REVIEW ARTICLE

Steroidogenic cytochrome P450 enzymes as drug target

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Abstract

Human cytochrome P450 (CYP) enzymes are composed of 57 individual enzymes that perform monooxygenase activities. They have diverse physiological roles in metabolizing xenobiotics and producing important endogenous compounds, such as steroid hormones and vitamins. At least seven CYP enzymes are involved in steroid biosynthesis. Steroidogenesis primarily occurs in the adrenal glands and gonads, connecting each reaction to substrates and products. Steroids are essential for maintaining life and significantly contribute to sexual differentiation and reproductive functions within the body. Disorders in steroid biosynthesis can frequently cause serious health problems and lead to the development of diseases, such as prostate cancer, breast cancer, and Cushing's syndrome. In this review, we provide current updated knowledge on the major CYP enzymes involved in the biosynthetic process of steroids, with respect to their enzymatic mechanisms and clinical implications for the development of new drug candidates.

Keywords Cytochrome P450 · CYP11A1 · CYP17A1 · CYP19A1 · CYP11B1 · CYP11B2

Introduction

Cytochrome P450 (CYP, P450) enzymes are a superfamily of monooxygenases with heme as the prosthetic group [1]. They catalyze the metabolism of diverse xenobiotic and endogenous compounds including steroids, vitamins, and fatty acids [2]. These enzymes exist in the endoplasmic reticulum and mitochondria, and their catalytic activities are supported by NAD(P)H and redox partners such as P450 oxidoreductase (POR), adrenodoxin (ADX) and adrenodoxin reductase (ADR) [3]. The human genome contains 57 CYP genes, many of which are involved in steroid biosynthesis and metabolism [2, 4]. At least seven CYP enzymes are involved in steroid biosynthesis; CYP11A1, CYP17A1, CYP19A1, CYP11B1, CYP11B2, CYP21A2, and CYP51A1 (Fig. 1) [2]. Steroidogenesis primarily occurs in the adrenal glands and gonads, connecting each reaction to substrates and products. Steroids are critical for life and contribute significantly to sexual differentiation and reproductive functions within the body [5]. However, abnormal steroid levels can also cause diseases such as prostate cancer, breast cancer, and Cushing's syndrome [6–8]. To treat these diseases, it is necessary to reduce steroid levels, and drugs targeting the key enzymes involved in each steroid biosynthesis step have been developed (Table 1). However, several shortcomings are associated with these drugs, particularly their selectivity and cross-inhibition. To date, various attempts have been made to address these issues. This review focuses on steroidogenic CYPs as potential drug targets for diseases treatment. Additionally, the efficacy and adverse effects of approved drugs and candidate compounds are explored.

CYP11A1

CYP11A1 is involved in the initiation step of hormonal steroid biosynthesis from cholesterol (Fig. 1). It is located in the mitochondrial membrane and catalyzes the conversion of cholesterol to pregnenolone. Cholesterol is first transported into the mitochondria by translocator protein and steroidogenic acute regulatory protein. Subsequently, CYP11A1 performs two successive hydroxylations on C22 and C20 of cholesterol and cleaves the cholesterol side chain between C20 and C22 (Fig. 2). The activity of CYP11A1 requires two cofactor proteins in the mitochondrial membrane: ADX and ADR. Pregnenolone, a product of CYP11A1, is a

