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Review

Targeting cytochrome P450 enzymes: A new approach in anti-cancer drug development

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Abstract—Cytochrome P450s (CYPs) represent a large class of heme-containing enzymes that catalyze the metabolism of multitudes of substrates both endogenous and exogenous. Until recently, however, CYPs have been largely overlooked in cancer drug development, acknowledged only for their role in phase I metabolism of chemotherapeutics. The first successful strategy targeting CYP enzymes in cancer therapy was the development of potent inhibitors of CYP19 (aromatase) for the treatment of breast cancer. Aromatase inhibitors ushered in a new era in hormone ablation therapy for estrogen dependent cancers, and have paved the way for similar strategies (i.e., inhibition of CYP17) that combat androgen dependent prostate cancer. Identification of CYPs involved in the inactivation of anti-cancer metabolites of vitamin D_3 and vitamin A has triggered development of agents that target these enzymes as well. The discovery of the over-expression of exogenous metabolizing CYPs, such as CYP1B1, in cancer cells has roused interest in the development of inhibitors for chemoprevention and of prodrugs designed to be activated by CYPs only in cancer cells. Finally, the expression of CYPs within tumors has been utilized in the development of bioreductive molecules that are activated by CYPs only under hypoxic conditions. This review offers the first comprehensive analysis of strategies in drug development that either inhibit or exploit CYP enzymes for the treatment of cancer.
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Contents

1. Introduction

Cyotochrome P450s (CYPs) are a large ubiquitous family of proteins containing a single iron protoporphyrin IX prosthetic heme group. The majority of CYPs (designated Class I and II) act as versatile monooxygenases. These enzymes catalyze a multitude of reactions, including the hydroxylation of alkanes to alcohols, conversion of alkenes to epoxides, arenes to phenols, sulfides to sulfoxides and sulfones, and the oxidative split of C–N, C–O, C–C or C–S bonds.

Functionally, CYPs can be classified into two groups: those with specific roles in the metabolism of endogenous molecules such as hormones, and those that non-specifically process exogenous molecules (drugs, chemicals, natural products, etc.). Both classes of CYPs offer potential targets in chemotherapeutic and chemopreventative strategies.

Most CYPs were once considered liver specific enzymes, but now their extrahepatic expression has been well established. It has long been known that CYP17 and CYP19—the enzymes responsible for the production of androgens and estrogens, respectively—are expressed in the testes, ovaries, and adrenals. However, CYP19 has subsequently been found expressed locally in the adipose tissue of the breast, and indirect evidence suggests CYP17 may be expressed in adipose tissue as well.[1,2] It is also now well established that expression of CYPs responsible for the metabolism of anti-cancer metabolites of vitamin A (all-*trans*-retinoic acid; ATRA) and vitamin D (1α,25-dihydroxyvitamin D_3; 1,25-D_3) are induced by their substrates in target cells (including cancer).[3–11] Members of CYP families 1, 2, and 3 have also been identified in both healthy and cancerous extrahepatic tissues.[12–19] The enzymes in these families are involved in the metabolism of xenobiotic substances such as carcinogens, pro-carcinogens, and chemotherapeutics.[20–23] Of note, CYP1B1 and, more recently, CYP2W1 have been identified as having tumor-specific expression.[15,17,18,24,25]

These observations have led to a greater appreciation for the role of CYPs in tumor formation and development. Targeting of these enzymes with natural or synthetic small molecules offers potential benefits in cancer prevention and therapy. Because crystal structures for nearly all CYPs are yet to be determined, drug design strategies rely on the knowledge of substrate structure and the enzyme's mechanism of action. Strategies to target these enzymes include: (i) designing molecules that inhibit the enzymes; (ii) designing prodrugs that are activated by the enzymes; (iii) immuno-based therapies that target immune responses toward the enzymes; (iv) genetic therapy strategies to express specific CYPs in cancer cells.[26] This review will focus on the small molecule based approaches (i and ii) being employed to target CYPs involved in hormone, vitamin, and xenobiotic metabolism for the treatment and prevention of cancer (Fig. 1).

2. CYPs and hormone dependent cancer

2.1. Aromatase (CYP19)

The development of aromatase inhibitors for the treatment of breast cancer (BCa) represents the paradigm of success of CYP inhibition in cancer therapy. For years, the standard pharmacological treatment for hormone dependent BCa in post-menopausal women was blocking estrogen (E) binding to the estrogen receptor (ER) with the anti-estrogen tamoxifen. ER is a nuclear hormone receptor that is normally activated by E to recruit co-activators and induce transcription of target genes. By blocking E binding to the ER, tamoxifen prevents E-induced proliferation. Unfortunately, tamoxifen, although an important advance in BCa therapy, has many drawbacks. First, it is a partial ER agonist in many tissue types,[27] which has been correlated with a threefold increase in the incidences of endometrial cancer in patients receiving the drug.[28] Furthermore, resistance to tamoxifen therapy inevitably results.[27] An alternative approach to tamoxifen treatment is inhibition of estrogen synthesis. The target for such therapy is the enzyme aromatase, which catalyzes the rate limiting step in the conversion (aromatization) of androgens to estrogens (Fig. 2). Aromatase is expressed in many tissues throughout the body, including adipose and muscle, which are the main sites of estrogen synthesis in post-menopausal woman.[1] Therefore, surgery to remove endocrine glands is ineffective, and inhibition of estrogen production requires a systemic pharmacological approach.

The first successful aromatase inhibitor, *4-hydroxyandrostenedione* (4-OHA, *Formestane*), was demonstrated by Harry and Angela Brodie and colleagues to have efficacy against breast cancer tumors in 1977.[29] Since then, several selective inhibitors of aromatase have been developed (Fig. 3). These include fellow steroidal inhibitor *6-methylenandrosta-1,4-diene-3,17-dione* (*exemestane*) as well as two non-steroidal triazoles, *letrozole* (*femara*) and *anastrozole* (*arimidex*). All four of these inhibitors are approved for the treatment of BCa, and have been shown in clinical trials to be more effective as a first line therapy than tamoxifen for post-menopausal women with hormone-sensitive BCa.[30] A great deal of work is still being done in this field to optimize the efficacy of aromatase inhibitors both alone and in combination with other treatments, but these studies are beyond the scope of discussion here. Despite the obstacles that lay ahead, it seems clear that aromatase inhibitors represent the first successful class of cancer therapeutics specifically designed to target a CYP enzyme.

