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Inhibitors of human 20α-hydroxysteroid dehydrogenase (AKR1C1)

Ossama El-Kabbani[a,*], Urmi Dhagat[a], Akira Hara[b]

[a] Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia
[b] Laboratory of Biochemistry, Gifu Pharmaceutical University, Daigaku-Nishi, Gifu 501-1196, Japan

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ABSTRACT

Human 20α-hydroxysteroid dehydrogenase (AKR1C1), a member of the aldo-keto reductase (AKR) superfamily, is one of four isoforms (with >84% amino acid sequence identity) existing in human tissues. AKR1C1 most efficiently reduces biologically active progesterone and 5α-pregnan-3α-ol-20-one into their corresponding 20α-hydroxysteroids among the isoforms. The enzyme also accepts endogenous and xenobiotic non-steroidal carbonyl compounds as the substrates. In addition to the up-regulation of the AKR1C1 gene in cancer cells, the enzyme's over-expression in the cells of lung, ovary, uterine cervix, skin and colon carcinomas was reported to be associated with resistance against several anticancer agents. Thus, AKR1C1 may be a marker of the above cancers and a target of poor prognosis in cancer therapy. The recently determined X-ray crystal structures of AKR1C1/$NADP^+$/20α-hydroxyprogesterone and AKR1C1/$NADP^+$/3,5-dichlorosalicylic acid ternary complexes have provided a strong foundation for structure-based design methods to improve inhibitor selectivity and potency. In this review we provide an overview of the different types of AKR1C1 inhibitors and an update on the design of potent and selective inhibitors based on the crystal structure of the enzyme-inhibitor complex.

Article from the Special issue on Targeted Inhibitors.

1. Introduction

20α-Hydroxysteroid dehydrogenase (20α-HSD, EC 1.1.1.149) plays an important role in controlling the cellular concentration of progesterone (an active progestin) by catalyzing its NADPH-dependent reduction into 20α-hydroxyprogesterone. The role is demonstrated in mice deficient for 20α-HSD, which sustains high progesterone levels and display a delay in parturition of several days [1]. In addition to the well-known effects on the endocrine and reproductive systems, progesterone exerts a wide range of actions depending on the target tissues through non-genomic signalling as well as the nuclear progesterone receptors-A and -B [2,3]. For example, progesterone is naturally present as a neurosteroid in the human brain where it up-regulates the γ-aminobutyric acid type A ($GABA_A$) receptor. Additionally, its metabolite 5α-pregnan-3α-ol-20-one is a potent positive modulator of the $GABA_A$ receptor, consequently producing anxiolytic, sedative, hypnotic and anaesthetic effects [2,3]. Progesterone and/or 5α-pregnan-3α-ol-20-one also exert neuroprotective effects after brain injury in clinical trials [3,4], and in experimental models of peripheral nerve crush injury [5] and diabetic neuropathy [6]. Although progesterone treatment in traumatic brain injury has moved on to phase III in clinical trials [4], the possibility exists that inhibitors of human 20α-HSD may exert similar effects on neuronal disorders by preventing the inactivation of neuroactive steroids.

Human 20α-HSD belongs to the aldo-keto reductase (AKR) superfamily, and is named AKR1C1 that is one of four isoforms (with >84% amino acid sequence identity) existing in human tissues. The other isoforms including AKR1C2 (type 3 3α-HSD), AKR1C3 (type 2 3α-HSD and type 5 17β-HSD) and AKR1C4 (type 1 3α-HSD) display distinct positional and stereochemical preferences for steroid substrates from AKR1C1, despite that the four AKR1C isoforms have overlapping catalytic properties [7]. With the exception of liver-specific AKR1C4, the other three isoforms are expressed in many human tissues, in which their expression extents are different from each other [7]. AKR1C1 mRNA expression is highest in the lung, followed by the liver, testis, mammary gland, endometrium and brain. Recent reports show the expression of AKR1C1 in the kidney [8], adipose cells [9], skin [10], osteoblasts [11] and optic nerve head astrocytes [12]. Among the isoforms, AKR1C1 most efficiently reduces biologically active progesterone and 5α-pregnan-3α-ol-20-one into their corresponding 20α-hydroxysteroids [7,13,14]. The enzyme also accepts endogenous and xenobiotic non-steroidal carbonyl compounds as the substrates [15,16]. Among them prostaglandin D_2 is reduced to

