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Type 5 17 β -Hydroxysteroid Dehydrogenase/Prostaglandin F Synthase (AKR1C3): Role In Breast Cancer and Inhibition by Nonsteroidal Antiinflammatory Drug Analogs

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Abstract

Aldo-keto reductase (AKR) 1C3 catalyzes the NADPH dependent reduction of Δ^4 -androstene-3,17-dione to yield testosterone, reduction of estrone to yield 17 β -estradiol and reduction of progesterone to yield 20 α -hydroxyprogesterone. In addition, it functions as a prostaglandin (PG) F synthase and reduces PGH₂ to PGF_{2 α} and PGD₂ to 11 β -PGF₂. Immunohistochemistry showed that AKR1C3 is over expressed in invasive ductal carcinoma of the breast. Retroviral expression of AKR1C3 in MCF-7 breast carcinoma cells shows that each of the assigned reactions occur in a breast cell microenvironment. Steroid and prostaglandin conversions were monitored by radiochromatography. Prostaglandin conversion was validated by a second method using HPLC coupled to APCI-MRM/MS. The combined effect of the AKR1C3 catalyzed 17- and 20-ketosteroid reductions will be to increase the 17 β -estradiol : progesterone ratio in the breast. In addition, formation of PGF₂ epimers would activate F prostanoid receptors and deprive PPAR γ of its putative anti-proliferative PGJ₂ ligands. Thus, AKR1C3 is a source of proliferative signals and a potential therapeutic target for hormone dependent and independent breast cancer. Two strategies for AKR1C3 inhibition based on non-steroidal anti-inflammatory drugs were developed. The first strategy uses the Ullmann coupling reaction to generate *N*-phenylanthranilate derivatives that inhibit AKR1C enzymes without affecting PGH₂ synthase (PGHS) 1 or PGHS-2. The second strategy exploits the selective inhibition of AKR1C3 by indomethacin, which did not inhibit highly related AKR1C1 or AKR1C2. Using known structure activity relationships for the inhibition of PGHS-1 and PGHS-2 by indole acetic acids we obtained *N*-(4-chlorobenzoyl)-melatonin as a specific AKR1C3 inhibitor ($K_I = 6.0 \mu\text{M}$) that does not inhibit PGHS-1, PGHS-2, AKR1C1, or AKR1C2. Both strategies are informed by crystal structures of ternary AKR1C3•NADP⁺•NSAID complexes. The identification of NSAID analogs as specific inhibitors of AKR1C3 will help validate its role in the proliferation of breast cancer cells.

Keywords

Aldo-keto reductase; steroid hormone metabolism; prostaglandin metabolism; indomethacin; *N*-phenylanthranilic acids

1. Introduction

In post-menopausal women, the proliferation of hormone dependent breast cancer cells is driven by the local production of estrogen [1,2]. Aromatase inhibitors have shown that

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inhibition of estrogen production is an effective strategy for the treatment and prevention of hormone dependent breast cancer. In addition to aromatase, the reductive 17β -hydroxysteroid dehydrogenases (17β -HSDs) are essential for the production of 17β -estradiol from Δ^4 -androstene-3,17-dione (androstenedione, Scheme 1A)[1,2]. For instance, reduction of androstenedione by a 17β -HSD will yield testosterone, which can then be aromatized to 17β -estradiol (the most potent endogenous estrogen). The primary 17β -HSD involved in this reaction in peripheral tissues is believed to be aldo-keto reductase (AKR) 1C3 (type 5 17β -HSD)[3,4]. If aromatase converts androstenedione to estrone, this is then reduced to 17β -estradiol. The reductive 17β -HSDs generally associated with this reaction are types 1, 7, and possibly 12, but there is evidence that AKR1C3 can do this reaction as well [4]. Given the success of aromatase inhibitors against breast cancer and the contribution of the reductive 17β -HSDs to 17β -estradiol formation, there is considerable interest in developing inhibitors of these enzymes.