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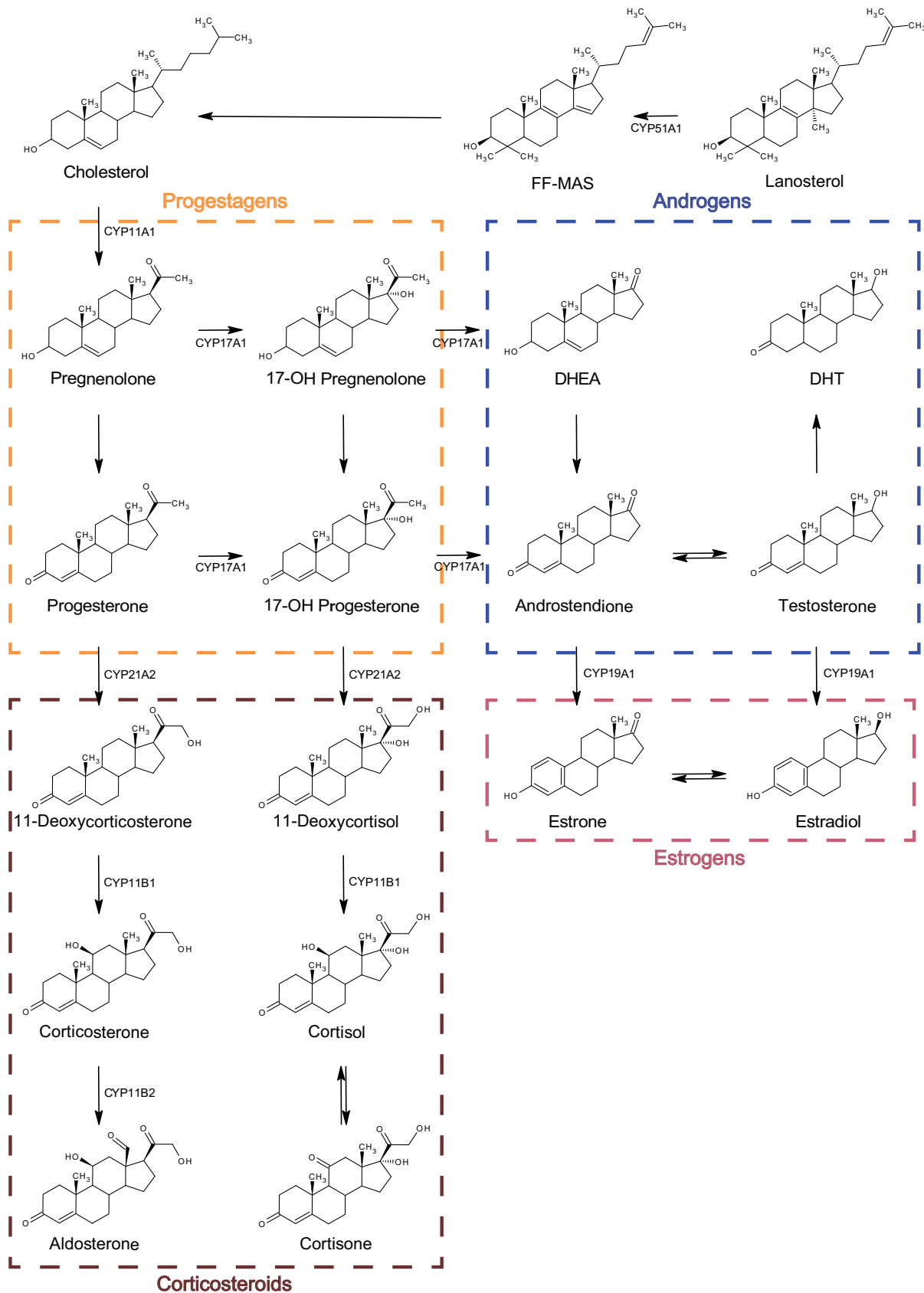
◄**Fig. 1** Steroid biosynthesis pathway. FF-MAS (follicular fluid meiosis-activating sterol, 14-demethyl-14-dehydrolanosterol), DHEA (dehydroepiandrosterone), and DHT (dihydrotestosterone)

precursor of all steroid hormones. Inhibiting this catalytic step can suppress subsequent steroidogenic reactions and induce a deficiency in steroid hormones [9]. There have been attempts to leverage steroid levels by co-treatment with a physiological dose of essential steroids to treat diseases [10]. ODM-208 was chosen as a candidate inhibitor after screening and utilizing the crystal structure of CYP11A1 bound to cholesterol (Fig. 3) [10]. ODM-208 inhibits pregnenolone biosynthesis and suppresses downstream of steroid biosynthesis, with IC_{50} values in the nanomolar range [10]. It has more potent efficacy than the other non-selective inhibitors. In patients with castration-resistant prostate cancer (CRPC) co-treated with dexamethasone and fludrocortisone, ODM-208 exhibited a reduction in the levels of pregnenolone and testosterone [10].

