Minireview

Cytochrome P450 Enzymes as Drug Targets in Human Disease

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Running title: Targeted inhibition of P450s

Number of words in

Abstract: 109
Significance Statement: 31
Discussion: N/A

Number of references: 57
Number of tables: 1
ABSTRACT

Although the mention of cytochrome P450 (P450, CYP) inhibition usually brings to mind unwanted variability in pharmacokinetics, in several cases P450s are good targets for inhibition. These P450s are essential but in certain disease states it is desirable to reduce the concentrations of their products. Most of the attention to date has been with human P450s 5A1, 11A1, 11B1, 11B2, 17A1, 19A1, and 51A1. In some of those cases, there are multiple drugs in use, e.g., exemestane, letrozole, and anastrozole with P450 19A1, the steroid aromatase target in breast cancer. There are also several targets that are less developed, e.g. P450s 2A6, 8B1, 4A11, 24A1, 26A1, and 26B1.

SIGNIFICANCE STATEMENT

The selective inhibition of certain P450s that have major physiological functions has been shown to be very efficacious in certain human diseases. In several cases the search for better drugs continues.
Introduction

Cytochrome P450 (P450, CYP) enzymes are the major catalysts involved in the metabolism of drugs and xenobiotics (Rendic and Guengerich, 2015) as well as steroids, fat-soluble vitamins, and some other endogenous substrates (Guengerich, 2015). Many of the reactions with endogenous substrates are critical to life, as evidenced by transgenic rodent studies (Gonzalez, 2003) and by the characterization of inherited diseases (Miller and Auchus, 2011). The inhibition of P450s is a major concern in drug development (Correia and Hollenberg, 2015), and the U.S. Food and Drug Administration (FDA) and other regulatory agencies have developed protocols for the in vitro and in vivo inhibition of P450s (and other enzymes involved in drug metabolism and transport) prior to registration, often expecting at least some in vitro data prior to an IND application (Food and Drug Administration, 2022, 2023). When P450 inhibition is mentioned, the usual thought is this context, i.e., avoiding P450 inhibition.

However, there are several disease situations in which the level of a chemical in the body is associated with a disease, and it is desirable to reduce its concentration. Accordingly, there are at least five well-developed situations in which lowering the P450 activity is a very viable strategy in curing a disease (Table 1). Several of these involve interfering with steroid metabolism, which is dominated by P450s (Fig. 1). There are also some P450s that have been considered as drug targets, but the development of inhibitors has not progressed as far. Following is an overview of some of the major targets and a few potential new ones. Also see a very recent review by Foti in this journal (Foti, 2023).

P450 5A1 (thromboxane synthase)
This enzyme has long been a target in the treatment of several cardiovascular diseases (Table 1). P450 5A1 is one of the two mammalian P450s that normally operate in a mode involving rearrangement of an oxidized substrate (prostaglandin endoperoxide) to a new, more stable product in the absence of the input of electrons and molecular oxygen. A drug lead was actually used in the original purification of the enzyme (Hecker et al., 1986). The goal of treatment is to lower thromboxane levels and inhibit platelet aggregation (Table 1).

**P450 11A1**

This P450, the cholesterol side chain cleavage lyase, is the entry point from cholesterol to all mammalian steroids (Fig. 1). Although this enzyme has been considered as a target before (Jarman et al., 1988; Olakanmi and Seybert, 1990; Guengerich, 2015), one might question the logic of inhibiting it. However, Karimaa et al. (Karimaa et al., 2022) have proposed a strategy for treating castration-resistant prostate cancer by blocking all endogenous steroid production with a drug and then supplementing patients with the necessary steroids (e.g., prednisone). There is precedent for the approach with abiraterone and P450 17A1 (vide infra), although there are major side effects with the steroid supplementation. The candidate ODM-208 (Karimaa et al., 2022) showed promise in pre-clinical species and is now in Phase I/II clinical trials.

