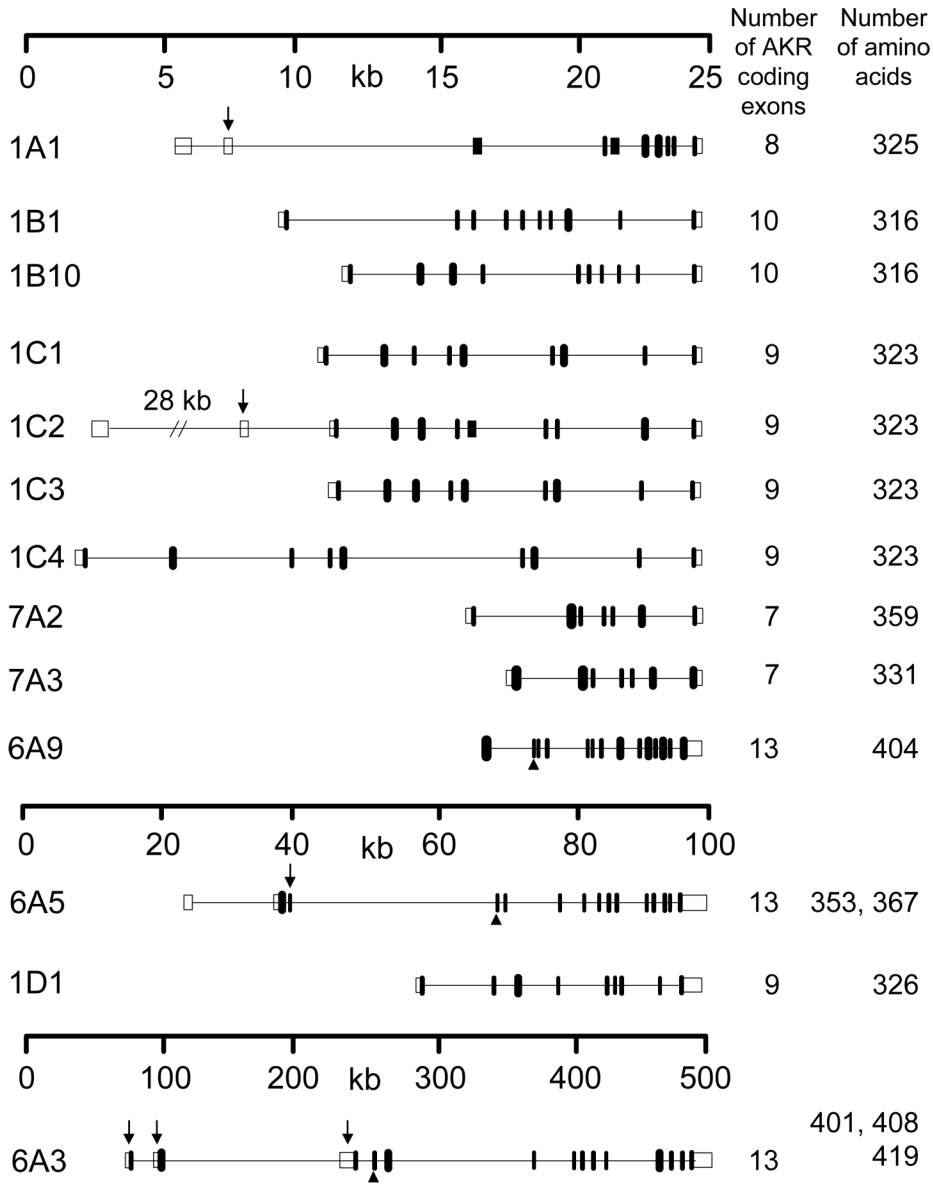


Fig. 7. Intron-exon structure of human AKR genes

Open boxes represent the noncoding exons and filled boxes indicate the coding exons. Arrows point to the exons subject to alternative splicing. The arrowheads show the start of the AKR domain in the Kvβ (AKR6) family. The number of exons coding for the AKR domain and the amino acid length of the corresponding protein are shown in adjacent columns.