2.2. 17α-Hydroxylase,C17,20-lyase (CYP17)

The clinical success of aromatase inhibitors raises the question of whether a similar strategy could be employed for the treatment of androgen dependent cancers such as prostate cancer (PCa). In 1941, Charles Huggins and colleagues first demonstrated the benefits of hormone deprivation therapy in prostate cancer.[31,32] To this
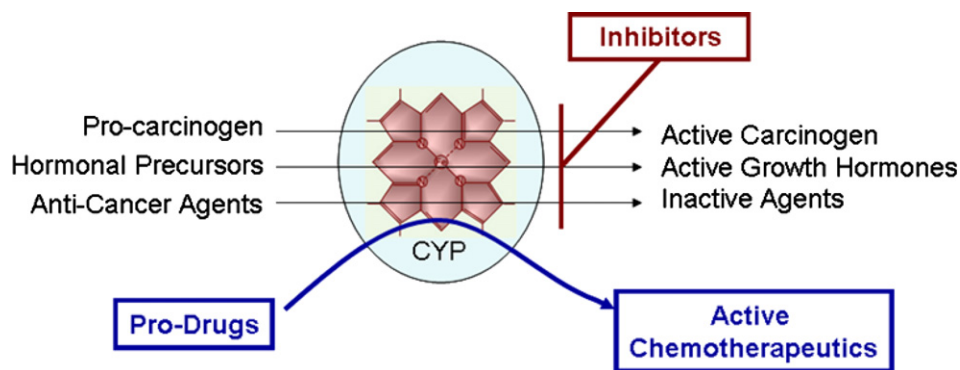
Figure 1. Potential strategies targeting CYPs for cancer therapy and prevention.
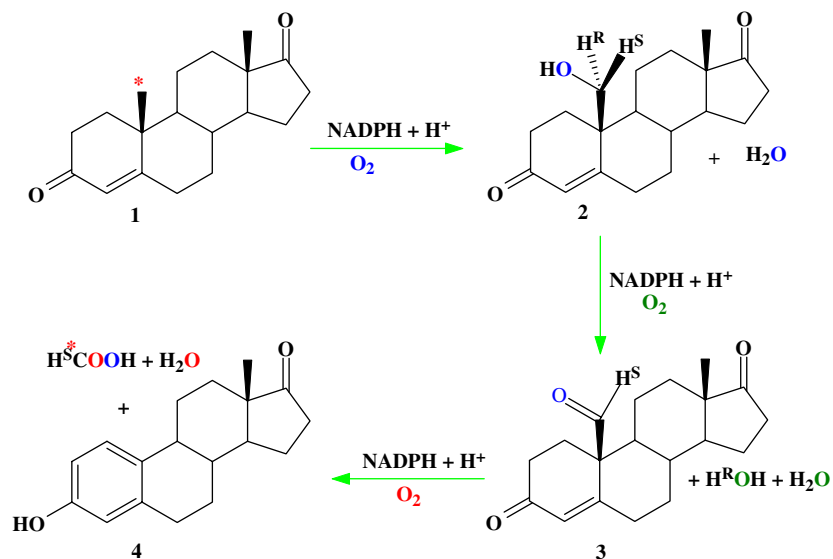
Figure 2. Biosynthesis of estrogens from androgens catalyzed by CYP19 (aromatase).

4-hydroxyandrostinedione

(Formestane)

6-Methylandrosta-1,4-diene-3,17-dione

(Exemestane)

Letrazole

(Femara)
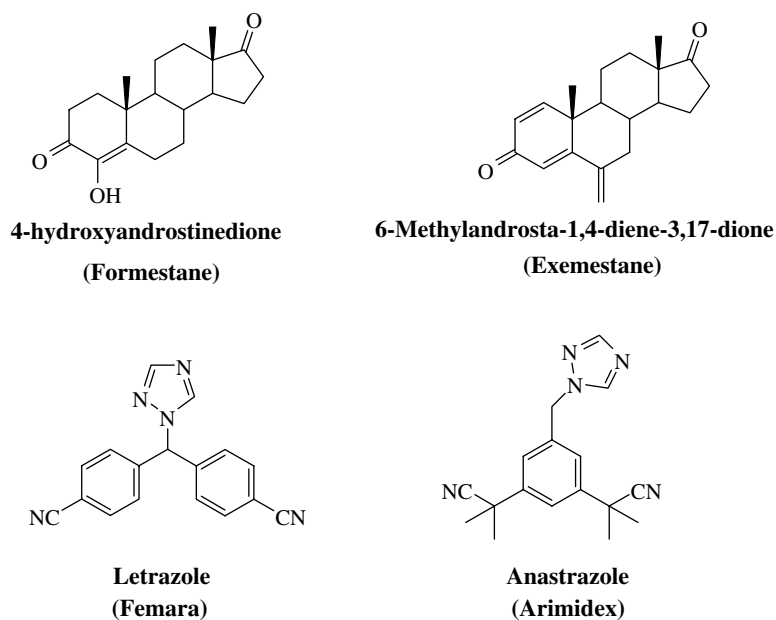
Anastrazole

(Arimidex)

Figure 3. Chemical structures of aromatase inhibitors.

day, androgen ablation remains the standard treatment for advanced PCa.

At the molecular level, androgens, mainly testosterone (T) and dihydrotestosterone (DHT), bind to the androgen receptor (AR) in target cells and initiate transcription of genes involved in cell proliferation and survival.[33,34] Generally, androgen withdrawal therapy is carried out via treatment with LHRH or GnRH agonists and anti-androgens (AR antagonists, e.g., bicalutamide, flutamide). Unfortunately, LHRH and GnRH agonists fail to prevent synthesis of testosterone by the adrenal glands (site of about 10% of total androgen production) and anti-androgens can act as weak agonists in prostate cancer cells expressing mutated and/or over-expressed AR.[35] In addition, combination therapy with anti-androgens seems to fail to extend survival rates in patients with advanced PCa, with response times ranging for only 12–33 months.[36] It has been shown that patients developing resistance to anti-androgen therapy maintain levels of T and DHT in their cancerous tissue at levels sufficient to activate the AR, and that an increase in AR mRNA was the only change consistently associated with anti-androgen resistance.[35,37] Taken

together, these results suggest that androgens play a role even in so called hormone-refractory PCa. Furthermore, recent experiments suggest the possibility of androgen production in adipose tissue.[2] Thus, compounds that can systemically inhibit the production of androgens, similar to the systemic inhibition of estrogen production in BCa, may prove to be more effective in the treatment of PCa.

The last step in the production of T requires two sequential reactions both catalyzed by the same enzyme, 17α-hydroxylase/17,20-lyase (CYP17; Fig. 4).[38] Therefore, CYP17 has become the target of interest for systemic inhibition of androgen production. *Ketoconazole* (Fig. 5), an anti-fungal agent that non-specifically inhibits a broad range of CYP enzymes, has been used clinically as a second line therapy for advanced hormone-refractory PCa[39] and showed efficacy in patients no longer responding to treatment with the anti-androgen flutamide.[40] Owing to its lack of specificity for CYP17, *ketoconazole* treatment is, unfortunately, limited by toxicity. Clinical trials using low dose *ketoconazole* (LDK) co-treated with glucocorticoids and chemotherapeutics or androgen withdrawal
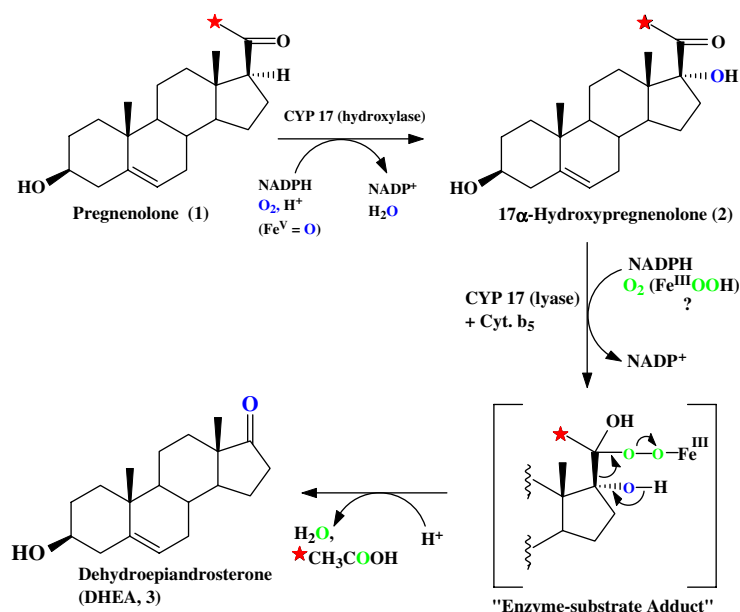