ODM-208, devoid of heme liganding properties, has IC50 values of 15-108 nM (Karimaa et al., 2022). Inhibition was enhanced by pre-incubation with P450 11A1 and NADPH, indicating that this is a time-dependent inhibitor or, probably more likely, that an oxidation product is a better inhibitor. (The oxidation of drugs by P450 11A1 is not unprecedented (Zhang et al., 2012).)
P450 11B1 and 11B2

Cushing’s disease involves the over-production of cortisol due to P450 11B1 (Fig. 1) (Boscaro et al., 2000; Yin et al., 2012; Emmerich et al., 2013; Emmerich et al., 2017). Reduction of aldosterone levels is the goal in the inhibition of P450 11B2, which is thus a target in treating hypertension. A number of drugs have been used to inhibit P450 11B1, including mifepristone (Chu et al., 2001). One of the issues in developing inhibitors is the high sequence similarity of P450s 11B1 and 11B2, differing only in 32 residues (94% identity). Nevertheless, it has been possible to develop molecules that are very selective for both P450 11B1 and 11B2 (Hu et al., 2014). Newer inhibitors of P450 11B1 are being considered (Emmerich et al., 2017). Some of these inhibitors, although selective for P450 11B1 vs. 11B2, also strongly inhibit P450s 17A1 and 19A1, which may or may not be desirable depending upon the situation (Hu et al., 2014).

P450 17A1 (steroid 17α-hydroxylase/17,20-lyase)

The reactions catalyzed by P450 17A1 are shown in Figs. 1 and 2. Dehydroepiandrosterone (DHEA) is readily oxidized to androstenedione by a dehydrogenase, which is then reduced to testosterone and then to dihydrotestosterone, both potent androgens. The main disease issue is prostate cancer, although breast cancer, polycystic ovary syndrome, Cushing’s disease, and glioblastoma are all under consideration (Wróbel et al., 2023). The only drug in this group approved for use is abiraterone acetate (Zytiga®), a pro-drug form that is cleaved to abiraterone (Fig. 3). Alternate therapies include targeting the androgen receptor (e.g., enzalutamide), and some drugs inhibit both P450 17A1 and the receptor, e.g. gleterone (TOK-001, VN/124-1) (Hu and Hartmann, 2014). YXG-158 has been reported to inhibit P450 17A1
and also to promote degradation of the androgen receptor, although it was actually the 17-hydroxylation reaction being assayed and not the lyase (Wang et al., 2023).

Crystal structures of P450 17A1 with abiraterone have been available since 2012 (DeVore and Scott, 2012), and structures with abiraterone metabolites show a second binding site (Petrunak et al., 2023). Although it has been reported that abiraterone acts by slow, tight-binding inhibition (Cheong et al., 2020), pre-steady-state assays have shown that this is not the case and inhibition is essentially immediate upon binding (Guengerich et al., 2021).

Abiraterone inhibits with high affinity (IC$_{50}$ and $K_i$ in low nM range). A recurring issue is the specificity of inhibition. One concern is cross-inhibition of P450 21A2 (e.g., abiraterone), which has been addressed in the design of some new candidates (Fehl et al., 2018). Some new inhibitors have been published (Fig. 3) (Padmakar Darne et al., 2022; Wang et al., 2023).

In addition to the issue of cross-inhibition with other P450s, a problem with inhibiting P450 17A1 is that the 17α-hydroxylation reaction is needed to provide 17α-hydroxyprogesterone for use in cortisol production. The inhibitors developed to date all inhibit both reactions (17α-hydroxylation and lyase), and thus patients need to be supplemented with steroids such as prednisone, which has major side effects (Bird and Abbott, 2016). Although several leads have been purported to be selective, they have not been in assays done in other laboratories (Petrunak et al., 2017; Guengerich et al., 2021). The search for new steroidal and non-steroidal inhibitors of P450 17A1 has been reviewed in detail recently (Wróbel et al., 2023). BMS-737 has been reported to have 11-fold selectivity for the lyase activity over 17-hydroxylation (Padmakar Darne et al., 2022).

