Chapter 12

# Steroidal CYP17 Inhibitors for Prostate Cancer Treatment: From Concept to Clinic

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Additional information is available at the end of the chapter

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## 1. Introduction

The successful application of therapeutic strategies to block the known growth stimulation property of estrogen in breast cancer, namely the aromatase (CYP19) inhibitors formestane (4-OH) and exemestane (Aromasin) [1], has paved the way for the investigation of inhibitors of other P450 enzymes that might impart the growth of hormone-dependent cancers [2]. Cytochrome P450 17$\alpha$-hydroxylase,$C_{17,20}$-lyase (CYP17) is at the crossroads of androgen and corticoid biosynthesis and has become a valuable target in prostate cancer (PC) treatment [3-8]. Androgens, which are produced in steroidogenic tissues, bind to the androgen receptor (AR) and initiate transcription which in turn results in the synthesis of prostate-specific proteins, as well as in cell proliferation. Systemic ablation of androgen by castration, either surgical or chemical, is highly effective in treating PC when the disease is hormone-dependent [3]. However, within 18-24 months following the onset of primary hormonal therapies, the disease becomes androgen-refractory by mechanisms in which AR-mediated signaling and gene expression is still active despite castrate androgen levels [9]. The FDA approved the combination of docetaxel (Taxotere) *1* and prednisone for the treatment of castrate-resistant PC (CRPC) which improves survival time in about 18 months [10, 11], and cabazitaxel (Jevtana) *2* [12], a novel taxane derivative, for metastatic CRPC (mCRPC) which has progressed following docetaxel therapy (Fig. 1). The immunotherapy Sipuleucel-T (Provenge) is also approved for the treatment of asymptomatic or minimally symptomatic mCPRC. In April 2011, abiraterone acetate (Zytiga) *3* became the first steroidal CYP17 inhibitor to be approved by the FDA for the treatment of docetaxel-resistant mCRPC (Fig. 1) [13, 14]. Following abirateroneacetate *3*, galeterone (TOK-001) *4* (Fig. 1), another steroidal CYP17 inhibitor,

with AR antagonistic and ablative activities, is currently undergoing Phase I/II clinical trials for the treatment of chemotherapy-naive CRPC [15, 16].