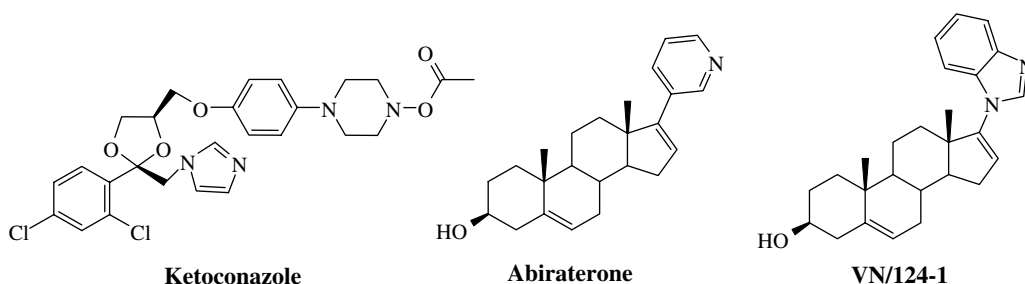**Figure 4.** Biosynthesis of androgens.

Figure 5. Inhibitors of CYP17. Ketoconazole is a broad range CYP inhibitor while abiraterone and VN/124-1 are designed as specific CYP17 inhibitors.

therapies have shown similar efficacy to high dose *ketoconazole* (HDK) with reduced toxicity.[41–43]

Despite the potential therapeutic benefits of LDK, development of more potent and selective inhibitors of CYP17 would clearly offer a therapeutic advantage over *ketoconazole*. One such molecule, *abiraterone* (Fig. 5), has already entered phase II clinical trials. At the 2007 American Association for Cancer Research Annual Meeting, Attard et al. reported exciting phase II results for *abiraterone acetate* (prodrug of *abiraterone*) showing PSA responses ($\geqslant 50\%$) in 11/18 hormone-refractory patients and a drop in circulating T levels from castrate levels (<50 ng/dl) to undetectable levels (<1 ng/dl).[44] Our laboratory has done extensive research in this field as well, and has developed several molecules that inhibit both CYP17 and the AR directly. One of these compounds, a 17-benzoimidazole called *VN/124-1* (Fig. 5), has shown superb anti-cancer properties both in vitro and in vivo.[45] In fact, *VN/124-1* is the only CYP17 inhibitor/anti-androgen to date that has been shown to inhibit prostate cancer tumor growth in vivo more effectively than castration.[45] *VN/124-1* has recently been licensed to Tokai Pharmaceuticals Inc., Boston, MA, USA, with hopes of following *abiraterone* into the clinic within a few years. A more comprehensive review of this subject can be found in the several reviews written by our group and others regarding the development of CYP17 inhibitors.[46–49]

3. Inhibiting vitamin metabolism

3.1. 25-Hydroxyvitamin D$_3$-24-hydroxylase (CYP24)

Vitamin D is synthesized in the skin upon exposure to UVB radiation. One of vitamin D's active metabolites, 1,25-D$_3$ (clinical formulation is known as calcitriol), acts as hormone, and like E and T, it binds to a nuclear receptor, the vitamin D receptor (VDR), and initiates transcription. Unlike T and E, however, 1,25-D$_3$ has gained attention as an anti-cancer agent because of its ability to inhibit proliferation, promote differentiation, and induce apoptosis in many cancer cell types including colon and prostate.[50–53] The role of 1,25-D$_3$ in cancer prevention is also supported by epidemiological studies that reveal a negative correlation between occurrence of certain cancers and sunlight exposure.[54–56] This connection has been particularly well documented in colon cancer where it has recently been suggested by Grant and Garland that as much as 20–30% of colorectal cancer incidences are due to insufficient exposure to sunlight.[57] Deactivation of 1,25-D$_3$ occurs via hydroxylation at C-24 catalyzed by CYP24 (Fig. 6).[58] CYP24 is mainly expressed in the kidney, however, its expression has been demonstrated in other tissue types both healthy and cancerous[3,59–63] and has been identified as a possible oncogene.[64] CYP24's role in tumor development and initiation is further supported by its apparent over-expression in lung and colon cancer compared to the corresponding healthy tissue.[62,63] Importantly, CYP24's expression is inducible by treatment with 1,25-D$_3$.[3] This negative feedback mechanism limits the amount of 1,25-D$_3$ present in tumor cells, and consequently the effectiveness of 1,25-D$_3$ therapy. Therefore, regulation of CYP24 enzymatic activity may potentiate the anti-cancer benefits of 1,25-D$_3$. In addition, clinical benefits of 1,25-D$_3$ have been limited due to hypercalciuric and/or hypercalcemic side effects at therapeutically necessary concentrations.[51] Targeting CYP24 provides the opportunity to increase endogenous levels of 1,25-D$_3$, or reduce the effective dose of exogenous 1,25-D$_3$, a therapeutic strategy that may help overcome the deleterious side effects associated with 1,25-D$_3$ treatment.

Strategies to limit CYP24 action include down-regulation of the enzyme's expression, as well as inhibition of the enzyme itself. *Genestein* (Fig. 7a), a naturally occurring isoflavonoid with anti-cancer properties,[65] has been shown to inhibit the transcription of CYP24 as well as CYP27B1 (25-hydroxyvitamin D-1α-hydroxylase),[61,66] which catalyzes the hydroxylation at C-1 of 25-hydroxyvitamin D$_3$ to 1,25-D$_3$.[67] Interestingly, co-treatment with the histone deacetylase inhibitor trichostatin A increased inhibition of CYP24 expression while restoring expression of CYP27B1.[66] Because CYP27B1 has been shown to be expressed in cancer cells,[68,69] this co-treatment offers a unique strategy to maximize the amount of 1,25-D$_3$ present in tumors by suppressing its metabolism while leaving its synthesis unhindered. *Genestein*, and various synthetic derivatives, have been evaluated extensively in pre-clinical studies and have shown promising results as chemopreventative and adjuvant chemotherapies[65,70] prompting the need for evaluation of these compounds in clinical trials.