Abbreviations: HSD, hydroxysteroid dehydrogenase; AKR, aldo-keto reductase; AKR1C1, human 20α-hydroxysteroid dehydrogenase; AKR1C2, human type 3 3α-hydroxysteroid dehydrogenase; AKR1C3, human type 2 3α-hydroxysteroid dehydrogenase; AKR1C4, human type 1 3α-hydroxysteroid dehydrogenase; AKR1B1, human aldose reductase; $GABA_A$, γ-aminobutyric acid type A; PAH, polycyclic aromatic hydrocarbon.

* Corresponding author. Tel.: +61 3 9903 9691; fax: +61 3 9903 9143.
E-mail address: ossama.el-kabbani@monash.edu (O. El-Kabbani).

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9α,11β-prostaglandin F_2 by AKR1C1 [17,18], although the k_{cat}/K_m value ($0.24\,min^{-1}\,\mu M^{-1}$) is lower than that ($1.27\,min^{-1}\,\mu M^{-1}$) of AKR1C3 that is also called prostaglandin F synthase. In addition, 4-hydroxy-2-nonenal, a cytotoxic aldehyde derived from lipid peroxidation, is reduced more highly by AKR1C1 among the four AKR1C isoforms [19]. The role of AKR1C1 in the detoxification of lipid peroxidation products is further supported by our unpublished results that 4-oxo-2-nonenal, another major cytotoxic product of lipid peroxidation [20], is the best substrate for AKR1C1, showing lower K_m (0.16 μM) and higher k_{cat}/K_m values ($28\,min^{-1}\,\mu M^{-1}$) than those for steroidal substrates and the k_{cat}/K_m value ($2.1\,min^{-1}\,\mu M^{-1}$) of human aldose reductase (AKR1B1), as the previously known efficient 4-oxo-2-nonenal reductase [21]. The four AKR1C isoforms are implicated in the metabolic activation of carcinogenic polycyclic aromatic hydrocarbons (PAHs), because they catalyze the $NADP^+$-linked oxidation of *trans*-dihydrodiol metabolites of PAHs, which yield reactive and redox active PAH quinones [15,16]. The enzyme exhibiting this oxidase activity is classified as dihydrodiol dehydrogenase (DD, EC 1.3.1.20) in the IUBMB Enzyme Nomenclature, and AKR1C1–1C4 have been also called DD1, DD2, DD3 (or DDX) and DD4, respectively.

AKR1C1 and/or its mRNA are reported to be highly expressed in tumors and carcinoma cells from lung [22,23], endometrial [24,25], ovarian [26], hepatocellular [27], gastric [28] and skin [29] cancers, although in several studies AKR1C1 and AKR1C2 were not distinguished because of their high amino acid sequence identity (97%). The up-regulation of the AKR1C1 gene is also observed in stem cells in lung carcinoma A549 cells [30] and thyroid cancer stem-like cells [31]. Furthermore, a recent report shows fibrosarcoma formation in nude mice by subcutaneous injection of NIH3T3L1 cells expressing AKR1C1, suggesting that oncogenic metabolite(s) produced in the above activities of the enzyme participate in neoplastic transformation [32]. Thus, AKR1C1 may be a marker of the above cancers and a target of poor prognosis in cancer therapy [14], although the mechanism underlying cancer proliferation and tumorigenesis is not clear. In addition to the up-regulation of AKR1C1 gene in cancer cells, its over-expression in cells of lung [33,34], ovary [26,33,35], uterine cervix [33,36], skin [29] and colon [37] carcinomas was reported to be associated with resistance against several anticancer agents (cisplatin, carboplatin, adriamycin, doxorubicin, bleomycin and methotrexate). These anticancer agents, except for methotrexate, generate reactive oxygen species, which lead to cell dysfunction and apoptosis via diverse signalling mechanisms, as well as up-regulation of the AKR1C1 gene through activation of an anti-oxidant response element [38,39]. The over-expressed AKR1C1 has been suggested to reduce the generation of reactive oxygen species, detoxify free radicals and anthracycline drugs, or alter DNA repair and apoptosis [26,29,33–36]. In methotrexate resistance, the enzyme counteracts the S-phase accumulation of the cells and apoptosis caused by the drug [37]. Although the exact mechanisms of resistance to anti-cancer agents by AKR1C1 warrant further investigations, the enzyme is required as a survival factor in cancer cells exposed to anticancer agents, as well as in cancer cell proliferation. Due to the associated up-regulation of AKR1C1 with cancers and anticancer drug resistance, inhibition of AKR1C1 represents an approach to develop novel drugs for improving poor prognosis in cancer therapy and new adjuvant therapy for cancer chemotherapeutic drug resistance.