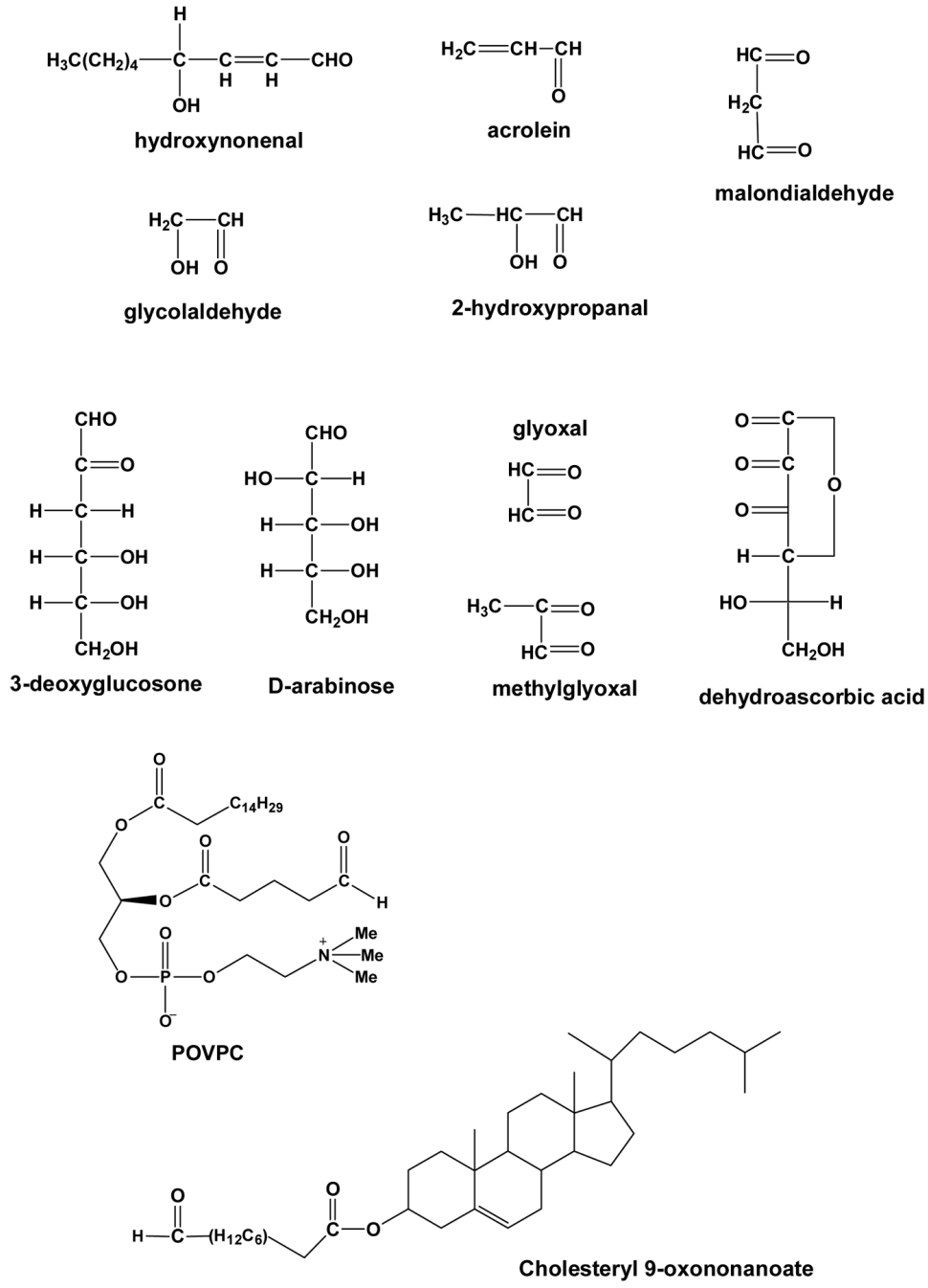


Fig. 8. Major reactive endogenous carbonyls

Chemical structures of the endogenous carbonyls identified as lipid peroxidation products, or carbohydrate-derived advanced glycosylation end-product (AGE) precursors are shown. At the bottom are representative structures of core aldehydes generated during the oxidation of phospholipids (e.g. 1-palmitoyl-2-arachidonyl phosphatidylcholine) or cholesterol esters containing unsaturated fatty acid side chains. POVPC: 1-palmitoyl-2-oxovaleroyl phosphatidyl choline.

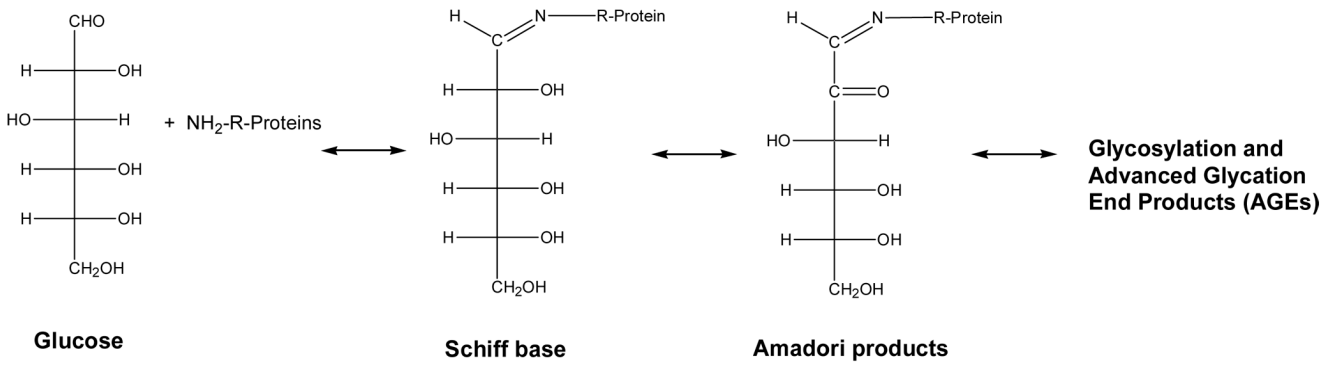


Fig. 9. Glycation of the amino groups of the proteins by reactive aldehydes and formation of advanced glycation end-products (AGEs)

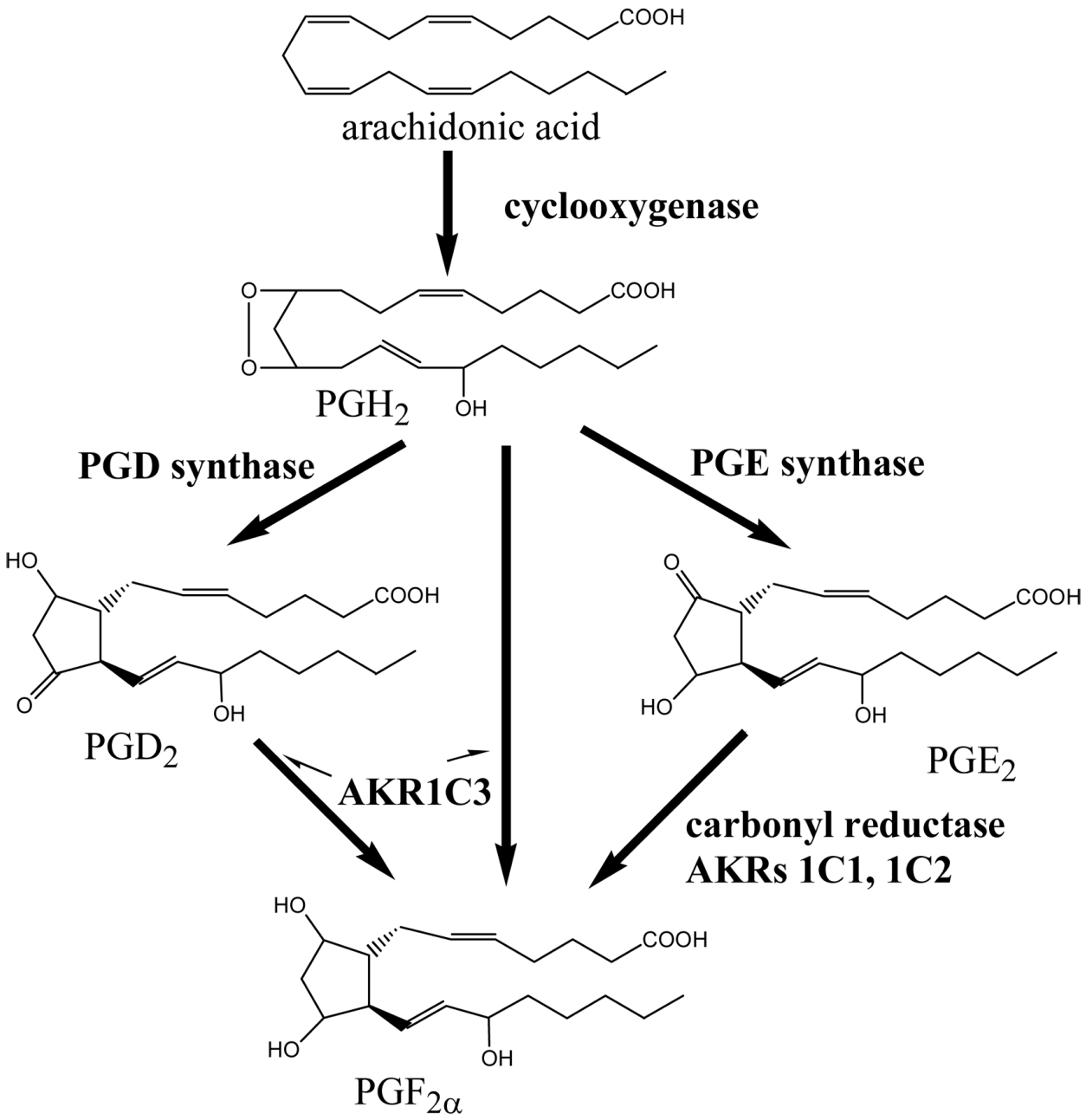


Fig. 10. Prostaglandin biosynthesis

Members of the AKR family catalyze the transformation of PGH_2, PGD_2 and PGE_2 into PGF_2.