Recently, Sundaram et al. described the ability of the synthetic 1,25-D$_3$ analogue, *QW-1624F$_2$-2* (Fig. 7a), to inhibit the expression of CYP24.[71] *QW-1624F$_2$-2* was originally described by Posner et al. to mimic the actions of 1,25-D$_3$ through binding of the VDR and activation of transcription.[72] Importantly, however, it lacks the calcemic side effects of 1,25-D$_3$. QW-162F$_2$-2 seems to be as effective as 1,25-D$_3$ in inhibiting cell growth, inducing its effect through modulation of cell cycle and apoptotic proteins.[73] The molecule has also been shown to inhibit neuroblastoma xenografts in nude mice more effectively than the 1,25-D$_3$ analogue, EB1089 (1α,25-dihydroxy-22,24-diene-24,26,27-trishomovitamin D$_3$).[74] Furthermore, *QW-1624F$_2$-2* can inhibit the expression of CYP24 even in the presence of 1,25-D$_3$ and has been shown to act synergistically with 1,25-D$_3$ to inhibit cell proliferation.[71] These pre-clinical results are compelling and demonstrate a need to develop *QW-1624F$_2$-2* as a chemotherapeutic agent.

Obviously, small molecules that can bind to CYP24 and inhibit the enzyme's activity directly may also prove to be effective treatments for some forms of cancer. Currently, the non-selective CYP inhibitor *ketoconazole* (Fig. 5) is being used as an adjuvant therapy for hormone-refractory prostate cancer, mainly for its inhibition of CYP17 (discussed earlier). However, the compound has also been shown to inhibit CYP24 and act synergistically with vitamin D$_3$ analogues in cell culture[75,76] and is being tried in combination with calcitriol
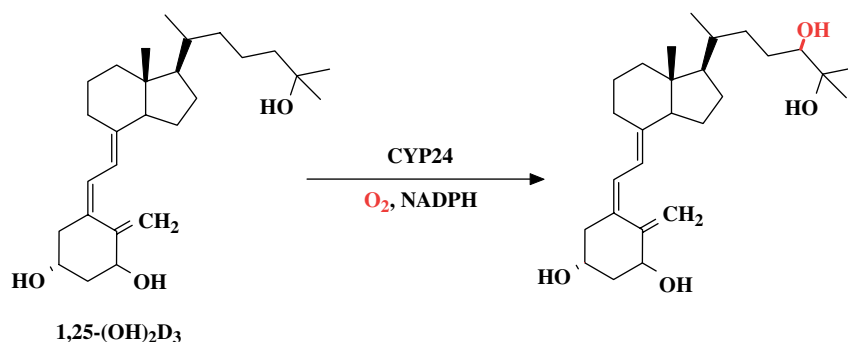
Figure 6. Metabolism of 1,25-D$_3$ via C-24 hydroxylation catalyzed by CYP24.
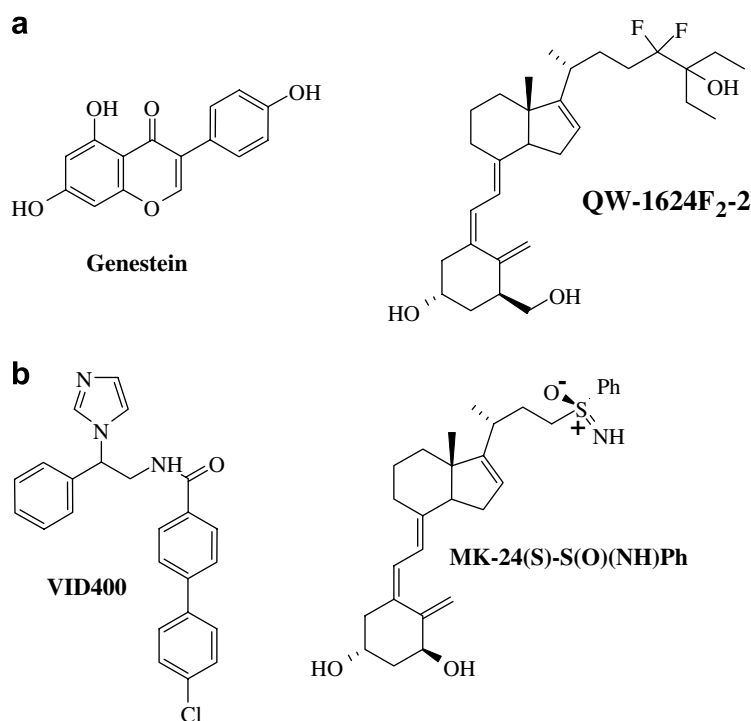

Figure 7. CYP24 Inhibitors. (a) Genestein and QW1624F$_2$-2 inhibit CYP24 expression; (b) VID400 and MK-24(S)-S(O)(NH)Ph inhibit CYP24 enzyme activity.

in a phase I clinical trial.[77] *Liarozole* (Fig. 9), a CYP inhibitor initially designed to inhibit CYP26, has also been shown to inhibit 1,25-D$_3$ hydroxylation and act synergistically with 1,25-D$_3$ in androgen independent DU-145 prostate cancer cells.[78] Unfortunately, both *liarozole* and *ketoconazole* are more potent inhibitors of CYP27B1 than they are of CYP24, greatly limiting their potential efficacy.

Due to these limitations, selective CYP24 inhibitors offer a therapeutic advantage, and several groups have developed compounds to this end.[76,79–81] Schuster and colleagues have developed potent azol containing inhibitors of CYP24, and their lead compound, *VID400* (Fig. 7b), is highly selective for CYP24 over CYP27B1 (IC$_{50}$'s of 15 and 616 nM, respectively).[82] *VID400* is currently undergoing pre-clinical development as an anti-proliferation agent.[79,82,83] A different class of CYP24 inhibitors, sulfone analogues of 1,25-D$_3$, have recently

been developed by Posner and colleagues.[81,84] Their lead compound, a NH phenyl sulfoximine called *MK-24(S)-S(O)(NH)Ph* (*MK*; Fig. 7b), has shown great specificity for CYP24 with an IC$_{50}$ of 7.4 nM, compared to CYP27B1 (IC$_{50}$ = 554 nM) and CYP27A1 (IC$_{50}$ > 1000 nM). *MK* was recently shown to be effective in pre-clinical models of lung cancer, working synergistically with 1,25-D$_3$ to inhibit growth of the non-small cell lung cancer cell line 128-88T.[63]

3.2. ATRA hydroxylase (CYP26)

All-*trans*-retinoic-acid (ATRA) is the most active biological metabolite of vitamin A. Through its interaction with nuclear retinoic acid receptors (RAR), ATRA induces cellular differentiation of epithelial cells,[85] and is being used for the treatment and prevention of several types of cancer.[86–89] However, despite ATRA's pre-clinical efficacy and its clinical success in the treatment of

acute promyelocytic leukemia,[90] the overall clinical efficacy of ATRA against human cancer has been disappointing.[91,92] ATRA's success seems to be limited by the development of resistance in patients.[93] Like 1,25-D_3, this resistance seems to be due, in part, to the rapid metabolism of ATRA in vivo via C-4 hydroxylation (Fig. 8).[94,95] This realization has inspired researches to develop new classes of drugs designed to inhibit the metabolism of ATRA. Such drugs are often termed retinoic acid metabolism blocking agents (RAMBAs).