In addition to the cancer chemotherapy, AKR1C1 inhibitors would be desirable to act as progestational agents to maintain early and late pregnancy, and potentiate the action of neurosteroids that positively regulate the $GABA_A$ receptor as described early in this review. Although inhibitors specific to a therapeutic target are generally required for drug development, there is a growing need for the development of compounds that selectively inhibit AKR1C1 activity, particularly in the metabolism of the neurosteroids. AKR1C1 decreases the concentrations of active neurosteroids by efficiently reducing progesterone, 5α-pregnan-3α-ol-20-one and its precursor 5α-pregnane-3,20-dione to their corresponding inactive 20α-hydroxy metabolites, whereas AKR1C2 is involved in synthesis of the active neurosteroid 5α-pregnan-3α-ol-20-one from 5α-pregnane-3,20-dione [13,14]. Because AKR1C1 and AKR1C2 share 97% sequence identity, there is a difficulty in attaining specificity between the two isoforms.

To date, a variety of competitive and non-competitive inhibitors have been identified through high-throughput virtual screening and synthetic efforts. The X-ray crystal structures of AKR1C1 in complex with the substrate 20α-hydroxyprogesterone [PDB ID 1MRQ] and the potent inhibitor 3,5-dichlorosalicylic acid [PDB ID 3C3U] have been reported providing an excellent basis for structure-based design to improve inhibitor selectivity and potency [40,41]. Here we provide an overview of the different types of AKR1C1 inhibitors and our efforts in the design of potent and selective inhibitors based on the crystal structure of the enzyme-inhibitor complex.

2. Non-competitive inhibitors of AKR1C1

In 2002, Usami et al. found that benzodiazepines, compounds known to inhibit enzymes involved in the metabolism of neurosteroids, were non-competitive inhibitors of AKR1C1 [42]. The inhibition potency of a few representative benzodiazepines was tested against the four AKR1C isoforms. Due to the high sequence identity between AKR1C1 and AKR1C2 most of these compounds exhibited similar inhibition spectra for the two isoforms. Medazepam was the only inhibitor which potently inhibited AKR1C1 over the other AKR1C isoforms (Table 1).

In an attempt to find new AKR1C1 inhibitors with novel scaffolds, Brozic et al. synthesized a series of pyrimidine, phthalimide and anthranilic acid derivatives, and evaluated their inhibitory potencies towards AKR1C1 [43]. The two most potent inhibitors from this study were the pyrimidine derivative *N*-benzyl-2-(2-(4-methoxybenzyl)-6-oxo-1,6-dihydropyrimidin-4-yl)acetamide (IC_{50} = 17.9 μM) and the anthranilic acid derivative 2-(2′,3-dichloro-N-methylbiphenyl-4-ylcarboxamido)benzoic acid (IC_{50} = 35 μM). Both these compounds exhibited non-competitive inhibition with respect to the substrate 1-acenaphthenol.

3. Competitive inhibitors of AKR1C1

A study on the inhibitory effects of a series of *N*-phenylanthranilic acid derivatives and steroid carboxylates on the AKR1C isoforms reported A-ring substituted *N*-phenylanthranilates as potent competitive inhibitors with respect to the substrate 1-acenaphthenol [44]. Some of the steroid carboxylates, like lithocholic acid (IC_{50} = 9.2 μM), and other *N*-phenylanthranilic acids inhibited AKR1C1 at low μM concentrations, but are not selective inhibitors of this isoform.