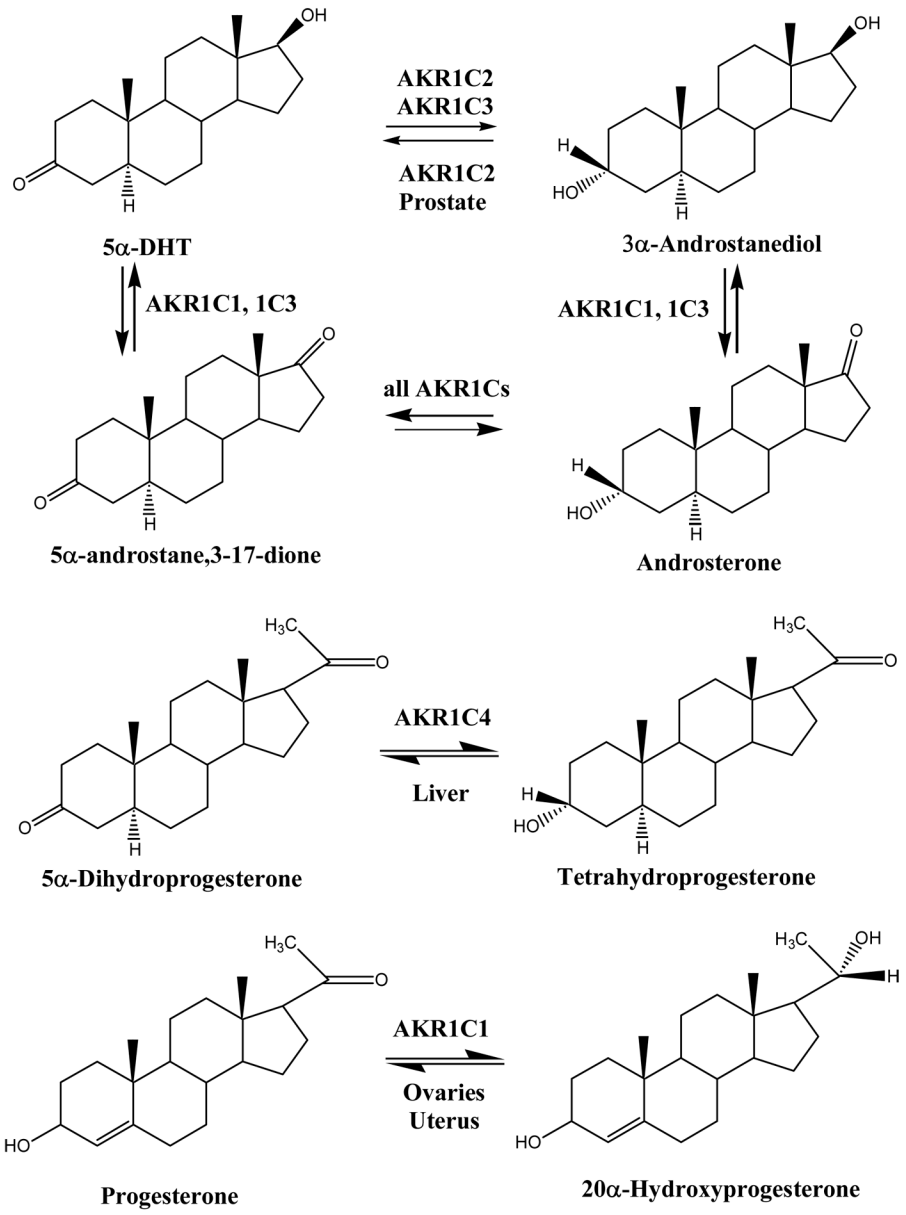


Fig. 11. Representative steroid transformations catalyzed by the AKR1C family of enzymes

Several AKR1C enzymes catalyze oxidation-reduction reactions at the 3, 17, and 20 positions of the steroid molecule.

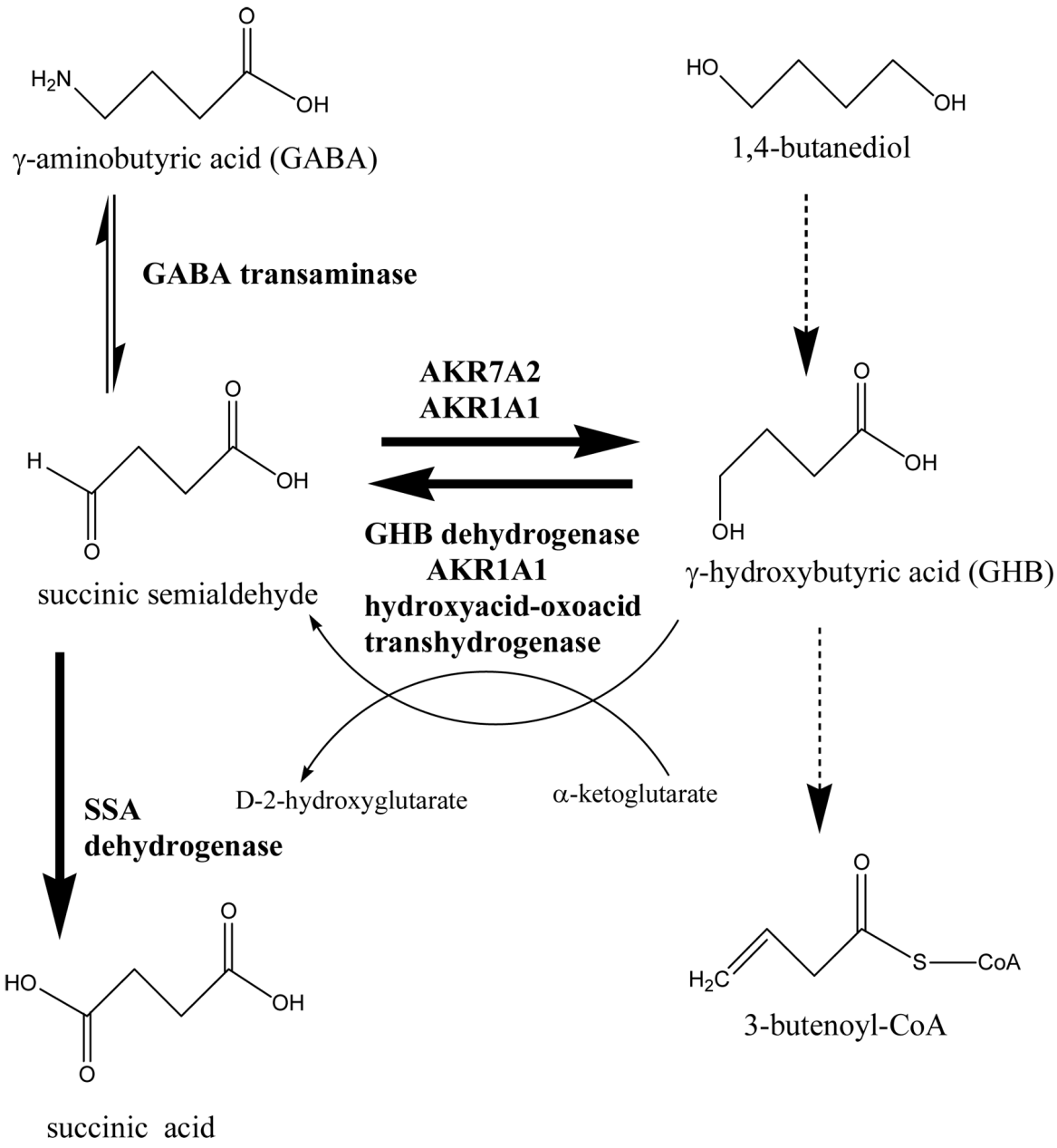


Fig. 12. Catabolism of the neurotransmitter GABA

Members of AKR families 1 and 7 catalyze the interconversion between succinic semialdehyde and γ-hydroxybutyrate (GHB).