Many CYPs have been identified that show the ability to metabolize ATRA via 4-hydroxylation including CYP2C8, CYP3A4, and CYP2C9.[96–99] However, the specificity of these enzymes for ATRA is quite low.[96–98] CYP26A1 and CYP26B1 have recently been identified as members of a new family of P450 enzymes that seem dedicated to ATRA metabolism.[10,11] Furthermore, CYP26A1 expression has been shown to be induced upon treatment with ATRA in cancer cells,[4–9] and expression of the enzyme limits induction of apoptosis by ATRA.[5] This phenomenon appears to be implicated in clinically acquired resistance to ATRA. In addition, certain cancers including acute promyelocytic leukemia, prostate, breast, and non-small lung carcinomas express CYP26A at constitutively high levels.[5–7,100–102] This has led to a great deal of interest in the specific targeting of RAMBAs toward CYP26. However, non-specific inhibition of all enzymes involved in ATRA metabolism is an alternative strategy that hinges on the idea that non-specific metab-

olism of ATRA would prevent accumulation of the hormone to levels sufficient enough to induce the expression of CYP26.

Liarazole (Liazal™; Johnson and Johnson Pharmaceutical Research and Development; Fig. 9) is the first and only RAMBA to be evaluated clinically in patients with cancer. Interestingly, *liarazole* is a relatively weak inhibitor of CYP26 ($IC_{50} \sim 2.2$–$6.0\ \mu M$).[103–107] *Liarazole* showed promise in pre-clinical models of prostate cancer[106,108] and clinically as a second line therapy following failure of androgen deprivation.[109] However, *liarazole's* usefulness as a cancer therapy is unfortunately limited by its lack of specificity for CYP isozymes responsible for ATRA metabolism, as well as its moderate potency against CYP26 and is no longer being developed as an anti-cancer agent.[10] Follow-up compounds *R*115866 and *R*116010 (Fig. 9) are far more potent and selective inhibitors of CYP26 and have shown efficacy in pre-clinical cancer models.[10] Work by our group, researchers at Allergan Sales Inc., and OSI Pharmaceuticals Inc. have yielded novel compounds classified as azolyl retinoids, benzeneacetic acid derivatives, and 2,6-disubstituted naphthalenes, respectively (Fig. 9). All of these compounds have shown strong inhibition of CYP26. Additionally, the azolyl retinoids and the 2,6 disubstituted naphthalenes have shown anti-cancer properties in pre-clinical models.[10,110,111] In fact, one of our RAMBAs, *VN/14-1*, inhibits the growth of letrozole resistant breast cancer cells more potently
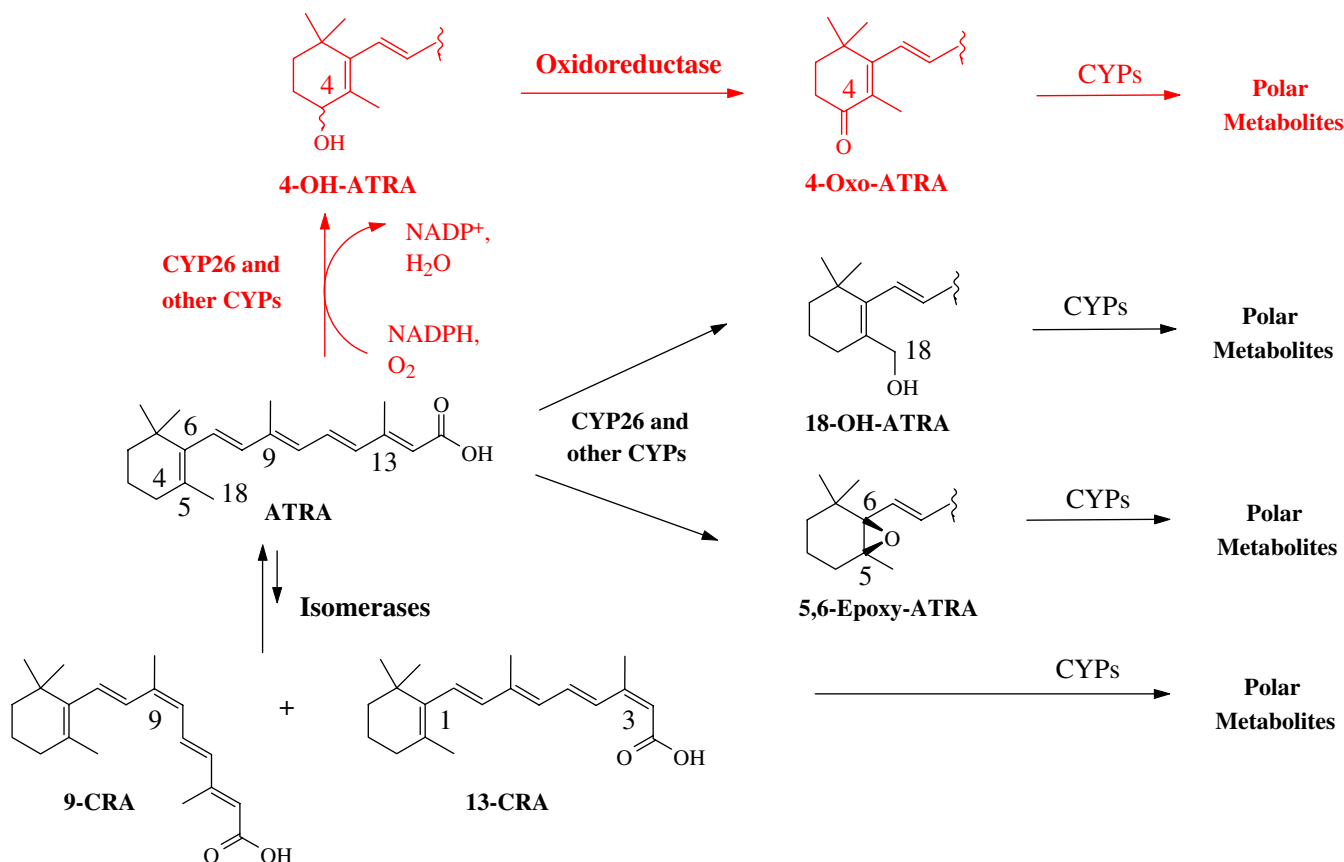