Brozic et al. investigated the inhibitory action of phytoestrogens (plant-derived and non-steroidal constituents of diets) on AKR1C1 and investigated their effect on the key progesterone metabolizing enzyme [45]. Among a wide variety of tested compounds such as flavones, flavanones, isoflavones, plant organic acids and synthetic estrogens, 7-hydroxyflavone, 3,7-dihydroxyflavone and flavanone naringenin were identified as the most potent inhibitors of AKR1C1, showing IC_{50} values of 2.3 μM, 4.9 μM and 2.6 μM, respectively. Possible binding of the flavones in the active site of the enzyme was proposed by a molecular docking study.

Since compounds that have a steroidal core structure exhibit residual steroidogenic activity, Stefane et al. synthesized a series of cyclopentane derivatives and investigated their inhibition

Table 1
Inhibitors of AKR1C1.

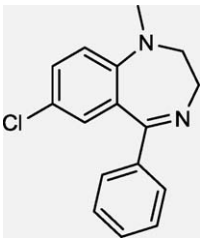
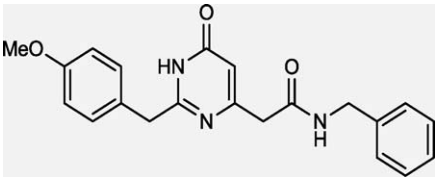
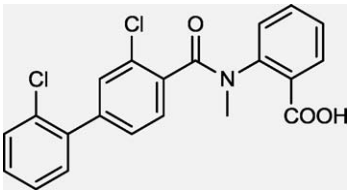
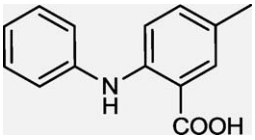
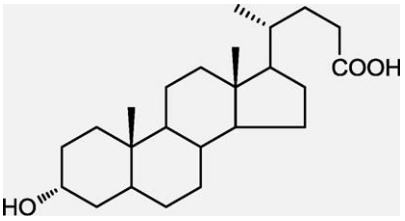
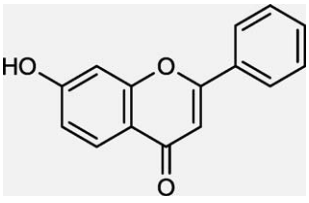
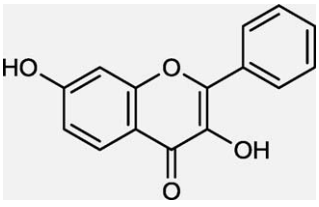
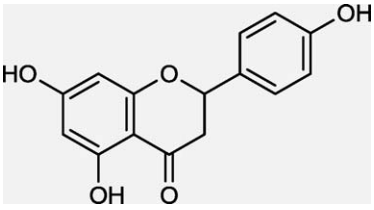
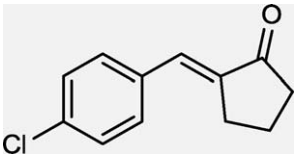
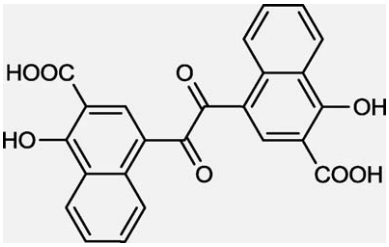
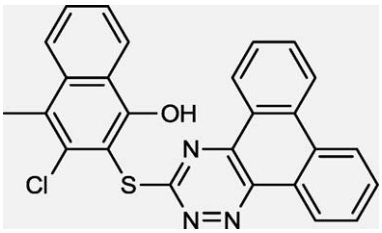
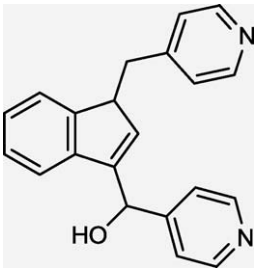
Inhibitor classification	Compound name	Structure	IC_{50} value (μM) and inhibition pattern
Benzodiazepine	Medazepam/7-Chloro-2,3-dihydro-1-methyl-5-phenyl-1*H*-1,4-benzodiazepine		4.7 Non-competitive
Pyrimidine derivative	*N*-Benzyl-2-(2-(4-methoxybenzyl)-6-oxo-1,6-dihydropyrimidin-4-yl)acetamide		17.9 Non-competitive
Anthranilic acid derivative	2-(2′,3-Dichloro-N-methylbiphenyl-4-ylcarboxamido)benzoic acid		35 Non-competitive
N-Phenylanthranilic acid derivative	5-Methyl-*N*-phenylanthranilic acid		3.2 Competitive
Steroid carboxylate	Lithocholic acid		9.2 Competitive
Flavones	7-Hydroxyflavone		2.3 Competitive
	3,7-Dihydroxyflavone		4.9 Competitive
	Naringenin		2.6 Competitive