AKR1C3 performs several reactions that may lead to the development of breast cancer. Homogenous recombinant AKR1C3 catalyzes the reduction of androstenedione to testosterone, progesterone to 20α -hydroxyprogesterone and, to a lesser extent, estrone to 17β -estradiol [3]. These reactions have also been observed when AKR1C3 was transfected into human embryonic kidney (HEK-293) cells [4,5]. The combined effect of these activities in breast cancer cells would be to increase the 17β -estradiol to progesterone ratio, and hence increase estrogen receptor (ER) α and decrease progesterone receptor (PR) signaling, but this remains to be tested. While the increase in estrogenic signaling will clearly contribute to the development of breast cancer [1,2], the relationship between progesterone signaling and breast cancer is not as well understood. Genes encoding aromatase, her-2/neu, prostaglandin H_2 synthase (PGHS) 2 and matrix metalloproteinases are suppressed by PR activation, so progesterone inactivation by AKR1C3 could further contribute to the development of breast cancer *in vivo* [6,7].

AKR1C3 is also involved in the reduction of prostaglandins, which could generate hormone-independent proliferative signals (Scheme 1B). Purified recombinant AKR1C3 stereospecifically and efficiently converts prostaglandin (PG) H_2 to $PGF_{2\alpha}$ and PGD_2 to $9\alpha, 11\beta$ - PGF_2 [8,9]. Of the known endogenous substrates, AKR1C3 exhibits the highest catalytic efficiency towards the prostaglandins, particularly PGD_2 . The PGF_2 isomers bind to the F prostanoid receptor and induce MAPK signaling cascades that lead to cell proliferation [10]. In addition, by removing PGD_2 , AKR1C3 prevents its spontaneous dehydration and rearrangement to form the anti-proliferative and anti-inflammatory J_2 series prostaglandins, including 15-deoxy- $\Delta^{12,14}$ - PGJ_2 (15dPGJ₂). 15dPGJ₂ covalently reacts with a cysteine residue in the ligand-binding domain of PPAR γ , resulting in its activation [11]. It also reacts with residues in the DNA-binding domains of NF κ B and ER α , preventing them from binding to DNA [12,13]. The resulting increase in PPAR γ -dependent and decrease in NF κ B-dependent and ER α -dependent gene transcription is predicted to inhibit the proliferation of breast cancer cells.

We have been exploring the role of AKR1C3 in breast cancer and developing non-steroidal anti-inflammatory drug (NSAID) analogues as selective inhibitors of AKR1C3. We will describe work from our lab and others showing that AKR1C3 is expressed in breast cancer. It will also describe our recent work using an AKR1C3 over-expressing MCF-7 hormone dependent breast cancer cell line to examine the roles of AKR1C3 in steroid hormone and prostaglandin signaling [14]. Finally, we will discuss our work developing NSAID analogues as isoform specific inhibitors of AKR1C3 [15,16].

2. AKR1C3 is expressed in human breast cancer

Using semi-quantitative RT-PCR, we have shown that the normal breast expresses very high levels of AKR1C3 relative to other tissues [3]. Using immunohistochemistry with an isoform specific antibody, we observed that AKR1C3 expression is even higher in the tumor of a patient with ER and PR positive invasive ductal carcinoma, as compared to surrounding normal tissue [17]. Work from Sasano's group has shown an 18-fold increase in the median AKR1C3 mRNA levels in breast cancer patients as compared to those without [18]. They have also detected AKR1C3 with immunohistochemistry in 53% of breast carcinomas [2]. Another RT-PCR analysis of 669 breast cancer cases by Oduwole et al found significantly higher expression of AKR1C3 in breast tumors than in normal tissue [19]. They also found that patients with the highest levels of AKR1C3 expression had a worse overall prognosis. Jansson et al observed that patients with ER⁺ tumors that overexpress AKR1C3 had a higher rate of late recurrence [20]. These results provide evidence for an association between AKR1C3 expression and breast cancer, which raises the question of how AKR1C3 might alter breast cancer cell signaling and proliferation.

3. AKR1C3 catalyzes steroid hormone reduction reactions in MCF-7 cells

In order to explore the potential of AKR1C3 to contribute to proliferative signaling in breast cancer, we developed an MCF-7 cell line that over-expresses AKR1C3 using a pLNCX retroviral vector (MCF-7-AKR1C3 cells). Using this cell line, we explored the effects of AKR1C3 expression on the metabolism of radiolabeled steroid hormones (Table 1)[14]. Parental cells primarily metabolized [¹⁴C]-androstenedione to 5 α -androstenedione and almost no 17 β -HSD activity was observed. The MCF-7-AKR1C3 cells exhibited much higher 17 β -HSD activity and converted over 20% and 10% of 0.1 and 5 μ M [¹⁴C]-androstenedione into testosterone, respectively, after 24 h. 5 α -DHT was also formed as a minor metabolite.