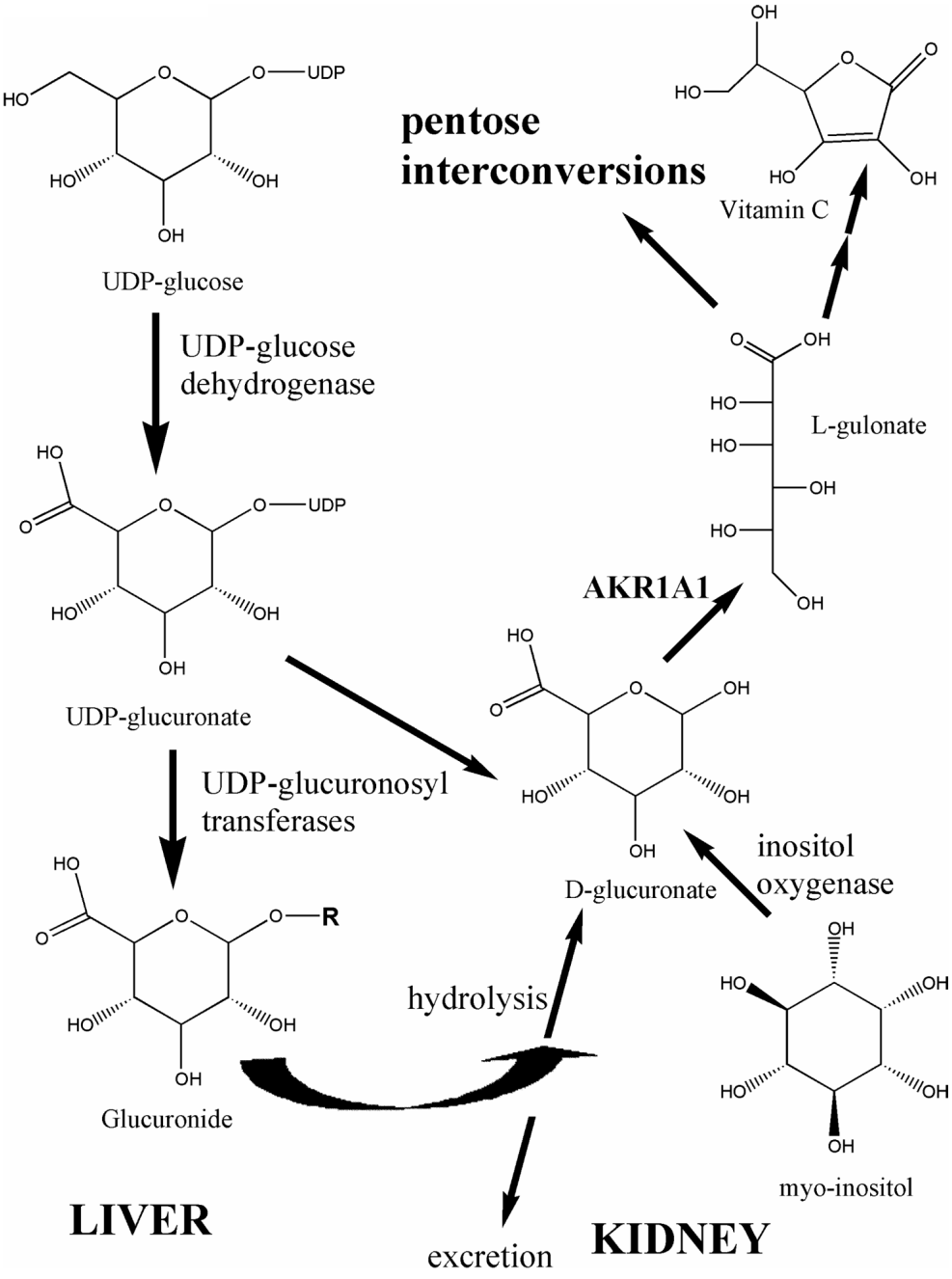


Fig. 13. Biochemical pathways for the biosynthesis of ascorbic acid
AKR1A catalyzes the reduction of D-glucuronate to L-gulonate.