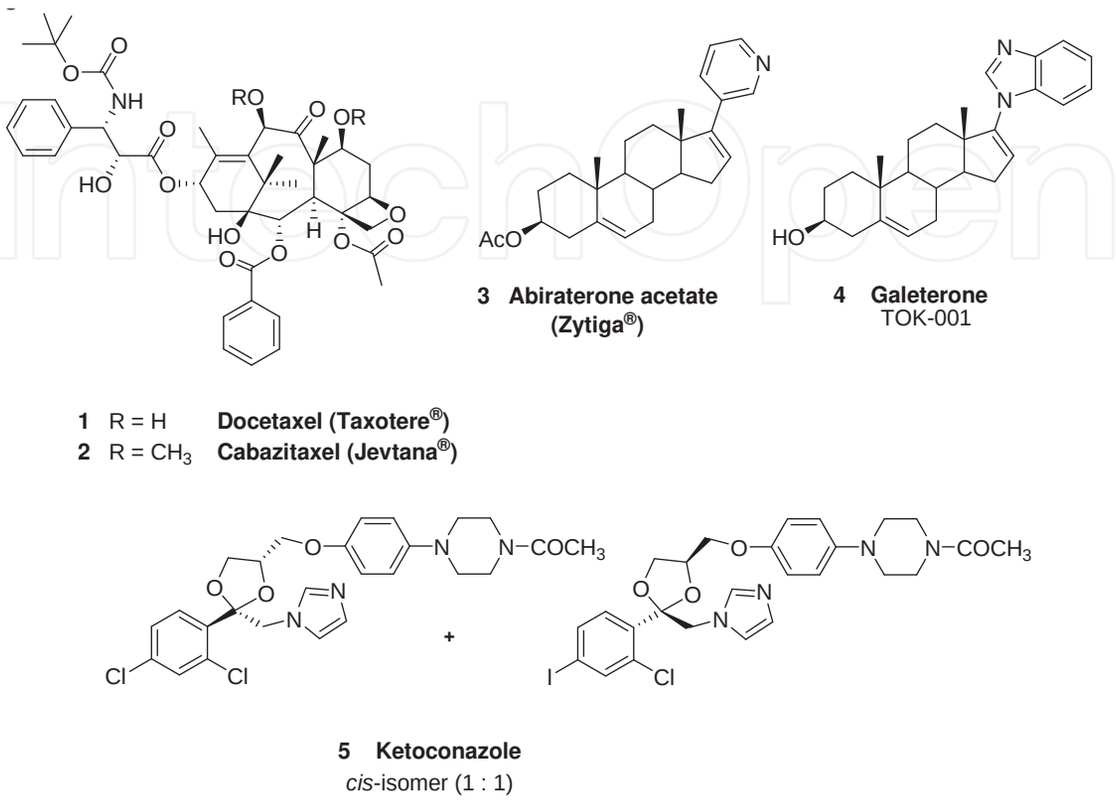


**Figure 1.** Compounds used in the clinical practice for PC treatment, and galeterone4, currently undergoing clinical trials for the treatment of chemotherapy-naive CRPC.

The first reports on steroidal CYP17 inhibitors date back to about 40 years ago [3, 8, 17-20]. Many different chemistries have been exploited in their development which has been complicated by the fact that no 3D structure of the enzyme is available. Nonetheless, structure-activity analysis has revealed the general features of a good inhibitor and recent docking and modeling studies have further shed some light on the way these molecules interact with the enzyme's active site [21, 22]. Moreover, additional effects of these compounds on other PC-related targets have been studied and disclosed. This chapter will tell the success story of the development of steroidal CYP17 inhibitors from their early discovery days to their very recent introduction into the clinics for the treatment of advanced PC.

## 2. The CYP17 enzyme: One active site, two activities

The eukaryotic class II cytochrome P450 enzyme CYP17 is an endoplasmic reticulum membrane bound multifunctional protein with 17$\alpha$-hydroxylase and $C_{17,20}$-lyase activities, both engaged on a single active site (Fig. 2) [23-28].

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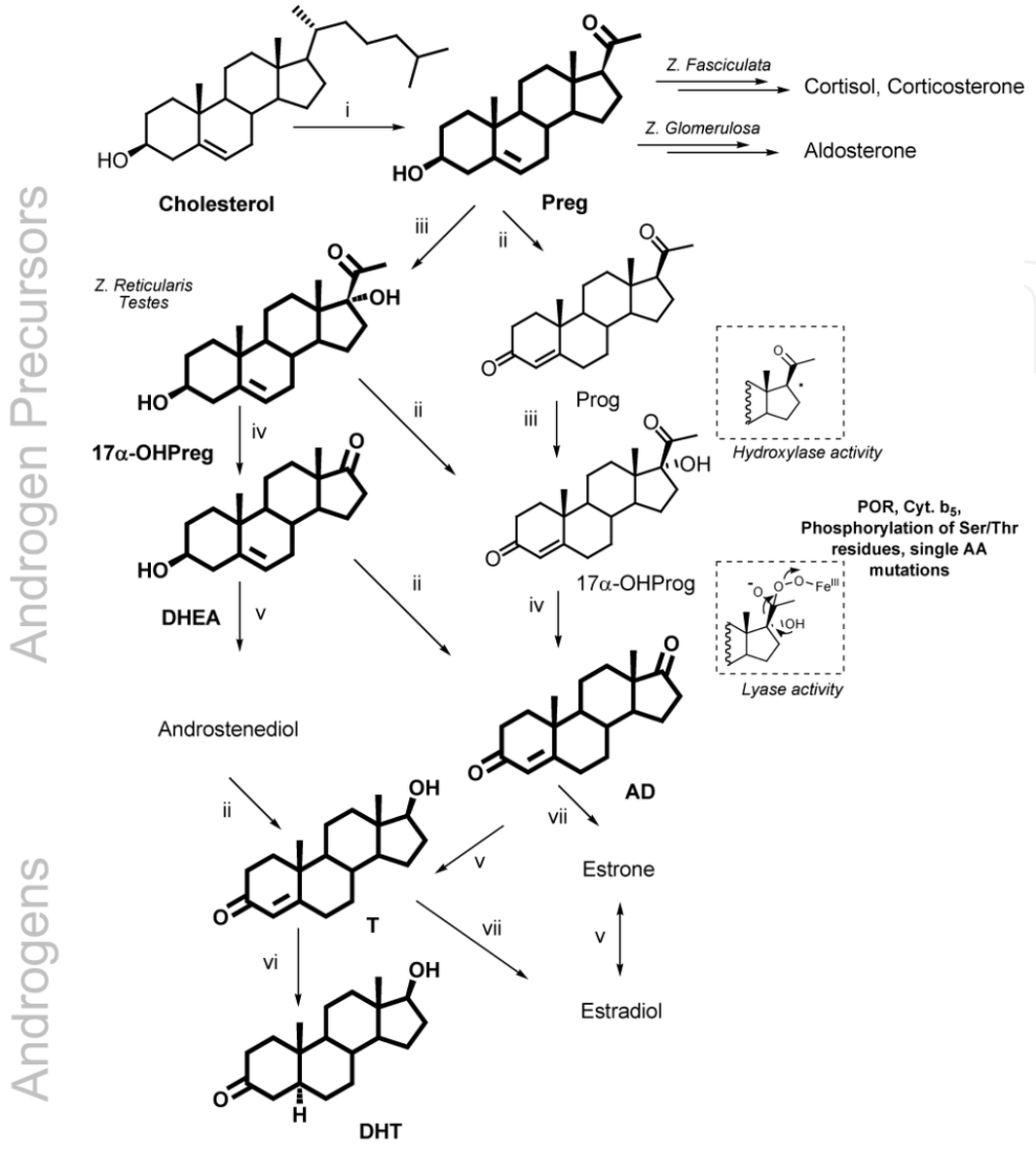