**P450 19A1 (steroid aromatase)**
The development of aromatase inhibitors as a means of treating breast and other hormonal cancer was pioneered by the late Angela Brodie (Brodie et al., 1990). Cancers that are fueled by estrogens are retarded by either suppression of estrogen synthesis or by attenuation of estrogen receptors. Several 3rd-generation aromatase inhibitors are in wide use clinically, both steroidal and non-steroidal (Fig. 4).

These are very high affinity drugs and have been very efficacious in estrogen receptor-positive breast cancers (and some other hormonal cancers). Most of the side effects of treatment are related to estrogen deprivation (e.g., problems with calcium homeostasis), which has tended to discourage the development of new drugs in this field. One other problem is that the site of synthesis of estrogens is an issue (Simpson, 2003), i.e., in post-menopausal women the major source of estrogen synthesis is peripheral tissues (e.g., breast adipose) but in pre-menopausal women the source is the ovaries, and estrogen deprivation is not as effective for treating breast cancer.

P450 51

There are two aspects here. The first is human P450 51A1, the lanosterol 14α-demethylase, a key enzyme involved in cholesterol synthesis. This enzyme is not only expressed in liver but also in other tissues that synthesize cholesterol, and mRNA is very high in testes (at least in rats) (Stromstedt et al., 1998). In mice, embryonic knockouts are lethal, as might be expected (Keber et al., 2011). In principle, P450 51A1 could be a target for hypercholesterolemia and was an interest in some pharmaceutical companies (Trzaskos et al., 1995). However, 3-hydroxymethylglutarate (HMG) CoA reductase is highly sensitive to feedback regulation when cholesterol is depleted, and HMG CoA reductase itself has proven to be a much better target with
the success of statins. There is some consideration of inhibitors of human P450 51A1 in the context of treating cancer, at least based on in vitro results (Friggeri et al., 2019).

Of greater interest for inhibition are the CYP 51 enzymes of fungi, yeast, and other parasites (Lepesheva et al., 2018). Many of these organisms are life-threatening and have widespread incidence in the global health picture. The enzyme is needed for the synthesis of important sterols that these organisms use for membrane-synthesis (e.g., ergosterol). The enzyme is a major target for anti-fungals (Table 1), and these drugs are used to treat relatively simple maladies (e.g., athlete’s foot-tinea pods) as well as life-threatening fungal systemic fungal infections common in immunocompromised people (Chen et al., 2020). P450 51 enzymes are also targets being developed for various parasites, e.g., trypanosomiasis, leishmania (Lepesheva et al., 2004; Lepesheva and Waterman, 2007; Friggeri et al., 2014).

The difficulty in the development of effective drugs is exemplified by studies with posaconazole (Hargrove et al., 2017) (Fig. 5). The inhibitor posaconazole is much more effective than an earlier lead molecule, SCH 51048, differing only in the addition of a hydroxyl group in the isopentyl side chain. Long after the introduction of posaconazole, the X-ray structure of Candida albicans P450 51A was determined (Fig. 5). In the structure, the additional hydroxyl group protrudes from the active site and, at least in this structure, does not appear to interact with the protein. Posaconazole has been reported to have higher intrinsic activity, with the reason being still unclear, and broader inhibition of different fungal species (Nomeir et al., 2008). It was also more soluble (facilitating iv use), exhibited greatly enhanced pharmacokinetic properties (e.g., $C_{p,\text{max}}$ and AUC) in multiple species, and was considerably better in increasing survival in multiple pre-clinical species (Bennett et al., 2006; Nomeir et al., 2008).
Other P450s as potential targets

Several other human P450s have been considered as potential targets for drugs to treat various health issues (Table 1) (Guengerich, 2015), but work has not been as extensive as with the other P450s discussed here.