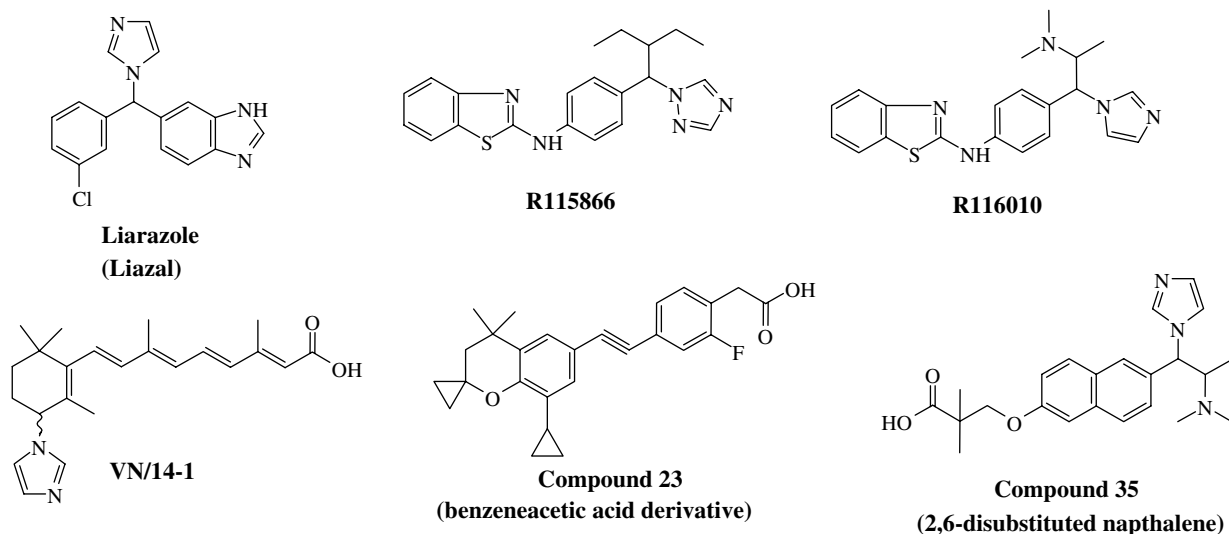**Figure 8.** Metabolism of ATRA by CYP26.

Liarazole
(Liazal)

R115866

R116010

VN/14-1

Compound 23
(benzeneacetic acid derivative)

Compound 35
(2,6-disubstituted napthalene)

Figure 9. Retinoic acid metabolism blocking agents.

than parental letrozole sensitive cells.[112] These results indicate the potential usefulness of RAMBAs for hormone-refractory cancers. Despite promising published pre-clinical results, no clinical trials have been undertaken to date with any RAMBA other than liarazole. However, plans are underway to advance our novel RAMBAs alone and in combination with histone deacetylase inhibitors for clinical trials in breast and prostate cancer patients. For more information on the development and utility of RAMBAs, please consult our group's recent review on the subject (Njar et al., Ref. 10).

4. Exogenous metabolizing CYPs and cancer

4.1. CYP1

Humans express three types of CYP1 enzymes: CYP1A1, CYP1A2, and CYP1B1. Members of this family are under the transcriptional regulation of the aryl hydrocarbon receptor (AhR) and are known to activate pro-carcinogens such as polycyclic aromatic hydrocarbons (PAHs). All members of the CYP1 family are expressed in extrahepatic tissues,[113] but CYP1B1 is unique in that it is over-expressed in many tumor types relative to normal tissues.[15,17,18] This insight has peaked a great deal of interest into CYP1B1.

Evidence for the role of CYP1B1 in tumorigenesis is supported not only by its increased expression, but also by its ability to activate several carcinogens in the chemical classes of PAHs, heterocyclic amines, aromatic amines, and nitropolycyclic hydrocarbons.[114,115] Furthermore, recent epidemiological studies have linked CYP1B1 polymorphisms to increased or decreased risk of certain cancers.[116–119] It is also likely that CYP1B1, along with CYP1A1, plays a role in advanced carcinoma, as well, as the ability of these enzymes to metabolize chemotherapeutic agents may help tumors avoid chemotherapeutic induced cytotoxicity.[120–122]

Of most importance, however, is CYP1B1's role in estradiol metabolism. It catalyzes the hydroxylation of estradiol primarily at the C-4 position. C-2 hydroxylation can also occur primarily through CYP1A2 and CYP3A4. However, C-4 hydroxylation seems to be the preferred pathway outside of the liver and may play an important role in estrogen-related tumorigenesis.[123] The reasons for this are twofold. First, 4-hydroxyestradiol is a strong ER agonist, with a binding affinity for the estrogen receptor 1.5-fold greater than estradiol.[124] Second, and more importantly, 4-hydroxyestradiol is subsequently converted to estradiol 3,4-quinone, which has been shown to bind DNA and form unstable adducts leading to mutations.[123,125]

An obvious strategy for chemoprevention would therefore be the inhibition of CYP1B1. Studies with CYP1A1, CYP1A2, and CYP1B1 knockout mice demonstrated that animals lacking any one of these genes developed normally and showed no noticeable deficiencies. Furthermore, CYP1B1 knockout mice showed strong resistance to 7,12-dimethylbenz[*a*]anthracene (DMBA) induced tumor formation.[126] These studies provide evidence for the potential efficacy and safety of a chemopreventative agent that blocks CYP1B1 expression or activity.

As previously mentioned, members of the CYP1 family are under the transcriptional control of AhR. AhR binds a diverse set of exogenous molecules, including carcinogens such as PAHs and polyhalogenated hydrocarbons which are present in air pollution and cigarette smoke.[127,128] Upon binding, AhR translocates to the nucleus where it binds aryl hydrocarbon nuclear translocator (ARNT). The AhR/ARNT dimer regulates transcription of target genes.[127] The ability of PAHs and other carcinogens to induce CYP1 family expression is illustrated by experiments demonstrating elevated CYP1A1 and CYP1B1 expression in lung and urothelial tissue of smokers compared to non-smokers.[129,130] Because CYP1A1 and CYP1B1 are necessary for the

activation of many carcinogens that induce CYP1 expression via AhR, treatment with antagonists of AhR seems to be a practical chemopreventative strategy.

Many natural products have shown promise for this indication. Recently, the flavonoid *kaempferol* (Fig. 10a) was shown to inhibit agonist binding to AhR, as well as agonist induced AhR/ARNT/DNA complex formation and induction of CYP1A1 expression.[131] *Kaempferol* also inhibited cigarette smoke condensate induced growth of immortalized lung epithelia cells (BEAS-2B) in a soft agar colony assay. In these studies, *kaempferol* inhibited the AhR with an IC_{50} of 28 nM and cellular responses were seen at 10 μM (~IC_{90}). Other natural inhibitors of CYP1 family expression include *5,7-dimethoxyflavone*, which inhibits both expression and activity of CYP1A1,[132] and the stilbene phytoestrogen *resveratrol* (Fig. 10a).[133]

In addition to AhR inhibitors, a vast array of compounds, both natural and synthetic, have been evaluated for their ability to directly inhibit CYP1 family enzymatic activity.[115,123,134–137] Molecules that have been identified as potent inhibitors come from diverse chemical families including synthetic aromatics, coumarins, flavonoids, and stilbenes. Table 1 shows the IC_{50} values of various compounds shown to inhibit members of the CYP1 family, and Figure 10b shows the structures of representative compounds. Unfortunately, it remains unclear whether the inhibition of CYP1 family members by these compounds translates into chemoprevention in vivo.