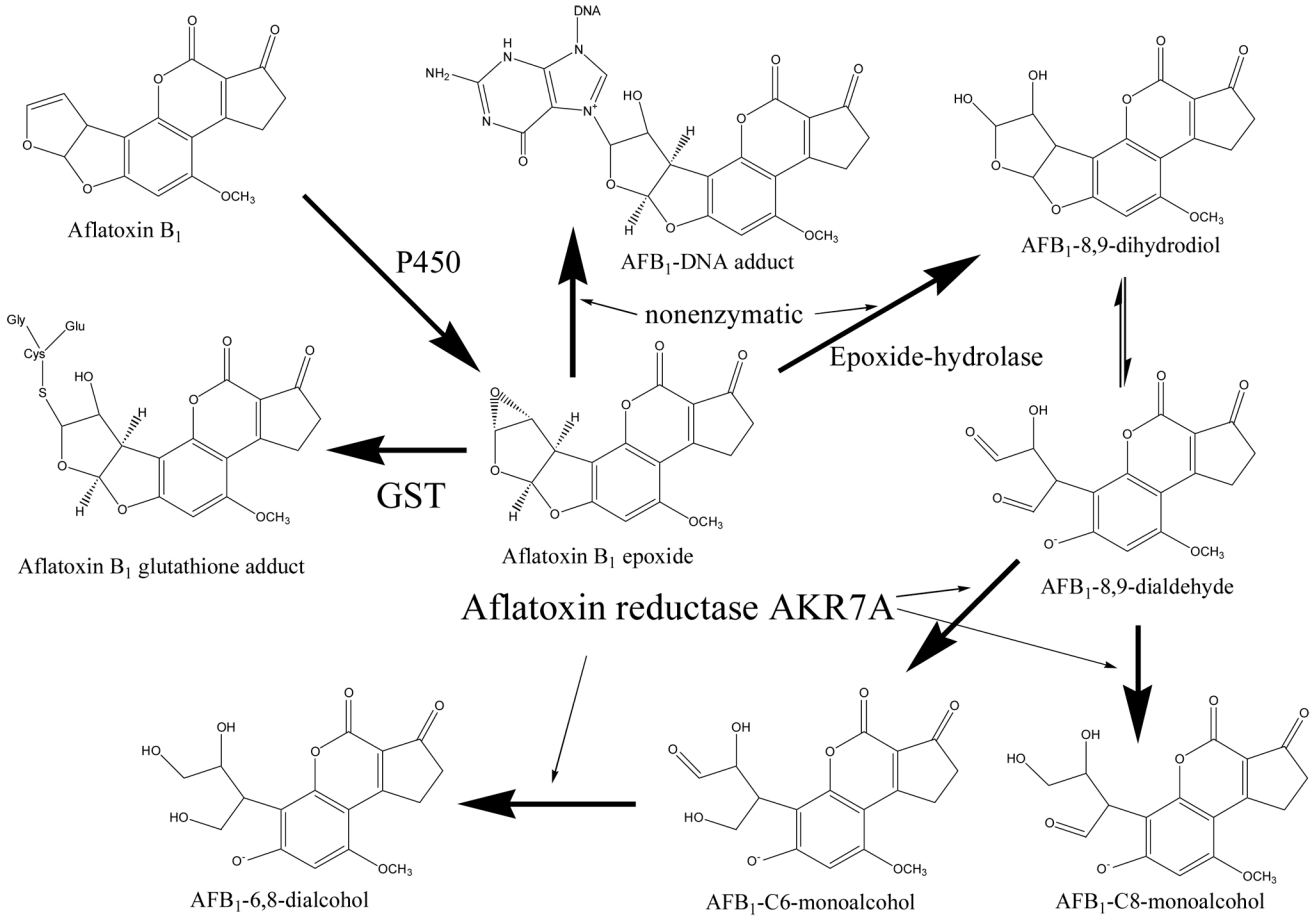


Fig. 14. Biochemical pathways for the metabolism of aflatoxin B_1

Aflatoxin reductases of the AKR7 family catalyze the reduction of AFB_1 dialdehyde, the cytotoxic intermediate of aflatoxin B_1 activation, to mono-and dialcohols as indicated.

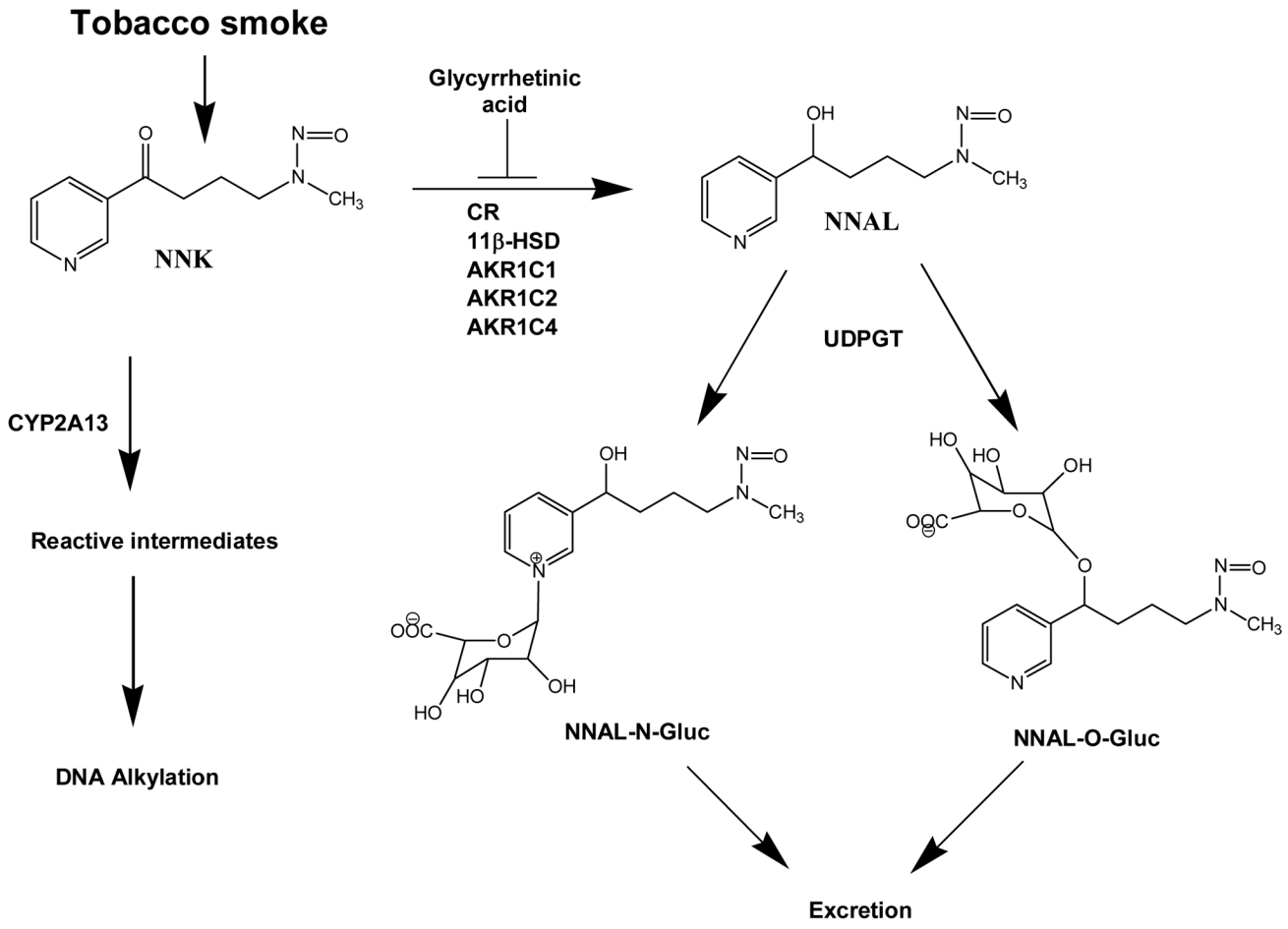


Fig. 15. Detoxification and bioactivation of tobacco-derived carcinogen 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK)

NNK can be oxygenated by cytochromes P450 or reduced by several enzymes of the AKR and HSD families to form nitrosamine alcohol (NNAL). Reduction represents detoxification pathway as NNAL is glucuronydated by uridine diphosphate glucuronosyl transferases (UDPGT) and is subsequently excreted.

Table 1

Coenzyme binding to AKRs

	AKR	Buffer	K_dNADPH (μM)	K_dNADP$^+$ (μM)	Reference
1	AKR1A1	5 mM K Phos, pH 7.5	0.13	0.36	(Barski *et al.*, 1995)
2	AKR1B1	5 mM K Phos pH 7.0	0.010	0.006	(Ehrig *et al*., 1994)
3	AKR1C2	10 mM K Phos pH 7.0	0.12	0.21	(Jin and Penning, 2006)
4	AKR6A3	200 mM K Phos pH 7.4	1.8	5.6	(Tipparaju et al., 2007)
5	AKR6A2	150 mM K Phos pH 7.4	0.12	0.36	(Liu *et al.*, 2001)

Table 2

Sequence homology between human, mouse, and rat AKRs

Human		Mouse		Rat	
Genbank Symbol	**AKR Symbol**	**Genbank Symbol**	**AKR Symbol**	**Genbank Symbol**	**AKR Symbol**
1A1	1A1	1a4	1A4	1a3	1A3
1B1	1B1	1b3	1B3	1b4	1B4
1B10	1B10	2310005E10Rik	--	1b10	--
[tcag7.1260][a]	--	1b8	1B8	1b8	1B13
		1b7	1B7	1b7	1B14
1C1	1C1	1c21	1C21	1c21	--
1C2	1C2				
1C3	1C3	1c18	1C18	1c18	1C8
1C4	1C4	1c6	1C6	1c6	--
homology not established					
[tAKR]					
[1CL1]					
		1c12	1C12	LOC364773	1C24
		1c13	1C13		
		1c19	1C19	*[RGD1562954]*	--
		1c20	1C20		
		1c14	1C14	LOC191574	1C9
				1c12_predicted	1C16
				RGD1559604	1C17
				AKR1cl1	1C15
pseudogenes				*[RGD1564865]*	
[LOC643789]					
[LOC648947]					
1D1	1D1	1d1	--	1d1	1D2

Human		Mouse		Rat	
Genbank Symbol	**AKR Symbol**	**Genbank Symbol**	**AKR Symbol**	**Genbank Symbol**	**AKR Symbol**
[1CL2]	1E2	1e1	1E1	1e1	--
KCNAB1	6A3	Kcnab1	6A8	Kcnab1	6A13
KCNAB2	6A5	Kcnab2	6A4	Kcnab2	6A2
KCNAB3	6A9	Kcnab3	6A14	Kcnab3	6A12
7A2	7A2	7a5	7A5	7a2	7A4
7A3	7A3			7a3	7A1
[AFAR3]					

Genes from different species placed on the same line represent homologs according to Homologene database, i.e. genes with the highest degree of sequence identity between species. On the left side of each column are the names of the genes according to NCBI database, whereas on the right side are their names according to the AKR database.