The parental MCF-7 cells metabolized [¹⁴C]-progesterone primarily to non-polar products, possibly glucuronides, and low levels of 5 α - and 20 α -reduced products were also observed. The MCF-7-AKR1C3 cells exhibited significantly more 20 α -HSD activity. 20 α -Hydroxyprogesterone and 20 α -hydroxy-5 α -dihydroprogesterone accounted for 27% and 7% of the total radioactivity, respectively, when 0.1 μ M [¹⁴C]-progesterone was incubated for 6 h. The MCF-7-AKR1C3 cells also exhibited very high levels of 20 α -HSD activity with 5 μ M [¹⁴C]-progesterone as substrate, where 20 α -hydroxyprogesterone accounted for 50% of the radioactivity by 24 h.

Surprisingly, at 0.1 μ M, [¹⁴C]-estrone was the best of the substrates assayed. The parental MCF-7 cells exhibited high endogenous 17 β -HSD activity towards estrone: 17 β -estradiol accounted for 17% of the radioactivity by 6 h with no other products formed. When MCF-7-AKR1C3 cells were incubated with 0.1 μ M [¹⁴C]-estrone, 17 β -estradiol was formed much faster, accounting for over 80% of the radioactivity by 6 h. However, AKR1C3 expression had almost no effect on the metabolism of 5 μ M [¹⁴C]-estrone. These results suggest that AKR1C3 acts as a high affinity low capacity enzyme towards estrone *in vivo*.

We examined whether the increased conversion of estrone to 17 β -estradiol in MCF-7-AKR1C3 cells would confer a proliferative advantage (Table 2)[14]. Parental cells have a modest proliferative response to picomolar concentrations of both estrone and 17 β -estradiol. Expression of AKR1C3 significantly increases the responsiveness of these cells to both estrogens. The similar response of parental cells to estrone and 17 β -estradiol suggests that a rapid equilibrium is established, in which picomolar concentrations of estrogens are inter-converted by endogenous reductive and oxidative 17 β -HSDs. The expression of AKR1C3

provides an additional reductive 17 β -HSD that shifts the equilibrium in favor of 17 β -estradiol, increasing the proliferative response of these cells.

4. AKR1C3 catalyzes PGD₂ reduction in MCF-7 cells

We used the MCF-7-AKR1C3 cells to examine the ability of AKR1C3 to convert [³H]-PGD₂ to 9 α ,11 β -PGF₂ [14]. In cell-free media, [³H]-PGD₂ disappeared, accompanied by the formation of PGJ₂ and 15dPGJ₂. In the presence of MCF-7 cells, [³H]-PGD₂ disappeared at a similar rate, but the levels of PGJ₂ and 15dPGJ₂ observed were much lower, with much of the radioactivity remaining in the aqueous fraction. This observation is consistent with Michael addition to the reactive cyclopentenone ring of the J prostaglandins by glutathione and other hydrophilic cellular nucleophiles. When MCF-7-AKR1C3 cells were used, the rate of [³H]-PGD₂ disappearance was slightly higher, accompanied by the formation of a PGF₂ product. At 24 h, this PGF₂ product accounted for 35% of the radioactivity in the media of the MCF-7-AKR1C3 cells treated with 0.1 μ M [³H]-PGD₂. Because the TLC separation used for the radiometric assay did not conclusively distinguish 9 α ,11 β -PGF₂ from PGF_{2 α} , we also examined metabolism of unlabeled PGD₂. In this approach, we used a method that employed pentafluorobenzyl derivatization of the prostaglandins, separation with chiral normal phase HPLC and analysis with electron capture APCI-MRM/MS [14,21]. Using this method, we confirmed that the reduction of PGD₂ in MCF-7-AKR1C3 cells was stereospecific, forming only 9 α ,11 β -PGF₂. Prostaglandin levels were quantified by reference to deuterated prostaglandin internal standards and were consistent with the levels observed radiometrically.