**Figure 2.** CYP17 and androgen physiology. i. P450 cholesterol side-chain cleavage ($P450_{scc}$); ii. 3β-Hydroxysteroid dehydrogenase, $\Delta^{4,5}$-isomerase; iii. CYP17 (OHase); iv. CYP17 (lyase); v. 17β-Hydroxysteroid dehydrogenase; vi. 5α-Reductase; vii. Aromatase (CYP19).

Alike other cytochrome P450 enzymes, this cysteinato-heme enzyme functions as a monooxygenase by activating and cleaving molecular dioxygen so that one of the atoms is inserted into its substrate while the other gives rise to a water molecule [29, 30]. P450 reductase transfer of electrons in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) is a requisite for both catalytic activities [29, 30]. Its natural substrates are pregnenolone (Preg) and progesterone (Prog) which are first hydroxylated at the 17 position and then their side chain is cleaved to afford 17-keto derivatives (dehydroepiandrosterone, DHEA and androstenedione, AD respectively), which are androgen precursors. The androgens (testosterone, T and dihydrotestosterone, DHT) that result from further metabolization of both DHEA and AD, bind to the AR and initiate transcription, triggering the synthesis of

specific proteins and also cell proliferation [31, 32]. Apart from male physiology, androgens are involved in PC development and progression, as at least 80% of human PCs respond favorably to androgen ablation therapy [33-35]. This dependence of PC on androgen signalling has been known for about 70 years [36, 37] and the use of strategies that effectively lower the levels of circulating androgens in PC patients has been the mainstay of PC therapy for several decades.

CYP17 is localized to the adrenals, testes, placenta and ovaries and plays a fundamental role in the synthesis of not only sex steroids but also corticosteroids. The testes are responsible for about 90-95% of the circulating androgens and the adrenals for the remaining 5-10% [38]. Human CYP17 is expressed from a single gene mapped to a specific sub-band of chromosome 10 at q24.3, in steroidogenic tissue [39-41]. This bifunctionality of the product of a single gene has been explained by modulation of the enzyme's $C_{17,20}$-lyase activity by several factors such as the presence of the electron carrier P450 oxidoreductase (POR) [42, 43], cytochrome b5 (cyt. b5) [44-48], the phosphorylation of serine/threonine residues [44, 49-51], and single amino acid mutations [52-55]. The effective ratio of $C_{17,20}$-lyase to 17$\alpha$-hydroxylase activities is under tight control during development in the human adrenal cortex, and becomes greatly elevated in adrenarche, where a rise in DHEA body concentrations is observed without concomitant increase in glucocorticoid or mineralocorticoid production [56]. Thus, production of the mineralocorticoid aldosterone occurs in the adrenal *zona glomerulosa* where CYP17 is absent. In the *zona reticularis* and in the gonads, the presence of both activities drives the production of sex steroids, whereas overexpression of 17$\alpha$-hydroxylase activity is fundamental for the production of glucorticoids in the *zona fasciculata*.