[a] Gene names in brackets and italicized represent predicted genes for which expression has not been confirmed.

Table 3

Carbonyl-containing Pharmaceuticals[†]

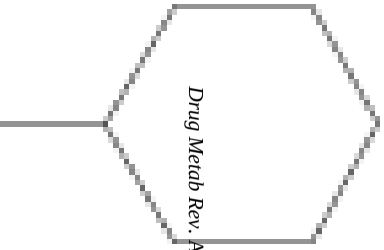
DRUG	CLASS	DRUG	CLASS
acetohexamide[*]	antidiabetic	L-691,121[*]	antiarrhythmic
befunolol[*]	antihypertensive	loxoprofen	NSAID
benfluron[*]	anticancer	menadione	vitamin (K3)
bromperidol[*]	antipsychotic	methylprednisolone	steroid
CS-670	NSAID	metyrapone	diagnostic
daunorubicin	anticancer	nabumetone	NSAID
dolasetron[*]	antiemetic	nafimidone	anticonvulsant
doxorubicin	anticancer	naftazone	vasoprotectant
E-10-oxo-nortriptyline	antidepressant	naloxone	opiate antagonist
epirubicin	anticancer	naltrexone[*]	opiate antagonist
ethacrynic acid	diuretic	oracin[*]	anticancer
fenofibrate	antihyperlipidemic	oxcarbazepine	antiepileptic
flobufen[*]	NSAID	oxisuran	immunosuppressive
haloperidol[*]	antipsychotic	pentoxifylline	hemorheological
HY-770[*]	muscle relaxant	S-1360	antiviral
idarubicin[*]	anticancer	TA-510	anti-inflammatory
iododoxorubicin	anticancer	timiperone[*]	antipsychotic
ketanserin[*]	antihypertensive	tolperisone	muscle relaxant
ketoprofen	NSAID	warfarin	anticoagulant
ketotifen	antiasthmatic	Z-10-oxo-nortriptyline	antidepressant

[†] Table provided by Dr. Jane Rosemond, GlaxoSmithKline, Research Triangle Park, NC, USA.

[*] Reduced product identified as a major metabolite

Table 4

c parameters of cytosolic reductases with pharmacological compounds

	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
	0.22	17[d]			1.3	2.6[d]	3.9	2.2[d]	0.5	4.9[d]		No act[d]

	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
	0.26	11[d]	1.1	1.3[b]		No act[d]	3.2	4.3[d]		No act[d]	0.36	65[d]

	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
			0.14	0.43[b]	0.160	147[e]	0.090	12.3[e]	0.15	1.72[e]		

	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
		No act[d]			0.52	26[d]	0.76	16.7[d]		No act[d]		No act[d]

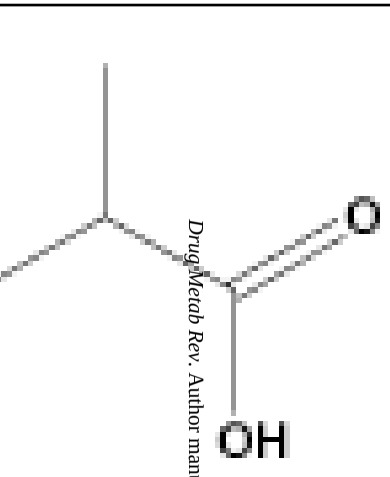
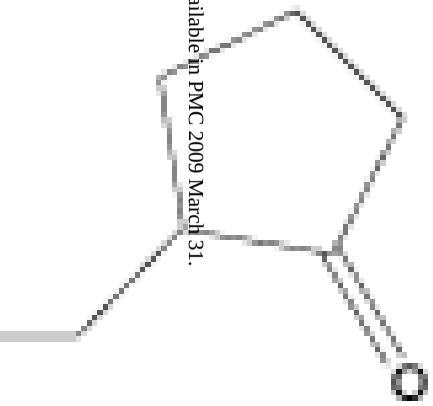
	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
		No act[d]			1.7	4.6[d]	1.1	2.9[d]		No act[d]		No act[d]

	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
					0.011	17	0.0075	284	No act[a]		No act[a]	

	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
					0.053	63	0.0036	1030		No act		No act
					0.0064	810	0.010	95.7		No act		No act
					0.0026	4416	0.0031	2061		No act		No act
			0.080	130[b]	0.064	110[c]	0.030	35[c]	0.22	90[c]	No sat	~30[c]

	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
		No act[d]			1.4 0.21	0.84[c] 21.9[d]	0.13 1.6	34[c] 4.0[d]	0.033 0.19	660[c] 12.2[d]		No act[c] No act[d]