5. Development of NSAID analogues as selective AKR1C3 inhibitors

5.1 NSAID screening

Given its potential role in breast cancer, AKR1C3 represents a promising therapeutic target. Specific inhibitors of AKR1C3 would also provide useful tools to explore its contribution to intercellular signaling pathways. We have focused our efforts on developing NSAID analogues as inhibitors of AKR1C3, based on our earlier observation that NSAIDs are potent inhibitors of rat 3 α -HSD (AKR1C9)[22]. The presence of crystal structures of AKR1C3 bound to flufenamic acid and indomethacin allows for rational design of inhibitors based on these two structures [23]. Inhibitors of AKR1C3 should not inhibit the related peripheral AKR1C1 and AKR1C2 isoforms or the PGHS enzymes if they are to dissect the roles of AKR1C3 in steroid hormone and prostaglandin signaling. AKR1C1 is primarily a 20-ketosteroid reductase and catalyzes the reduction of progesterone at the 20 position, while AKR1C2 is primarily a 3-ketosteroid reductase and reduces 5 α -dihydrotestosterone at the 3 position [3].

We examined a series of NSAIDs for their inhibition of the reduction of 9,10-phenanthrenequinone by AKR1C1, 1C2, and 1C3 (Table 3)[16]. We observed inhibition of the AKR1C enzymes at pharmacologically relevant concentrations with all of the NSAIDs used. These results raise the possibility that AKR1C inhibition might play a role in the pharmacological properties of these drugs. It is interesting to observe that salicylic acid, which rapidly forms from the deacetylation of aspirin *in vivo*, inhibits AKR1C1 and AKR1C2 at concentrations that are two orders of magnitude lower than those required to inhibit PGHS [24]. The inhibition of AKR1C1 by salicylic acid and aspirin has been recently reported by Dhagat *et al*, but their inhibition of AKR1C2 and AKR1C3 had not been reported [25].

There was surprising variability between AKR1C isoforms in their inhibition by different NSAIDs. Meclofenamic acid was a potent inhibitor of all three AKR1C isoforms [16].

Salicylates were potent inhibitors of AKR1C1 and AKR1C2, but only weakly inhibited AKR1C3 (unpublished data). Arylpropionic acids exhibited a strong preference for inhibition of AKR1C2 over AKR1C1, with an intermediate level of AKR1C3 inhibition [16]. Indomethacin inhibited AKR1C3 much more potently than AKR1C1 and AKR1C2. These observations suggested routes to AKR1C3 selective inhibitors.

5.2 *N*-Phenylanthranilic acids

We initially focused our efforts on the *N*-phenylanthranilic acid family of NSAIDs [15]. These compounds are among the most potent inhibitors of AKR1Cs and can be simply synthesized using the Ullman coupling reaction, in which any halogenated benzoic acid can be coupled to any aniline in the presence of Cu^{2+} . In addition, there are well-established structure-function relationships for PGHS inhibition, which indicate minor structural changes that would abolish their PGHS inhibition (Scheme 2A). These changes include A-ring substitution and movement of the carboxylic acid from the *ortho* position. We observed that mefenamic acid is a potent inhibitor of AKR1C3 and its two closely related family members, AKR1C1 and AKR1C2 (Table 2). The inhibition of the AKR1C enzymes occurred at much lower concentrations than those required to inhibit their putative PGHS targets. We then screened a series of *N*-phenylanthranilic acid derivatives with A-ring substitution and/or movement of the carboxylic acid to the *para* position for their inhibition of the AKR1C and PGHS enzymes. It was found that all of the compounds tested were potent competitive inhibitors of the three AKR1C isoforms ($K_i < 10 \mu\text{M}$) with very little or no inhibition of PGHS-1 and PGHS-2 ($\text{IC}_{50} > 200 \mu\text{M}$, Table 4). Two of the compounds we tested, 4-carboxy-2',4'-dinitrodiphenylamine and 4-benzoyl-benzoic acid exhibited a slight preference for inhibition of AKR1C3 over the other two isoforms, but none of the compounds provided enough selectivity between AKR1C isoforms to serve as an isoform-specific molecular probe.

Clues to the development of an AKR1C3 selective inhibitor based on *N*-phenylanthranilic acids are revealed by the comparison of the crystal structures of AKR1C2-NADP⁺-ursodeoxycholate (PDB 1IHI) and AKR1C3-NADP⁺-flufenamic acid (PDB 1S2C, Scheme 2A)[23]. When the two active sites of the ternary complexes are overlaid, it is apparent that ursodeoxycholate and flufenamic acid bind differently. The A-ring of flufenamic acid can tolerate bulky substituents because these would project into the steroid binding cavity of both AKR1C enzymes. Thus substituents to this ring are unlikely to provide AKR1C isoform specific inhibitors. By comparison, the B-ring of flufenamic acid projects into a unique sub-pocket present in AKR1C isoforms that is smaller and more hydrophobic for AKR1C2 than for AKR1C3 due to substitution of Phe 319, Phe 118, and Leu 308 in AKR1C2 with Tyr 319, Ser 118, and Ser 308 in AKR1C3. Polar substituents to the B-ring of *N*-phenylanthranilic acids and similar structures would occupy this subpocket and likely lead to isoform specific inhibitors.