An alternative strategy for CYP1 based chemotherapies involves the activation of an inactive prodrug to a cytotoxic compound. It has been reported that resveratrol can be activated to an active anti-cancer agent, *piceatannol*, by CYP1B1 within cancer cells.[138] Unfortunately, resveratrol has recently been shown to have limited anti-cancer activity in vivo in an athymic mouse cancer model.[139] New synthetic prodrugs specifically designed to be activated by CYP1A1 and CYP1B1 have been developed and have shown promise as novel approaches to cancer therapy.

One such compound, *phortress*, is a benzothiazole prodrug that has entered phase I clinical trials (Fig. 11a).[140] In the absence of cells, *phortress*, a hydrophilic lysil-amide, does not undergo hydrolysis, but is rapidly hydrolyzed in the presence of cells to its lipophilic amide parent compound *5F203*. *5F203* is then taken up by sensitive cells where it acts as a potent AhR agonist, leading to the induction of AhR target genes (i.e., CYP1A1 and CYP1B1). It is believed that CYP1A1 then metabolizes *5F203* to generate reactive electrophillic species, which ultimately leads to DNA damage and cell death. The drug has shown tremendous pre-clinical results both in vitro and in vivo against sensitive tumors.[140] Obviously, only AhR expressing cancer cells are susceptible to *phortress*, so patients will require screening to select those who might benefit from the drug.

Aminoflavone (5-amino-2,3-fluorophenyl-6,8-difluoro-7-methyl-4*H*-1-benzopyran-4-one; Fig. 11b) has recently followed *phortress* into phase I clinical trials. The
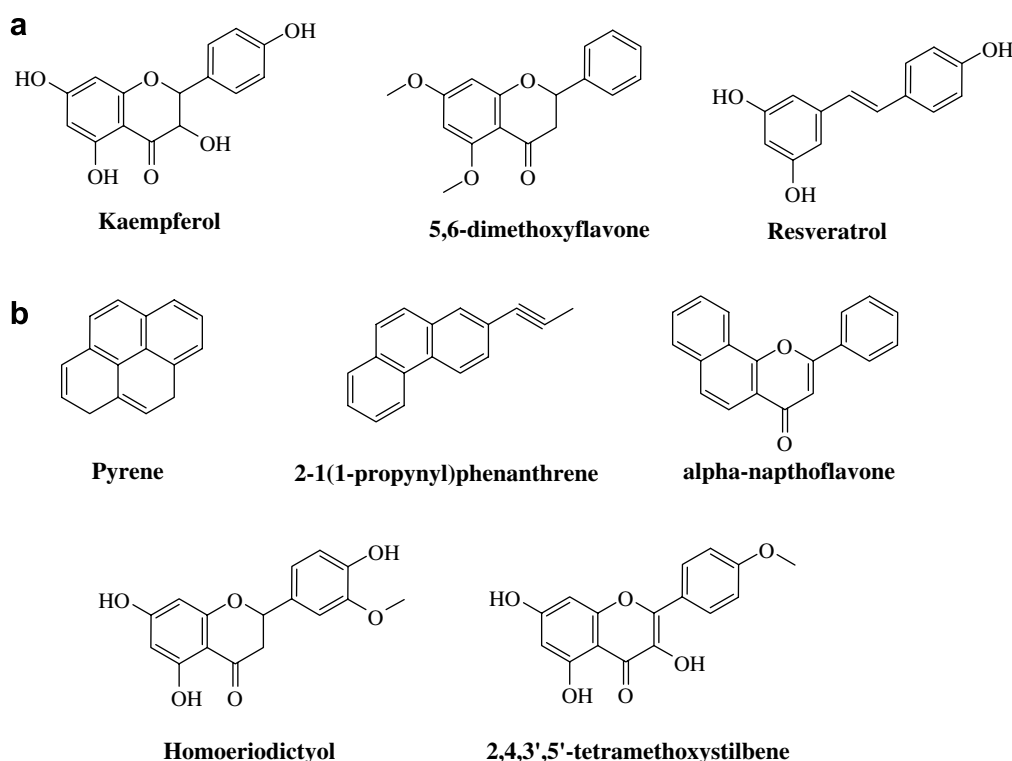

Figure 10. CYP 1 inhibitors. (a) Inhibitors of CYP1 family transcription (Ahr antagonists). (b) Inhibitors of CYP1 family enzyme activity.

Table 1. Inhibitors of the CYP1 family

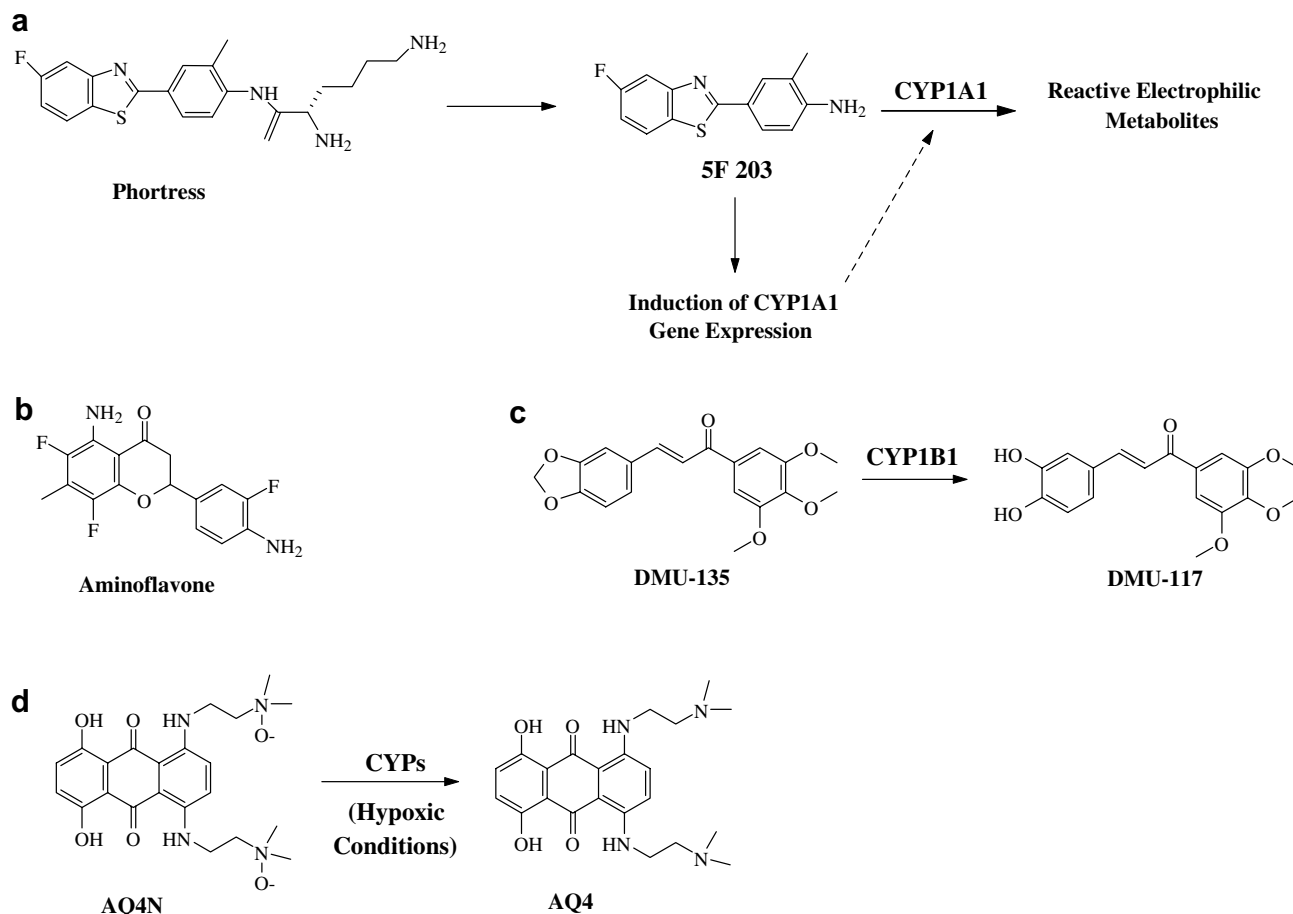| Compound	CYP1B1 IC$_{50}$ (nM)	CYP1A1 IC$_{50}$ (nM)	CYP1A2 IC$_{50}$ (nM)	Reference
Pyrene	2	41	7	134
2-(1-Propynyl) phenanthrene	30	150	60	134
3,3′,4,4′,5′-Pentachlorobiphenyl	11	ND	ND	135
α-Naphthoflavone	5	60	6	134
Acacetin	7	80	80	136
Homoeriodictyol	240	>4000	>4000	136
2,4,3′,5′-Tetramethoxystilbene	6	300	3000	137
2-[2-(3,5-Dimethoxy-phenyl)-vinyl]-thiopene	2	61	11	137