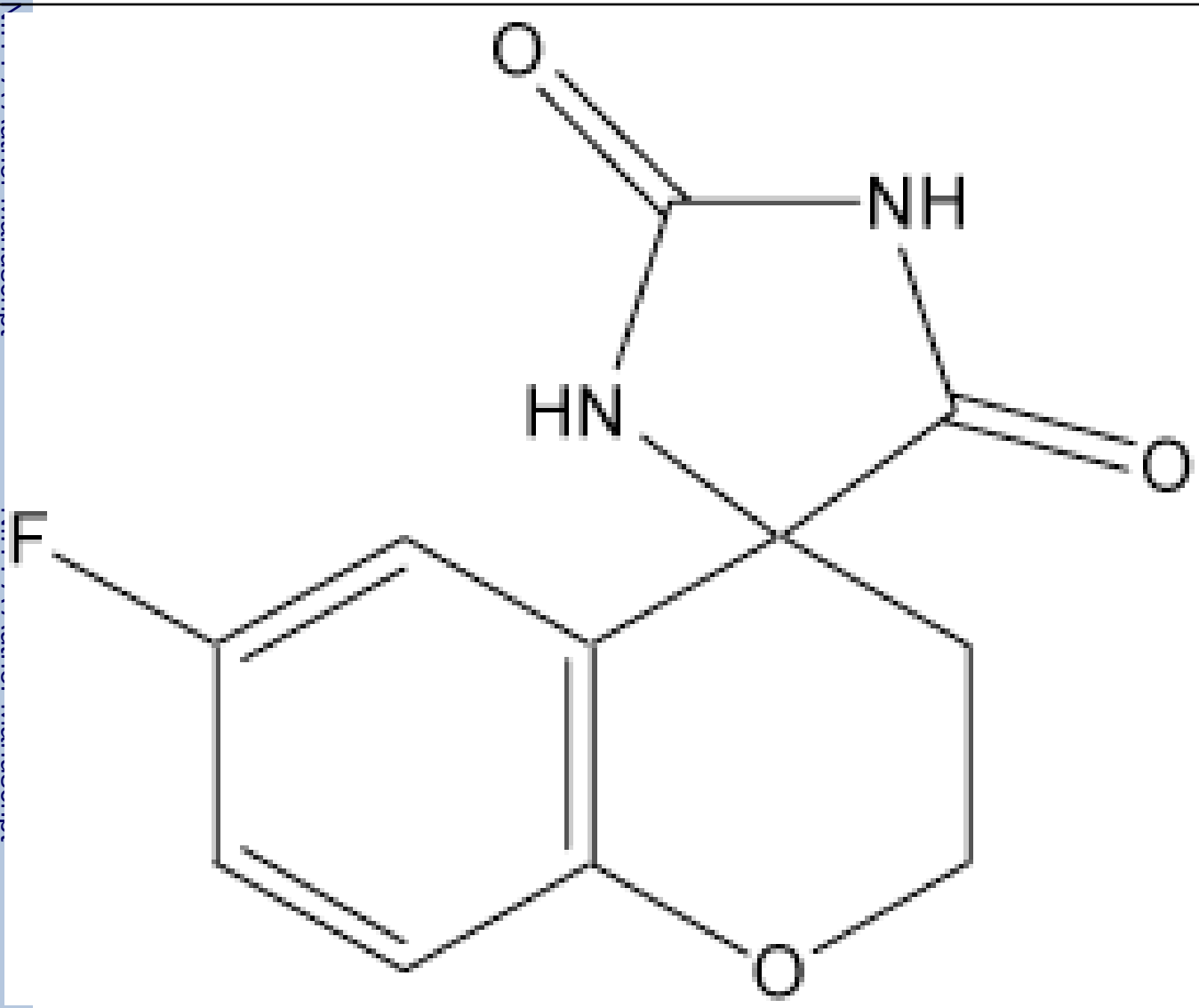
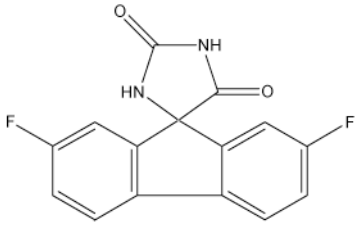
	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
		No act[d]			0.74	3.9[d]	1.9	3.7[d]	0.41	9.9[d]		No act[d]

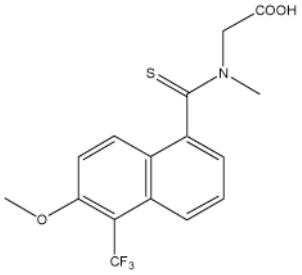
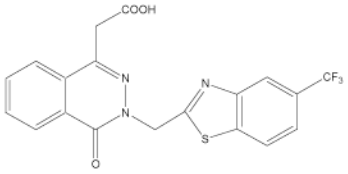
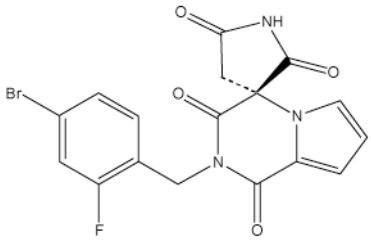
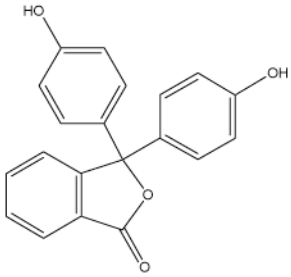
	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
		No act[d]				No act[d]	0.38	2.3[d]	0.5	4.9[d]		No act[d]
		No act[d]				No act[d]	1.1	2.9[d]	1.2	3.5[d]	38	7.9[d]

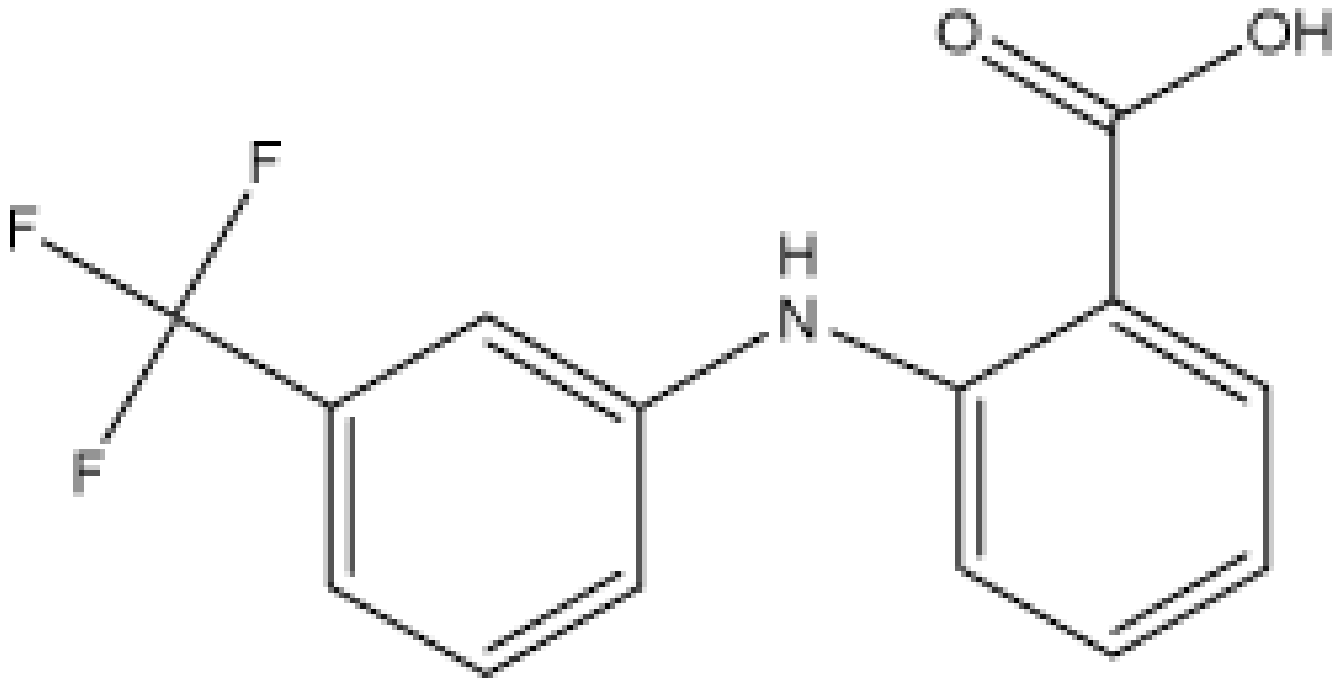
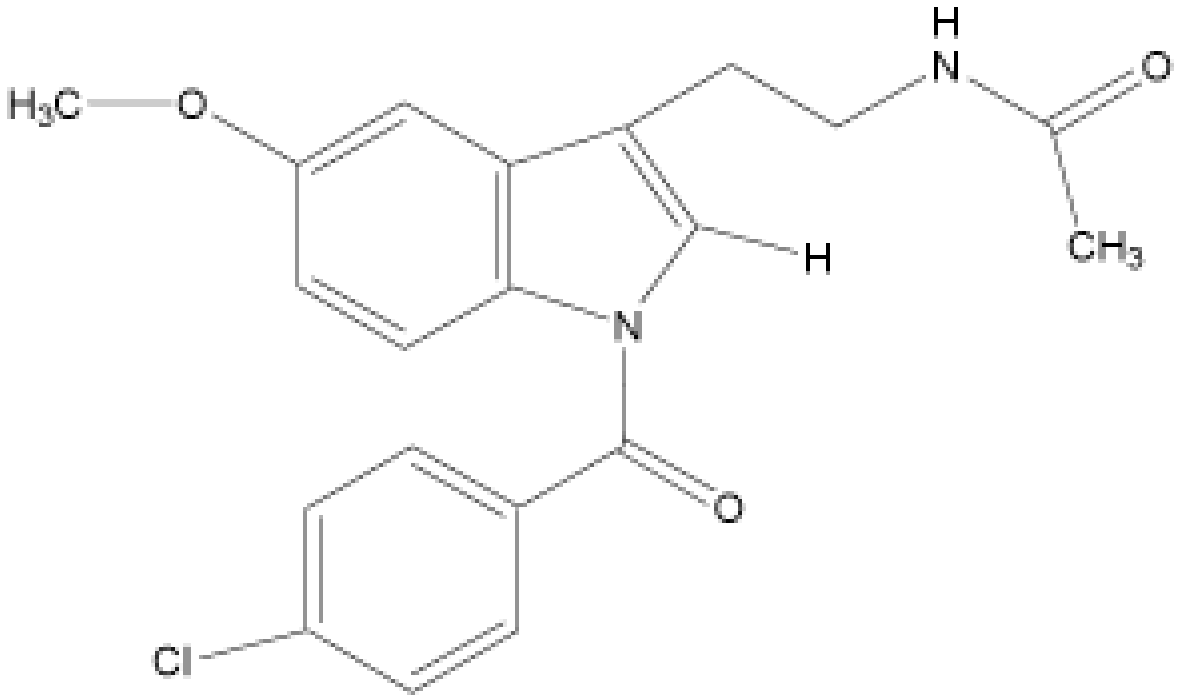
	AKR1A1		AKR1B10		AKR1C1		AKR1C2		AKR1C4		CR	
	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})	K_m (mM)	V_m/K_m (min^{-1} mM^{-1})
		No act[d]			0.19 1	8.5^{d} 0.51^{a}	0.62	2.2^{d} 0.91^{a}		No act[d]	1.2	22^{d} 105^{a}