5.3 Indole acetic acids

We then focused our attention on indomethacin, as this compound provided the best selectivity for AKR1C3 inhibition of the NSAIDs examined [16]. We used structure-activity relationships to guide the development of an AKR1C specific inhibitor based on indomethacin (Scheme 2B). The inhibition of the PGHS enzymes by indomethacin is largely dependent on the methyl group at the 2 position, which fits tightly into a small sub-pocket found in both PGHS isoforms, resulting in functionally irreversible inhibition [26]. Without this interaction, 2-desmethyl-indomethacin can be easily competed out of the active site of the PGHS enzymes by arachidonic acid. In addition, substituents to the acetic acid group limit PGHS-1 inhibition and can reduce the inhibition of PGHS-2 [27-29]. The crystal structure of the AKR1C3-NADP⁺-indomethacin complex (PDB 1S2A)[23], provides

structure-activity relationships for the inhibition of the AKR1C enzymes. The acetic acid group of indomethacin extends into the same sub-pocket of AKR1C3 that is occupied by the B-ring of flufenamic acid, suggesting that the addition of substituents to this region would further increase the selectivity for AKR1C3 over AKR1C2. The 2-methyl group is in an open region of the active site and does not interact with any residues, suggesting that its removal or modification would not effect AKR1C inhibition.

One compound that was predicted to retain its inhibition of AKR1C3 but not to inhibit either the PGHS enzymes or the other AKR1C isoforms was *N*-(4-chlorobenzoyl)-melatonin (CBM). This compound lacks the 2-methyl group that is critical for PGHS inhibition, and also has a reverse methyl amide in place of the carboxylic acid of indomethacin (Figure 1A). The synthesis of this compound and inhibition of PGHS-1 and PGHS-2 had been described by Kalgutkar et al, who observed that it did not inhibit either PGHS isoform [29]. We screened CBM for inhibition of the AKR1C enzymes and found that it was a potent and selective inhibitor of AKR1C3 [16]. It inhibited the reduction of 9,10-phenanthrenequinone by AKR1C3 with an uncompetitive pattern of inhibition and a K_i of 3.4 μ M, while it inhibited AKR1C1 and AKR1C2 by less than 10% at the highest concentration tested (60 μ M, Figure 1B). CBM inhibited the reduction of androstenedione by AKR1C3 with a competitive pattern of inhibition and a K_i of 6.0 μ M (Figure 1C). The differences in the pattern of inhibition of AKR1C3 by CBM with 9,10-phenanthrenequinone (turned over with high catalytic efficiency) and androstenedione (turned over with low catalytic efficiency) suggests that CBM binds differently during the inhibition of the reduction of these two substrates. The most likely explanation is that for androstenedione, CBM binds E·NADPH and blocks the binding of this substrate, but for 9,10-phenanthrenequinone it binds to E·NADP⁺ product complex and prevents the release of cofactor. It therefore appears that CBM is a selective inhibitor of AKR1C3 that could be useful to further investigate its roles in the development of breast cancer.

Summary

AKR1C3 likely plays important roles in the development of hormone-dependent, and possibly hormone-independent, breast cancer. It is highly expressed in normal breast and upregulated in breast cancer, where its expression is associated with a worse prognosis [3,17,19]. Its expression in MCF-7 cells leads to increased synthesis of 17 β -estradiol and 9 α , 11 β -PGF₂ and elimination of progesterone [14]. Consistent with a role in the development of hormone-dependent breast cancer, the increased synthesis of 17 β -estradiol due to AKR1C3 expression confers a proliferative advantage to these cells.