Table 1 (*Continued*)

Inhibitor classification	Compound name	Structure	IC_{50} value (μM) and inhibition pattern
Cyclopentane derivative	2-(4-Chlorobenzylidene)-cyclopentanone		35 Competitive
High throughput screening	4,4′-Oxalylbis(1-hydroxy-2-naphthoic acid)		9.6 Competitive
	3-Chloro-4-methyl-2-(phenanthro[9,10-e][1,2,4]triazin-3-ylthio)naphthalen-1-ol		25 Competitive
	Pyridin-4-yl(1-(pyridin-4-ylmethyl)-1*H*-inden-3-yl)methanol		19.6 Competitive

of AKR1C1 and AKR1C3 [46]. Most of these compounds displayed IC_{50} values in the μM range against AKR1C1. Of the 4-chlorobenzylidene cyclopentane derivatives evaluated, 2-(4-chlorobenzylidene)cyclopentanone was the most active inhibitor of AKR1C1 (IC_{50} = 35 μM and K_i = 17 μM), although it also inhibited AKR1C3 (K_i = 33 μM).

Recently, three structurally diverse inhibitors of AKR1C1 were discovered from virtual high-throughput screening studies [47]. These novel compounds which potently inhibit AKR1C1 were discovered by docking 1990 compounds from the National Cancer Institute "Diversity Set" using the program eHiTS for virtual screening. The three structurally diverse hits were 4,4′-oxalylbis(1-hydroxy-2-naphthoic acid), 3-chloro-4-methyl-2-(phenanthro[9,10-e][1,2,4]triazin-3-ylthio)naphthalen-1-ol and pyridin-4-yl(1-(pyridin-4-ylmethyl)-1H-inden-3-yl)methanol, showing IC_{50} values in the low μM range (Table 1). The compounds have potential for further optimization to improve potency and selectivity, and represent promising starting points for structural optimization in hit-to-lead development.

Our laboratory identified inhibitors of AKR1C1 from a virtual screening-based study, of which 3,5-diiodosalicylic acid was reported to be the most potent competitive inhibitor of the enzyme with a K_i value in the sub-μM range [48]. Additionally, we reported the first structure of AKR1C1 in ternary complex with the coenzyme and 3,5-dichlorosalicylic acid, an analogue of 3,5-diiodosalicylic acid discovered from a similarity search and found to be a more potent competitive inhibitor of AKR1C1, displaying a K_i value of 5.9 nM [41]. In an effort to further characterize the inhibitor-binding site we have compared the structures of the active-sites for the four AKR1C isoforms and used site-directed mutagenesis coupled with inhibitor binding studies to identify the role of several key residues in inhibitor binding and selectivity to the AKR1C isoforms in general, and to AKR1C1 in particular [41,48].

3.1. The inhibitor-binding site

The structure of AKR1C1 is comprised of an eight-stranded α/β-barrel with the $NADP^+$ bound in an extended conformation adjacent to an active site located at the C-terminal end of the barrel. Well defined electron density observed for the inhibitor molecule allowed unambiguous fitting of 3,5-dicholorosalicylic acid with its hydroxyl group pointing towards His222. The inhibitor-binding pocket is lined by side chains contributed by the eleven amino acid residues Tyr24, Leu54, Tyr55, Trp86, His117, His222, Glu224, Trp227, Leu306, Leu308 and Phe311. The inhibitor molecule is anchored from its carboxylate group that forms hydrogen bonds with the catalytic residues His117 (2.8 Å) and Tyr55 (3.1 Å), while the hydroxyl group is hydrogen bonded to His222 (2.8 Å) [41].