P450 2A6 catalyzes the oxidation of nicotine to cotinine and has been considered in the context of drugs for smoking cessation (Denton et al., 2005; Yano et al., 2006).

P450 8B1, a sterol 12α-hydroxylase, has been considered as a target for treatment of both Type 2 diabetes and nonalcoholic fatty liver disease. This enzyme catalyzes the synthesis of cholic acid and controls the ratio of cholic acid to chenodeoxycholic acid, thereby adjusting the hydrophobicity of bile. Some azoles are strong inhibitors but are not specific, and there are efforts to synthesize more selective inhibitors of this enzyme (Liu et al., 2023).

Single nucleotide variants in P450 4A11 influence ω-hydroxylation and have been associated with hypertension (Gainer et al., 2005). In principle, an inhibitor might provide benefit. A general issue is that simple inhibitors of ω-hydroxylation (HET0016) work on this reaction with multiple P450s (Correia and Hollenberg, 2015).

Inhibition of P450 24A1 has been considered in the context of preventing vitamin D degradation, as opposed to administration of vitamin D itself, in the treatment of cancer (King et al., 2010; Sakaki et al., 2014).

In a similar vein, inhibition of P450s 26A1 and 26B1 has been considered as an approach to raising levels of retinoic acid by blocking its degradation (Nelson et al., 2013).

P450 inhibition as a booster role in drug metabolism
With P450 3A4, the general goal in drug development is to avoid drugs that strongly inhibit the enzyme, because P450 3A4 is involved in so many cases (Bhutani et al., 2021). However, in some cases an important drug may have poor pharmacokinetic properties (e.g., short half-life)—or the drug may be very expensive. A P450 3A4 inhibitor can be given concurrently to retard drug metabolism, if that drug does not have any properties that would interfere with the therapy or otherwise have adverse effects. FDA has historically not been particularly favorable to such inhibitors, in that they introduce more complexity, but some “boosters” have been allowed. One such booster is cobicistat (Burger et al., 2020), which has been used in combination with HIV-1 protease inhibitors. The drug Paxlovid® is a combination of nirmatrelvir (a Covid-19 protease inhibitor) and HIV-1 protease inhibitor ritonavir, used for treating Covid-19 (Lamb, 2022).
Authorship Contributions

Wrote or contributed to the writing of the manuscript: Guengerich

Acknowledgements

I thank K. Trisler for assistance in the preparation of the manuscript and the editors for the opportunity to contribute this Minireview.

ABBREVIATIONS: P450 or CYP, cytochrome P450; FDA, United States Food and Drug Administration.

Conflict-of-interest statement: The author has no actual or perceived conflict of interest with the contents of this article.

Data Availability Statement: There are no datasets in this paper

Financial support: This work was supported by the National Institute of General Medical Sciences (Grant R01 GM118122) (to F. P. G.).

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Figure legends

Fig. 1. P450 enzymes involved in major reactions of steroid metabolism.

Fig. 2. Major P450 17A1 reactions: 17α-hydroxylation and 17α,20-lyase.

Fig. 3. Abiraterone and two new candidates for P450 17A1 inhibition (BMS-737 and YXG-158).

Fig. 4. P450 19A1 (aromatase) inhibitors.

Fig. 5. X-Ray crystal structure of *Candida albicans* P450 51A with bound posaconazole (Protein Data Bank 5FSA) (Hargrove et al., 2017). The drug is shown liganded to the iron in the (planar) heme (with the Cys-428 ligand below the plane of the porphyrin), and the arrow points to the extra hydroxyl group on the 3-pentyl side chain (compare posaconazole (SCH 56592) and SCH 51048) (Bennett et al., 2006; Nomeir et al., 2008).
Abiraterone

BMS-737

YXG-158

Fig. 3
Exemestane

Letrozole

Anastrozole
Fig. 5

SCH 51048

Posaconazole