Given that AKR1C3 may contribute to breast cancer cell proliferation, it represents a potential target for the treatment of breast cancer. Interestingly, AKR1C3 is potently inhibited by NSAIDs, which are protective against breast cancer [16,30]. While these compounds have traditionally been thought to work through inhibition of PGHS-2 activity, they also exhibit anti-cancer effects independent of PGHS [31]. AKR1C3 represents a potential alternative target for the anti-cancer effects of these drugs. A specific inhibitor of AKR1C3 might retain the anti-cancer effects of NSAIDs, without the gastro-intestinal toxicity and cardiovascular side-effects that limit their use.

By examining the inhibition of AKR1Cs by different classes of NSAIDs and NSAID analogues, we have made considerable progress in understanding how these compounds might lead to isoform specific AKR1C inhibitors. *N*-Phenylanthranilic acids are potent, but non-selective, inhibitors of all of the AKR1Cs. We continue to develop derivatives of these NSAIDs that will be selective for AKR1C3. Salicylates are excellent inhibitors of AKR1C1 and AKR1C2, and may provide a starting point for the development of a selective inhibitor

of AKR1C1. Indomethacin inhibits AKR1C3 at much lower concentrations than those required for inhibition of AKR1C1 and AKR1C2. One indomethacin analogue, CBM, also does not inhibit the PGHS enzymes and provides a molecular probe for examining the contribution of AKR1C3 to breast cancer signaling and proliferation.

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Abbreviations

AKR	aldo-keto reductase
PG	prostaglandin
APCI-MRM/MS	atmospheric chemical ionization-multiple reaction monitoring/mass spectrometry
PGHS	PGH ₂ synthase
17β-HSD	17 β -hydroxysteroid dehydrogenase
ER	estrogen receptor
PR	progesterone receptor
15dPGJ₂	15-deoxy- $\Delta^{12,14}$ -PGJ ₂
NSAID	non-steroidal anti-inflammatory drug (NSAID)
CBM	<i>N</i> -(4-chlorobenzoyl)-melatonin

Figure 1.

N-Chlorobenzoyl melatonin (CBM) exhibits uncompetitive inhibition patterns for the reduction of 9,10-phenanthrenequinone by AKR1C3 but competitive inhibition patterns for the reduction of androstenedione by AKR1C3 [16]. (A) Chemical structure of CBM. (B-C) Lineweaver–Burke plots demonstrating the pattern of inhibition by CBM for the reduction of (B) 9,10-phenanthrenequinone (PQ) or (C) androstenedione catalyzed by AKR1C3 [16].

A

Predicted Effect on Inhibition

Modification	AKR1C1 & AKR1C2	AKR1C3	COX-1	COX-2
A-Ring Substitution	No Change	No Change	Loss of Inhibition	Loss of Inhibition
B-Ring Substitution	Reduced Inhibition	Improved Inhibition	Improved Inhibition	Improved Inhibition
Movement of COOH	No Change	No Change	Loss of Inhibition	Loss of Inhibition
Substitution to Amine	No Change	No Change	Loss of Inhibition	Loss of Inhibition
Replacement of Heteroatom	No Change	No Change	Loss of Inhibition	Loss of Inhibition

B

Predicted Effect on Inhibition

Modification	AKR1C1 & AKR1C2	AKR1C3	COX-1	COX-2
Modification of Carboxylic Acid	Loss of Inhibition	No Change	Loss of Inhibition	Variable
Removal or Modification of 2-Methyl Group	No Change	No Change	Loss of Time-Dependent Inhibition	Loss of Time-Dependent Inhibition
<i>p</i> -Chlorobenzoyl Substitution	Loss of Inhibition	No Change	Loss of Inhibition	Loss of Inhibition
Removal of Bridge Carbonyl	Improved Inhibition	No Change	No Change	No Change

Scheme 1.

(A) Routes to 17 β -estradiol in the breast. The other reductive 17 β -HSDs include types 1, 7, & possibly 12; the oxidative isoforms include types 2 and 4. (B) Proposed role for AKR1C3 in prostaglandin signaling.

Scheme 2.

Predicted structure activity relationships of (A) *N*-phenylanthranilic acid derivatives and (B) indomethacin derivatives.