As the protein crystal structure data of CYP11A1 is available, its analysis can provide clues for the development of new drug candidates [11]. The crystal structure of CYP11A1 revealed that C20 and C22 of cholesterol are positioned just above the heme, and that this interaction was facilitated by enzyme residues such as W87, L101, F202, and I461 (Fig. 4A). The two methyl groups on the β face of cholesterol interact with S352 and a water molecule, forming a hydrogen bond with S352. This interaction facilitates the positioning of the reactive site of cholesterol closer to heme. Additionally, various residues assist in binding cholesterol rings to the active site. The 3β hydroxyl of cholesterol forms hydrogen bonds with two water molecules, creating an extensive hydrogen bonding network with residues such as H39, Y61, N210, Q377, and other water molecules (Fig. 4A) [11]. Rational design targeting of these key residues could potentially aid in the development of novel inhibitors that more efficiently modulate the initial step of steroid synthesis.

CYP17A1

CYP17A1 is primarily expressed in adrenal glands and gonads. It plays a crucial role in androgen synthesis by catalyzing the sequential 17α-hydroxylation of progesterone and pregnenolone, followed by a 17,20-lyase reaction. This reaction ultimately leads to the generation of androstenedione and dehydroepiandrosterone (DHEA) (Fig. 1). Androgens synthesized by CYP17A1 are essential for the differentiation and maturation of male reproductive organs. In addition, they are key factors in prostate cancer, promoting the survival and growth of prostate cancer cells. Therefore, androgen signaling is considered as an important therapeutic target for the treatment of prostate cancer [7]. Consequently,

CYP17A1 has been considered as a drug target for suppressing androgen synthesis. The first drug approved as a CYP17A1 inhibitor was abiraterone (Fig. 3). Abiraterone is a pregnenolone analog with a pyridyl substituent at the 17th carbon atom that inhibits the 17-hydroxylase and 17,20-lyase activities of CYP17A1 [12]. CYP17A1 binds abiraterone through a type II inhibitory interaction with CYP enzyme [13]. Abiraterone binds more tightly to CYP17A1 than progesterone [13]. Inhibition assays with purified CYP17A1 and the H295R cell line demonstrated that abiraterone inhibits the 17-hydroxylase and 17,20-lyase activities of CYP17A1 with IC_{50} values in the nanomolar range [14]. In clinical trial, the serum levels of testosterone, DHEA, and androstenedione decreased significantly after treatment with abiraterone, and patients exhibited > 50% reduction in prostate-specific antigen [15]. In the crystal structure of CYP17A1 with abiraterone, structural folding characteristics of the CYP enzyme are evident, with the abiraterone structure showing tight interactions with residues in the active site of CYP17A1 through a hydrogen bond network, along with water molecules (Fig. 4B) [13]. These tight interactions result in the significant inhibition of CYP17A1 enzyme activity. However, there are some concerns with the use of abiraterone in relation to the steroid biosynthesis pathway; abiraterone inhibits not only the 17,20-lyase reaction but also the 17-hydroxylase reaction, leading to interference in cortisol production (Fig. 1) [16]. When treated with abiraterone, cortisol levels decrease and adrenocorticotropic hormone (ACTH) secretion increases [17]. Side effects such as hypertension and hypokalemia may occur due to increased mineralocorticoid activity. Co-administration of an appropriate dose of prednisone as a glucocorticoid replacement can reduce ACTH levels and mitigate adverse effects caused by mineralocorticoids [15, 17, 18]. Targeting the active site may be challenging because of the shared active site of both hydroxylase and lyase reactions. Therefore, it is necessary to target auxiliary proteins that interact with CYP17A1. Cytochrome b_5 (b_5) is a small hemeprotein that performs various functions in the body [19, 20]. In particular, the b_5 protein shows selective enhancement in the lyase reaction of CYP17A1 (not in 17-hydroxylation), with the lyase reaction being mainly dependent on the presence of b_5 [21–24]. The b_5 increases the coupling efficiency of NADPH consumption, suggesting that this is one of the reasons for the activation of the lyase reaction by b_5 protein [25]. It has been suggested that b_5 operates as an allosteric regulator of CYP17A1 [21, 26–28], functioning without directly transferring electrons [28]. Previous studies have shown that R347 and R358 of CYP17A1 play critical roles in binding to b_5 [19, 21, 22]. An in vivo study showed that the loss of b_5 function results in lyase deficiency, leading to a decrease in androgen levels [29]. Therefore, compounds targeting R347 and R358 of CYP17A1 to inhibit binding to b_5 could