The crystal structure of CYP17 remains yet to be determined since purification from its membrane environment and subsequent reconstitution of activity *in vitro* has proved to be a difficult task [26, 29, 30]. However, the availability of some cytochrome P450 crystal structures, such as the ones from prokaryotic P450cam [57, 58], P450BM3 [59-61], and P450 CYPeryF [62], as well as the eukaryotic CYP3A4 [63] and AYP2C9 [64] among others [65], has been a valuable tool in building homology models. In addition, the high-resolution crystal structures of mammalian P450s that are significantly homologous to CYP17 and complexed to a variety of ligands [66] have now been uploaded onto the Protein Data Bank (PDB). A very recent model has been developed based on these crystal structures from closely related mammalian cytochrome P450s [21]. In another approach, a truncated, His-tagged version of human CYP17 was generated from a synthetic complimentary DNA and expressed in *E. coli* [22]. These models were used to dock known CYP17 inhibitors to the active site.

## 3. Steroidal CYP17 inhibitors

Clinical practice outcomes with ketoconazole *5* (Fig. 1), an orally administered non-steroidal imidazole antifungal agent that was first reported to cause gynecomastia in male patients [67-69], have further evidenced the value of inhibition of the steroid synthesis pathway as a therapeutic strategy for advanced PC. This compound is used clinically as the racemate of

the *cis*-isomer [17, 70], and is offered as secondary hormonal therapy to patients with CRPC, despite some significant gastrointestinal and hepatic side-effects when administered in high doses [71-73]. Following ketoconazole *5*, several non-steroidal compounds have been synthesized which displayed better inhibitory properties. In addition, modification of the original core of the enzyme's natural substrates has also afforded very potent steroidal inhibitors [3, 8, 17-20]. Based on the knowledge that was generated by this approach which was recently validated by computational studies, common features were established for optimal interaction between enzyme and substrate. Thus, a good inhibitor should possess a sufficiently large hydrophobic core, comparable to a steroid molecule, and bear electronegative groups at its external positions [74]. The presence of a heteroatom-containing group capable of coordination to the heme iron of CYP17, ofa planar $\alpha$-face to pack against the I helix; and in addition of hydrogen bonding groups such as the 3$\beta$-hydroxylto interact with conserved polar residues in a hydrogen binding network, has proved invaluable for optimal inhibition, as is the case of both abiraterone acetate *3* and galeterone *4* [22].

### 3.1. Androstanes

The first reports on CYP17 steroidal inhibitors date back to 1971 when Arth et al. synthesized and evaluated testosterone derivatives against rat testicular CYP17, following the observation that testosterone acetate *6* (Fig. 3, Table 1, entry 1) was a potent inhibitor of the enzyme [75]. Almost total abrogation of the enzyme's activity was observed after treatment with 1.5 μM of compounds *7*, *8*, and *10* (Table 1, entries 2-3, and 5), with the acetamide derivative *9* being less potent (Table 1, entry 4). Competitive inhibition of pig CYP17 was reported for the anabolic steroids mestanolone *11*, stanozolol*12*, and furazobol *13* (Fig. 3) [76]. Week inhibition in the high μM range was found with compounds *11* and *13* against the $C_{17,20}$-lyase activity whereas stanozolol *12* inhibited both enzyme activities with $IC_{50}$ values of 2.9 μM and 0.74 μM, for the 17$\alpha$-hydroxylase and $C_{17,20}$-lyase activities, respectively.

The irreversible inhibition of CYP17 by compound *14* (Fig. 3, Table 1, entry 6) was reported to occur due to the presence of a cyclopropylamino moiety capable of being activated by the enzyme by one-electron oxidation of the nitrogen atom, which causes ring opening to afford a $\beta$-iminium radical that covalently binds to the enzyme, while the compound is still bound in the active site [77]. Other related irreversible inhibitors reported include compounds *15-18* (Fig. 3, Table 1, entries 7-10) [78-81]. Compounds *15-17* were potent inhibitors of the human CYP17 at 0.8 and 1 μM, after preincubation with the enzyme (Table 1, entries 7-9). The ki values of the 4-amino derivatives *16-17* and of the sulfoxide derivatives *19-20* were determined using cynomolgous monkey and porcine testicular CYP17, respectively (Table 1, entries 8-9 and 11-12) [82]. Compound *18* also potently inhibited the activity of the monkey cynomolgous CYP17 at 0.1 μM, after preincubation with the enzyme (Table 1, entry 10) [80].