Table 1
Effect of AKR1C3 expression on the metabolism of steroid hormones and prostaglandins by MCF-7 cells

Substrate	Concentration (μ M)	% of Compound Metabolized by Cells at 6 h*	
		Parental MCF-7	MCF-7-AKR1C3
$[^{14}\text{C}]$ -Androstenedione	0.1	11 (1.9)	21 (7.5)
	5	4.6 (1.1)	6.9 (2.8)
$[^{14}\text{C}]$ -Progesterone	0.1	19 (1.2)	49 (27)
	5	5.4 (1.0)	19 (13)
$[^{14}\text{C}]$ -Estrone	0.1	17 (17)	82 (82)
	5	3.2 (3.2)	5.2 (5.2)
$[^3\text{H}]$ -PGD ₂	0.1	25 (0)	37 (14)
	5	21 (0)	35 (7.5)

* Values in parentheses represent the percentage of the radioactivity associated with the primary product formed by AKR1C3 (testosterone from androstenedione; 20 α -OH-progesterone from progesterone; 17 β -estradiol from estrone; 9 α ,11 β -PGF₂ from PGD₂).

Table 2
Proliferation of MCF-7 Cells in Response to Estrogens

Compound	Concentration	Cell Number (% of DMSO Treated Control*)	
		Parental MCF-7	MCF-7-AKR1C3
Estrone	1 pM	102 ± 3	112 ± 8
	10 pM	117 ± 12	172 ± 27
	33 pM	131 ± 4	204 ± 14
17β-Estradiol	1 pM	112 ± 4	167 ± 14
	10 pM	134 ± 4	213 ± 27
	33 pM	138 ± 5	218 ± 12

* Values expressed as means ± S.D. of three experiments performed in triplicate.

Table 3
Inhibition of the AKRIC catalyzed reduction of 9,10-phenanthrenequinone by NSAIDs [16]

Compound	IC ₅₀ values (μM)			Preference for AKRIC3 vs. AKRIC2		Preference for AKRIC3 vs. AKRIC1
	AKRIC1	AKRIC2	AKRIC3			
Indomethacin	96	50	2.3	22		42
Sulindac	10	6.6	3.4	1.9		2.9
Meclofenamic Acid	2.9	1.7	0.7	2.4		4.1
Zomepirac	>50	23	40	0.6		>1.25
Ibuprofen	29	1.9	9.9	0.19		2.9
Naproxen	280	1.2	1.4	0.86		200
Flurbiprofen	51	3.2	7.8	0.41		6.5
Aspirin	7.1	16	1200	0.013		0.0059
Salicylic Acid	2.7	7.4	770	0.0096		0.0035

Table 4
Inhibition of the AKRIC and PGHS enzymes by *N*-phenylanthranilic acid derivatives [32]

Inhibitor	IC ₅₀ values (μM)				
	AKRIC1	AKRIC2	AKRIC3	COX-1	COX-2
Mefenamic Acid	4.0±0.5 $K_I = 0.81$	0.96±0.1 $K_I = 0.22$	0.39±0.09 $K_I = 0.30^*$	33 $K_I = 3.90$	225
5-Methyl- <i>N</i> -phenylanthranilic Acid	3.2±0.1 $K_I = 0.88$	1.2±0.1 $K_I = 0.53$	11±0.4 $K_I = 7.3$	430	>> 1,000
4-Carboxy-2',4'-dinitrodiphenylamine	7.1±0.8 $K_I = 2.7$	3.7±0.2 $K_I = 1.3$	0.40±0.06 $K_I = 0.38$	>> 500	ND**
4-Chloro- <i>N</i> -phenylanthranilic Acid	8.2±0.9 $K_I = 3.10$	1.2±0.1 $K_I = 0.41$	3.0±0.2 $K_I = 2.9$	> 1,000	>> 1,000
4-Nitro- <i>N</i> -phenylanthranilic Acid	9.2±0.5 $K_I = 3.5$	2.1±0.4 $K_I = 0.75$	2.0±0.3 $K_I = 1.9$	>> 250	ND
4-Carboxy-2-aminodiphenylamine	49±8.9 $K_I = 19$	26±3.2 $K_I = 9.4$	11±1.5 $K_I = 10$	~ 1,000	>> 1,000
4-Chloro- <i>N</i> -(4-tolyl)-anthranilic Acid	8.3±0.5 $K_I = 3.1$	0.56±0.17 $K_I = 0.20$	1.5±0.4 $K_I = 1.4$	240	>> 1,000
4-Benzoyl-benzoic Acid	33±4.4 $K_I = 12$	37±2.5 $K_I = 13$	2.0±0.2 $K_I = 1.9$	>> 1,000	>> 1,000

* K_I values for AKRIC3 were computed assuming a K_M of 2 mM for 1-acenaphthenol.

** N.D., not determined.