Table 1 Cytochrome P450 enzymes involved in steroid biosynthesis and specific inhibitors

CYP enzymes	Target disease	Specific inhibitor	IC$_{50}$, nM	Cell line	References
CYP11A1	Prostate cancer	Opevesostat*	15	H295R	[10]
CYP17A1	Prostate cancer	Abiraterone[a]	9.4 (hydroxylase) 1.7 (lyase)	H295R	[14]
CYP19A1	Breast cancer	Exemestane[b]	900	MCF-7	[51]
		Letrozole[c]	0.35	MCF-7	[52]
		Anastrozole[d]	3.62	MCF-7	[52]
CYP11B1	Cushing's disease	Osilodrostat[e]	34.7	HAC15	[53]
		Levoketoconazole[f]	300	HAC15	[54]
CYP11B2	Secondary Hypertension	Baxdrostat* Lorundrostat*			

*Developmental stage

[a]FDA approved in 2011

[b]FDA approved in 1999

[c]FDA approved in 1998

[d]FDA approved in 2000

[e]FDA approved in 2020

[f]FDA approved in 2021
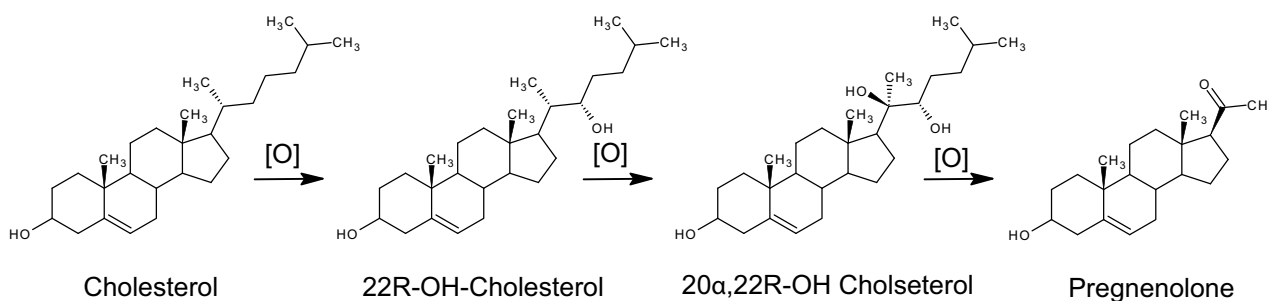
**Fig. 2** Multistep oxidation reactions of cholesterol by CYP11A1

be potential drug candidates for inhibiting the lyase reaction of CYP17A1 without interfering with the active site or hydroxylase reaction. Another concern regarding CYP17A1 inhibitors is their off-target effect that may inhibit several other CYP enzymes. Within the steroidogenic CYP family, abiraterone inhibits CYP21A2 at higher concentrations [30]. When CYP21A2 is inhibited, glucocorticoid and mineralocorticoid levels decrease, leading to increased ACTH secretion. This, in turn, can result in diseases such as adrenal hyperplasia and hyperkalemia [31, 32]. Therefore, various attempts have been made to develop new inhibitors with higher selectivity for CYP17A1 [32].
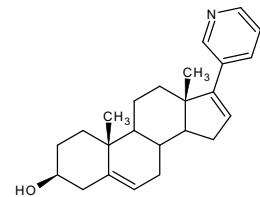
CYP19A1