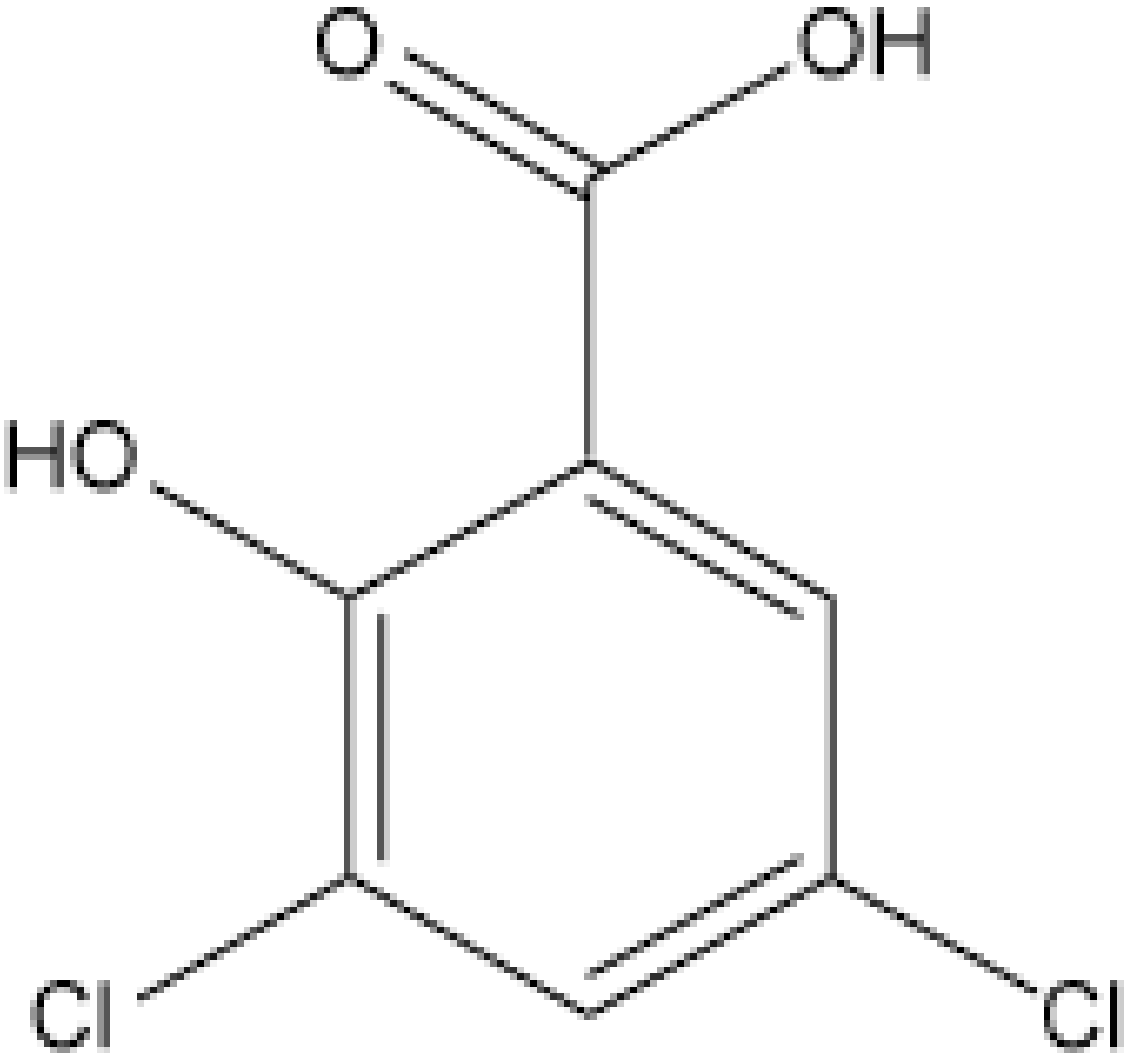
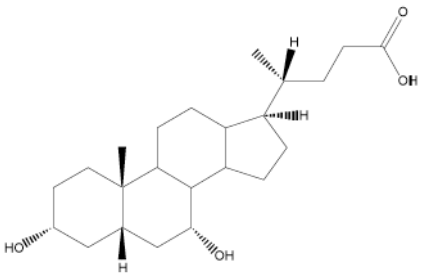
Table 5

Selected AKR Inhibitors[†]

Structure	AKR[*]
	Nonspecific 1A,
	1A1

Structure	AKR*
	1B1
	1B1
	1B1
	1C4>1C1>1C3>1

Structure	AKR*
	1C1, C2, C3
	1C3

Structure	AKR*
	1C1
	1C2

† The table is not intended to be a comprehensive list of AKR inhibitors; instead it is intended to show examples of the compounds that can be applied to inhibit selected AKR isoforms for research.

* Members listed in this column display more than 10-fold higher affinity for the inhibitor than other AKRs