Analysis of the inhibitor binding site revealed four non-conserved residues (Leu54, His222, Leu306 and Leu308) in the human AKR1C isoforms that are present within van der Waals contacts (<4.0 Å) from the inhibitor, and thus should be considered when designing specific inhibitors of AKR1C1. The His222Ser mutation in AKR1C1 resulted in a reduction in the potency of

Table 2
Comparison of K_i values for 3,5-dichlorosalicylic acid among AKR1C isoforms and AKR1C1 mutants.

K_i value (nM)[a]							
AKR1C1					AKR1C2	AKR1C3	AKR1C4
Wild-type	Leu54Val	His222Ser	Leu306Ala	Leu308Ala			
5.9 ± 0.8	85 ± 7.6 (14)	58 ± 10 (10)	270 ± 29 (46)	2800 ± 300 (470)	70 ± 2 (12)	94,000 ± 1300 (16,000)	24,000 ± 2100 (4100)

[a] The ratios of AKR1C1 mutant forms (Leu54Val, His222Ser, Leu306Ala and Leu308Ala) and AKR1C isoforms (1C2–1C4) to wild-type AKR1C1 are given in parentheses.

3,5-dicholorosalicylic acid (Table 2). This residue is present at the N-terminal end of loop B that undergoes an induced fit during the binding of coenzyme [40] and the effect of the mutation suggests that the hydrogen bond plays a role in binding the inhibitor in the proper orientation. Additionally, the replacement of His222 in AKR1C1 with Gln in AKR1C3 and AKR1C4 is likely to maintain the hydrogen bonding interaction between this residue and the inhibitor. In the inhibitor binding site, AKR1C1 and AKR1C2 differ only by one amino acid residue, which is Leu54 in AKR1C1 and is Val54 in AKR1C2 (Fig. 1). While the effect of the Leu54Val mutation on the 3,5-dicholorosalicylic acid inhibition was small (Table 2), it is not surprising that this mutation made the inhibition of AKR1C1 more similar to that of AKR1C2. It is noteworthy that the importance of the bulky side chain of Leu54 in AKR1C1 in dictating the binding confirmation of the steroidal substrate in the active site was pointed out in a previous study [40].

The analysis of the crystal structure together with the results obtained from the mutagenesis (Table 2) indicated that the greater than 4000-fold difference in inhibitor potency between AKR1C1 and the two isoforms AKR1C3 and AKR1C4 is derived mainly from the non-conserved interactions between the inhibitor and residues from the C-terminal loop. Mutations of the non-conserved Leu306 (Phe in AKR1C3 and Val in AKR1C4) and Leu308 (Ser in AKR1C3 and Met in AKR1C4) to Ala residues in AKR1C1 resulted in significant increases in the K_i value for 3,5-dichlorosalicylic acid (Table 2). Of the four corresponding residues in AKR1C3 and AKR1C4 only Met308 of AKR1C4 is present within van der Waals contacts of the modelled inhibitor. It should be noted that the role of Leu residues from the C-terminal loop of the AKR enzymes AKR1B1 and aldehyde reductase (AKR1A1) in inhibitor binding and selectivity has been thoroughly investigated [49–51]. Leu300 in AKR1B1 (Pro in AKR1A1) accounts for the difference in inhibitor potency for the two enzymes and may undergo an induced fit upon inhibitor binding to AKR1B1. In the case of AKR1C1, there was no apparent induced fit upon the binding of 3,5-dichlorosalicylic acid to the enzyme compared to the ternary structure with bound 20α-hydroxyprogesterone [40].