CYP19A1 catalyzes the aromatase reaction to convert androstenedione and testosterone into estrone and estradiol, respectively, via a sequential reaction (Fig. 5). Estrogens,

which are the products of the CYP19A1 reaction, are important hormones for reproductive development and growth in females. However, estrogen can cause breast cancer by inducing the division and proliferation of breast tissue [6]. Estrogen-dependent breast cancer can be treated by suppressing estrogen synthesis. Therefore, several approaches targeting CYP19A1, the key enzyme in estrogen synthesis, have been investigated and several clinical drugs have been developed. Exemestane, letrozole, and anastrozole are typical third generation CYP19A1 inhibitors (Fig. 3). They show high selectivity and potency in the suppression of CYP19A1 [33]. Exemestane, a steroidal inhibitor, inhibits CYP19A1 with IC$_{50}$ values in the nanomolar range and high affinity [34]. In patients, exemestane caused a significant reduction in the levels of estrogen in both plasma and urine, demonstrating fast absorption [35]. The key factor in these inhibition reactions is the C6-methylidene group, which represents a crucial distinction between exemestane and androstenedione. Exemestane exhibits binding characteristics similar to
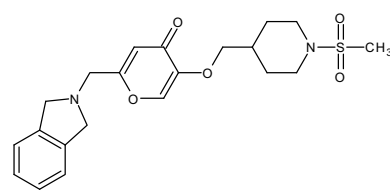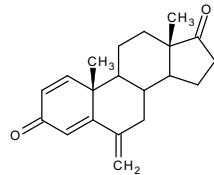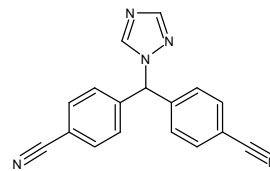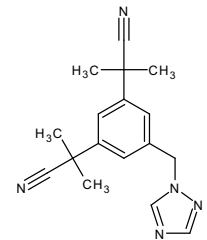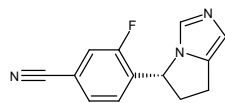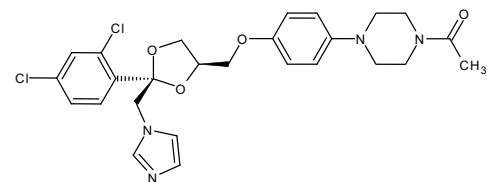
Opevesostat

Abiraterone
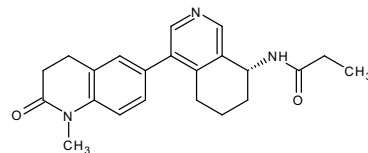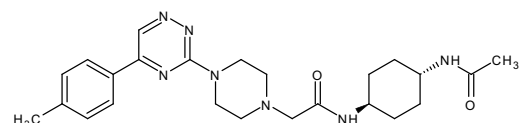
Exemestane

Letrozole

Anastrozole

Osilodrostat

Levoketoconazole

Baxdrostat

Lorundristat

those of androstenedione, a CYP19A1 substrate. The steroid nucleus is surrounded by hydrophobic residues, and the 3- and 17-keto oxygen atoms interact with 115R, 374 M, and 309D via hydrogen bonding. Several conformational changes in the access channel of CYP19A1 and additional hydrophobic interactions allow exemestane to bind to CYP19A1. These new interactions result in a higher affinity and more suitable structure for the CYP19A1 active site (Fig. 4C) [34]. Unlike exemestane, letrozole and anastrozole are non-steroidal inhibitors of CYP19A1. These inhibitors also decrease estrogen levels [36, 37]. The crystal structures of CYP19A1 with letrozole and anastrozole are not yet available. However, based on docking analysis, it has been speculated that the formation of a coordinate bond between the