Figure 11. Prodrugs activated by CYPs. (a) Phortress and its metabolite *5F203*. *5F203* activates Ahr inducing transcription of CYP1A1, which activates the drug to a reactive electrophilic species. (b) Aminoflavone is activated by CYP1A1 in a similar fashion as 5F203. (c) DUM-135 and its active metabolite DMU-117. DUM-135 is activated by CYP1B1 to form its active metabolite, DMU-117 (a tyrosine kinase inhibitor). (d) AQ4N and AQ4. AQ4N is activated by CYPs only under hypoxic conditions to form the active topoisomerase inhibitor AQ4.

compound is believed to function through a similar mechanism as *5F203*.[141] Interestingly, however, it has recently been shown that *aminoflavone* requires the expression of sulfotransferase A1 (SULTA1) to elicit a cellular response,[142] suggesting *aminoflavone* activation may be more complex than previously thought.

It remains unclear what the long term effects of treatment with *phortress* or *aminoflavone* will have on patients. As discussed earlier, activation of AhR and induction of CYP1 family proteins is believed to be involved in the development of some cancers. Inducing

CYP1 expression may induce more harm than benefit in the long run. Still, the developers of *phortress* have pointed out that the drug's cell specific activation of AhR differs greatly from that of carcinogens, such as PAHs, and pre-clinical safety results demonstrated relatively low toxicity.[140] Only time will tell if the exciting pre-clinical results of *phortress* and *aminoflavone* can translate to clinical efficacy.

Alternatively, prodrugs designed to be explicitly activated by the tumor specific CYP1B1 would offer the added safety advantage of not having to induce CYP1

enzymes. One such molecule, *DMU-135* (3,4-methylenedioxy-3′,4′,5′-trimethoxy chalcone; Fig. 11c) has recently been described.[143] *DMU-135* is converted by CYP1B1 within tumors to form its active metabolite, *DMU-117*, a potent non-selective tyrosine kinase inhibitor (and potentially a COX inhibitor as well). *DMU-135* is being developed as a chemopreventative agent and has shown the ability to prevent tumor gastrointestinal formation in the Apc^{Min+} mouse model without any sign of toxicity. *DMU-135* represents the first prodrug specifically targeted for activation by CYP1B1.

4.2. CYP2

Many members of the CYP2 family have been identified in extrahepatic tissue.[12,14,113] Of note, four are novel CYPs discovered by the Human Genome Project: CYP2S1, CYP2R1, CYP2U1, and CYP2W1.[144] These enzymes play an important role in xenobiotic metabolism, but are also important in endogenous metabolism as well. For example, CYP2S1 can metabolize ATRA, and CYP2R1 is a vitamin D hydroxylase.[144] The potential role of these enzymes in tumor development and progression remains unclear.

CYP2W1 is of particular interest, however, because it has recently been demonstrated as a tumor-specific CYP,[24,25] especially in gastric and adrenal cancers. Little is known about CYP2W1, but arachidonic acid and indole have recently been identified as potential substrates.[144,145] Despite our limited knowledge of the enzyme's function, its apparent tumor-specific expression is intriguing. Further research is needed to determine if this enzyme may be a potential target (either through inhibition or activation of prodrugs) for the prevention or treatment of gastric and adrenal cancers.

4.3. Bioreductive prodrug AQ4N

The topoisomerase inhibitor prodrug *AQ4N* (1,4-bisan5,8-dihydroxyanthracene-9,10-dione; Novacea®, Fig. 11d) exploits the expression of exogenous metabolizing CYPs in tumor cells in a unique manner. Though it does not target a specific CYP enzyme, the local expression of CYPs is required for its activation making it a notable strategy in CYP-based cancer therapy.

It is well established that hypoxic conditions often exist within the tumor microenvironment.[146] *AQ4N* is activated to its basic amine, *AQ4*, only in the hypoxic tumor microenvironment by CYP3A4, CYP1A1, and CYP1B1.[147] *AQ4N* is a weak DNA binder, but *AQ4* interacts tightly with DNA and can penetrate surrounding cells increasing its efficacy within a tumor.[147,148] The bioreduction of *AQ4N* is strongly inhibited by oxygen, ensuring its activation only occurs under hypoxic conditions. Because hypoxic cells are resistant to chemo- and radiotherapies, *AQ4N* is being developed mainly as an adjuvant treatment option.[149–151] *AQ4N* is entering phase Ib/IIa clinical trials.[152]

5. Conclusions

Great progress has already been made in the targeting of CYP enzymes in cancer therapy. For example, aromatase inhibitors have changed the way estrogen dependent cancers such as BCa are treated. This success has paved the way for similar strategies in inhibiting androgen production to combat AD prostate cancer. Molecules designed to block the CYPs responsible for 1,25-D$_3$ and ATRA metabolism (i.e., RAMBAs) offer advantages in vitamin therapy to fight several forms of cancer. The potential role of xenobiotic metabolizers CYP1A1 and CYP1B1 in the activation of carcinogens and inactivation of chemotherapeutics suggests a potential therapeutic benefits in inhibiting these enzymes. Furthermore, CYP expression in tumor cells is being exploited with prodrugs such as *phortress, aminoflavone, DMU-135*, and *AQ4N* that are activated by these enzymes within the tumor.

Of the drugs discussed, only aromatase inhibitors have achieved clinical success. It remains to be seen whether or not the pre-clinical excitement surrounding other CYP directed therapeutics will resonate in the clinic. Still, CYP directed drugs offer a desperately needed new approach in cancer chemotherapy.

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