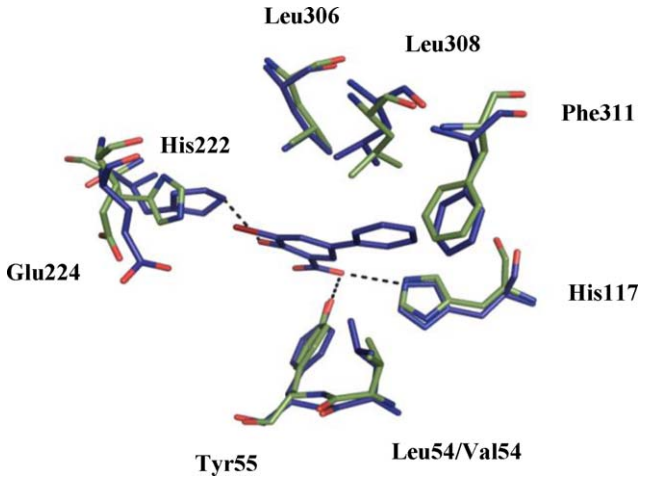
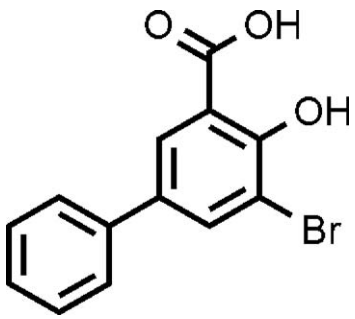


Fig. 1. The inhibitor 3-bromo-5-phenylsalicylic acid bound in the active site of AKR1C1. The inhibitor 3-bromo-5-phenylsalicylic acid was modelled in the active site of AKR1C1 (blue) and superimposed on AKR1C2 (green). The hydrogen bonds between the inhibitor and amino acid residues of AKR1C1 are shown as dashed lines.

3.2. Structure-based inhibitor design

Recently, our laboratory reported the first design, synthesis and evaluation of AKR1C1 inhibitors based on the published crystal structure of the AKR1C1 ternary complex [41,52]. While the enzyme-inhibitor interactions observed in the crystal structure remain conserved with the newly designed inhibitors, the additional phenyl group of the most potent compound, 3-bromo-5-phenylsalicylic acid (K_i = 4 nM), targets a hydrophobic pocket in the active site of AKR1C1 interacting with the nonconserved Leu54, resulting in improved specificity over AKR1C2 [52]. In addition, this compound potently inhibited the metabolism of progesterone by AKR1C1 in the cells, showing IC_{50} (concentration for 50% inhibition of this metabolism) value of 460 nM [52]. These results provide the frame work needed for the development of new inhibitors that are more specific to AKR1C1 for potential use in treatments against cancer and neurological disorders.

The crystal structure of AKR1C1 [41] together with a GRID [53] analysis of the inhibitor-binding site suggested that the replacement of the bromine atom at the 5-position of 3,5-dibromosalicylic acid with a phenyl (Scheme 1) is expected to enhance the inhibitor potency and selectivity for AKR1C1 over AKR1C2. Benzene rings and other aromatic systems are common moieties among compounds used as therapeutic agents, playing major roles ranging from providing steric bulk to forming an integral part of the pharmacophore [54]. The carboxyl and hydroxyl groups of this compound are hydrogen bonded to the side-chains of Tyr55, His117 and His222, and its phenyl moiety enters a hydrophobic selectivity pocket forming additional van der Waals interactions with residues Leu308, Phe311 and the non-conserved Leu54 (Val in AKR1C2). As previously observed in the structure of the ternary complex [41], the salicylic acid moiety is present within van der Waals contacts from Leu306. In addition, 3-phenyl-5-bromosalicylic acid that has the



Scheme 1. Chemical structure of the AKR1C1 inhibitor 3-bromo-5-phenylsalicylic acid. The potent inhibitor 3-bromo-5-phenylsalicylic acid (K_i = 4 nM) was designed based on the crystal structure of AKR1C1.

Table 3
Comparison of K_i values for 3,5-dibromosalicylic acid (DBSA), 3-bromo-5-phenylsalicylic acid (5-PBSA) and 3-phenyl-5-bromosalicylic acid (3-PBSA) among AKR1C isoforms.