triazole structure of the two inhibitors and the heme iron of CYP19A1 contributes to their high-affinity inhibition [38]. One of the adverse effects of CYP19A1 inhibitors is related to bones. In premenopausal women, the ovaries are the main glands responsible for estrogen secretion. In postmenopausal women, these glands no longer function in estrogen synthesis; instead, several peripheral tissues produce estrogens, which function as paracrine and intracrine regulators, but not as endocrine regulators [39]. In particular, CYP19A1 is expressed in bone tissues, such as osteoblasts and can produce estrogen, which is responsible for the maintenance of bone formation [40, 41]. Therefore, exemestane-induced inhibition can cause estrogen reduction in the bone, thereby increasing bone resorption. In a clinical study, exemestane
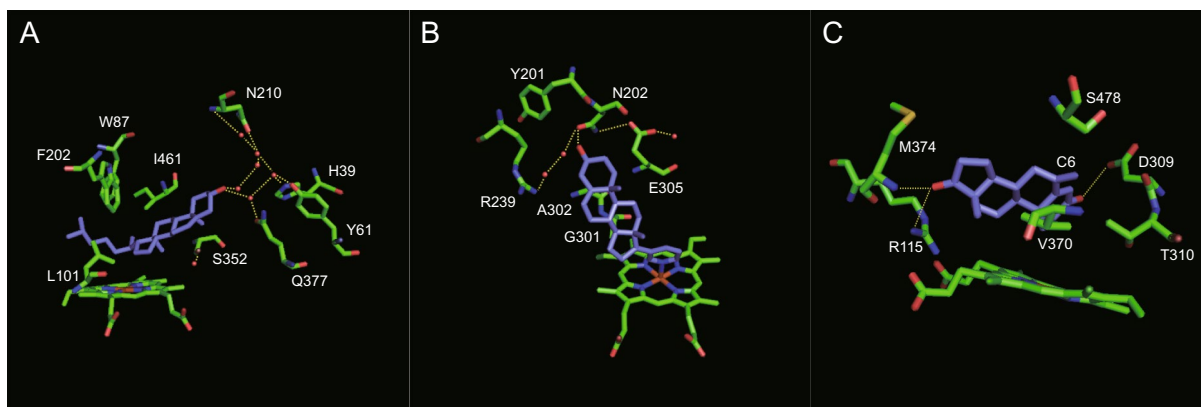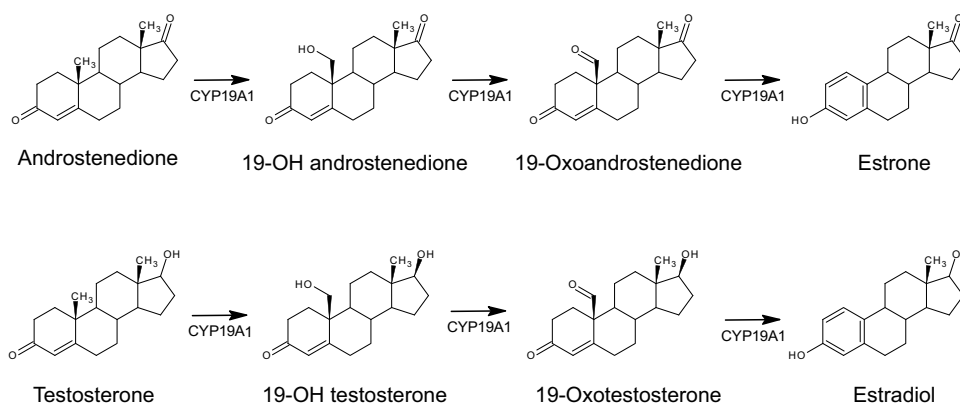
Fig. 4 Structural views of inhibitor binding in the active sites of steroidogenic CYP enzymes. **a** Binding of cholesterol to CYP11A1, **b** binding of abiraterone to CYP17A1, **c** binding of exemestane to CYP19A1

Fig. 5 Sequential enzyme reactions to produce estrogens by CYP19A

significantly reduced bone mineral density (BMD) and cortical thickness in various body parts [42]. Decreased BMD was also observed following treatment with letrozole, and anastrozole [43, 44].