Inhibitor	K_i value (nM)[a]			
	AKR1C1	AKR1C2	AKR1C3	AKR1C4
DBSA	9 ± 0.2	82 ± 2.3 (9)	23,000 ± 1100 (2600)	45,700 ± 5900 (5100)
5-PBSA	4 ± 0.4	87 ± 12 (21)	4200 ± 150 (893)	18,200 ± 2500 (3900)
3-PBSA	140 ± 17	1970 ± 13 (14)	21,000 ± 3400 (153)	No inhibition at 100 μM

[a] The ratios of AKR1C isoforms (1C2–1C4) to AKR1C1 are given in parentheses.

phenyl and bromo groups present at opposite positions was synthesized for comparison, and together with 3,5-dibromosalicylic acid the inhibitory potencies of the three compounds were measured against the four isoforms (AKR1C1 to AKR1C4). With the exception of 3-phenyl-5-bromosalicylic acid and AKR1C4, the three compounds inhibited the four isoforms competitively with respect to the substrate *S*-(+)-1,2,3,4-tetrahydro-1-naphthol, and their K_i values are shown in Table 3. 3-Phenyl-5-bromosalicylic acid showed the least potency for AKR1C1 and AKR1C2, likely due to short contacts and disruption of the hydrogen bonding interaction with His222. However, 3-bromo-5-phenylsalicylic acid showed improved potency and selectivity towards AKR1C1 compared to 3,5-dibromosalicylic acid due to the additional favorable interactions between the 5-phenyl ring and the residues lining the selectivity pocket (Fig. 1), and was a 21-fold more potent inhibitor of AKR1C1 than AKR1C2. Additionally, compared to 3,5-dichlorosalicylic acid [40], 3-bromo-5-phenylsalicylic acid displayed a 2.9-fold enhancement in the calculated binding energies of the AKR1C1-inhibitor complex (−114 kcal/mol versus −39 kcal/mol) and was a 2-fold more potent and selective inhibitor of AKR1C1 than AKR1C2 [52].

In summary, several classes of AKR1C1 inhibitors have been identified, including, benzodiazepines, benzofuranes, steroid carboxylates, flavones, and derivatives of pyrimidine, anthranilic acid and cyclopentane. The use of the recently determined crystal structure of AKR1C1 complexed with an inhibitor in conjunction with a GRID analysis of the inhibitor-binding site has allowed the design of a new salicylic acid-based inhibitor (3-bromo-5-phenylsalicylic acid) with improved potency (K_i = 4 nM) and selectivity (21-fold) over AKR1C2. Moreover, compound 3-bromo-5-phenylsalicylic acid significantly decreased the metabolism of progesterone in the cells with an IC_{50} value of 460 nM, which is comparable or superior to the IC_{50} values of the previously known two most potent inhibitors of AKR1C1, benzbromarone and 3′,3″,5′,5″-tetrabromophenolphthalein [13]. 3-Bromo-5-phenylsalicylic acid was designed to target a selectivity pocket in the active of AKR1C1 lined by the three apolar residues Leu54, Leu308 and Phe311. Leu308 is one of two nonconserved C-terminal residues (the other residue is Leu306) lining the binding site that are responsible for the greater than 4000-fold difference in salicylic acid-based inhibitor potency between AKR1C1 and the two isoforms AKR1C3 and AKR1C4 [41,52]. Since the active sites of AKR1C1 and AKR1C2 differ only by one amino acid residue, which is Leu54 in AKR1C1 and is Val54 in AKR1C2, and the current inhibitors show similar potency for the two enzymes, newly designed inhibitors that capture the maximum interactions with Leu54 in AKR1C1 are needed in order to improve their selectivity over AKR1C2. Thus, future developments of new derivatives of 3-bromo-5-phenylsalicylic acid are likely to improve on the selectivity of the currently known AKR1C1 inhibitors. Our results also illustrates that while large chemical database searches are useful in discovering new enzyme inhibitors, the use of the high-resolution crystal structure of an enzyme-inhibitor complex is an effective tool in optimizing the enzyme-inhibitor interaction by exploiting the small structural differences between the different enzyme isoforms.

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