CYP11B1 and CYP11B2

The main activity of CYP11B1 in the adrenal cortex is the conversion of 11-deoxycortisol to cortisol and 11-deoxy-corticosterone to corticosterone (Fig. 1). Excessive levels of cortisol, induced by various stimuli such as ACTH, can cause Cushing's syndrome and a crucial factor in the treatment of Cushing's syndrome is a reduction in cortisol levels [8]. Several candidate drug targeting CYP11B1 have been developed. Recently, osilodrostat and levoketoconazole have been approved as CYP11B1 inhibitors for the treatment of Cushing's syndrome (Fig. 3). In a clinical study, these agents showed a significant reduction in the mean urinary free cortisol below upper limit of normal in patients with Cushing's disease [45, 46]. Owing to the high similarity in amino acid sequences between CYP11B1 and

CYP11B2, the simultaneous inhibition of CYP11B2 can be a serious issue because CYP11B2 is involved in the synthesis of aldosterone. In practice, osilodrostat has been reported to potently inhibit both CYP11B1 and CYP11B2 in several cell lines [47].

CYP11B2 plays a key role in aldosterone synthesis from corticosterone (Fig. 1). Elevated levels of aldosterone, resulting from excessive activation of CYP11B2, are a representative cause of secondary hypertension. Therefore, CYP11B2 is considered a potential drug target for the treatment of hypertension. There are currently no approved drugs that selectively target CYP11B2; however, several agents for this purpose are currently under development. To this end, baxdrostat (Fig. 3), a candidate drug for CYP11B2 inhibition, showed 100 times higher selectivity for CYP11B2 than for CYP11B1 in in vitro tests [48]. Additionally, it significantly decreased aldosterone levels and demonstrated a dose-dependent reduction in systolic blood pressure without affecting cortisol levels [49]. Lorundrostat (Fig. 3), another candidate drug, exhibited similar effects [50].

Conclusion

Several inhibitors of steroidogenic CYPs have been approved as clinical drugs and have demonstrated significant suppression of steroid biosynthesis. However, there remain unmet needs for the treatment of diseases involving steroid biosynthesis. A third-generation inhibitor of CYP19A1 showed a simultaneous decline in BMD in postmenopausal women with a decrease in estrogen levels. Abiraterone also showed high efficacy in the reduction of androgens by inhibiting CYP17A1; however, co-treatment with prednisone was required to replace glucocorticoids. In addition, osilodrostat and abiraterone inhibit off-target CYPs, leading to a deficiency in glucocorticoids and mineralocorticoids.

Numerous candidate agents are currently under development with the aim of improved efficacy. However, several challenges remain in the development of drugs targeting CYPs. First, each steroidogenic reaction is interconnected with its substrates and products, indicating that the inhibition of a particular CYP can lead to unexpected effects. Consequently, some CYP inhibitors may require co-administration with other drugs to mitigate their side effects. Another concern is the similarity between various CYPs, which may inadvertently lead to the inhibition of unintended CYPs. Achieving high selectivity for the target CYP is crucial for addressing this issue. Crystal structure data plays a pivotal role in the development of drugs with enhanced selectivity.

The crystal structures of several steroidogenic enzymes, including CYP11A1, CYP17A1, CYP19A1, CYP11B1, and CYP11B2, have been determined. By comparing the binding of enzyme with substrates or other inhibitors using these crystal structure, new drugs can be designed, and modifications to increase selectivity can be explored. Additionally, a new strategy for CYP inhibition beyond competitive enzyme inhibition is necessary. Targeting the proteinaceous cofactors of CYPs, such as b_5, ADR, and POR, could offer promising avenues for inhibition. Identifying the residues responsible for binding between CYP and its cofactor could aid in the development of new drugs with heightened selectivity. By addressing these challenges and compensating for the related defects, CYP inhibitors with high efficacy and selectivity can be developed to treat various diseases caused by abnormal steroid levels.

Declarations

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