The introduction of heterocyclic moieties into molecules is a commonly used strategy in drug discovery and the design of potent steroidal CYP17 inhibitors based on this feature is an example of success. Thus, several androstane derivatives have been synthesized bearing a heterocycle ring at C17 either connected to it by a carbon (Fig. 4, Compounds *21-50*) or a nitrogen (Fig. 5, Compounds *53-60*) atom. In 1995, Jarman et al. reported the synthesis of

abiraterone *21* (Fig. 4), a 17-(3-pyridyl)androstane derivative and a potent irreversible inhibitor of human testicular CYP17 (Table 2, entry 1), about 16- and 9-fold more potent than ketoconazole *5* for the inhibition of the hydroxylase and lyase activities, respectively, with $IC_{50}$ values in the low nM range [86]. Its 3β-acetoxy derivative and prodrug, abiraterone acetate *3* (Table 2, entry 2) has helped to further evidence and establish the utility of specific CYP17 inhibition in metastatic PC (mPC) patients. In 2001, Hartmann et al. reported that the introduction of a pyrimidyl substituent at C17 originated compounds such as *22* and *23* (Fig. 4, Table 2, entries 3-4) which were more potent inhibitors of the human enzyme than both ketoconazole *5* and abiraterone *21*, under the same assay conditions, and that compound *23* effectively lowered T plasma concentrations to castrate levels after administration to mice [87, 88]. The thiazole and furan derivatives *24* and *25* were also synthesized and tested on the monkey cynomolgous enzyme (Fig. 4, Table 1, entries 13-14) [83, 85].

| Entry | Compound | Inhibitor concentration (μM) | % Inhibition[a] | Ki (nM) | $IC_{50}$ (μM) | Ref. |
|---|---|---|---|---|---|---|
| 1 | 6 | 1.5 | 65 | — | — | [75] |
| 2 | 7 | 1.5 | 95 | — | — | |
| 3 | 8 | 1.5 | 100 | — | — | |
| 4 | 9 | 1.5 | 85 | — | — | |
| 5 | 10 | 1.5 | 90 | — | — | |
| 6 | 14 | — | — | 90[b] | 4.6[c] | [77] |
| 7 | 15 | 0.8 | 64 | — | — | [78,79] |
| 8 | 16 | 1 | 84 | 339[b] | — | [80,81] |
| 9 | 17 | 1 | 86 | 286[b] | — | |
| 10 | 18 | 0.1 | 79[b] | — | — | [80] |
| 11 | 19 | — | — | 380[c, d] | 1.9[c] | [82] |
| 12 | 20 | — | — | 380[c, d] | 1.9[c] | |
| 13 | 24 | 0.1 | 58[b] | — | 0.063[b] | [83-85] |
| 14 | 25 | 0.1 | 53[b] | — | — | |

**Table 1.** Inhibition of CYP17 by androstane derivatives. [a]Human CYP17; [b]Determined on cynomolgous monkey testis enzyme; [c]Porcine testicular CYP17; [d]ki for compound 14 under the same assay conditions was 3620 nM.

A series of interesting effects on PC cells other than just CYP17 inhibition was reported by Brodie et al. for the imidazolyl, pyrazolyl, and isoxazolylandrostane derivatives *26-32* (Fig. 4, Table 2, entries 5-11). The isoxazolyl compound *32* was not only a non-competitive inhibitor of human CYP17 but also a competitive inhibitor of 5α-reductase, with potency similar to finasteride, while in addition bearing antiandrogenic activity [89-93]. Its effects were confirmed using PC xenograftmodels, however, its short half-life and rela-

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tively low bioavailability were reasoned to limit its efficacy *in vivo* [93-95]. Less successful attempts of CYP17 inhibitors design include the 5′-methyl-2′-thiazolyl androstane *33* (Fig. 4) which was a weak inhibitor of human CYP17 expressed in *E. coli* when compared to ketoconazole *5* [3]. In 2006, Wolfling et al. reported the synthesis of a series of dihydrooxazine derivatives *34-45* (Fig. 4) which low inhibitory activity of CYP17 is most likely due to the bulkiness of the C17 moieties and the absence of a double bond at C16 [96]. The same group later reported the synthesis of the oxazolidone derivative *46* (Fig. 4, Table 2, entry 12) which inhibited the activity of rat testicular $C_{17,20}$-lyase with an $IC_{50}$ value of 3 μM [97]. Similar inhibition of the enzyme was observed with the halogenated oxazoline derivatives *47* and *48* [98], and with the D-ring fused arylpyrazoline *51* (Fig. 4, Table 2, entries 13-14, and 17) [99]. The *N*-phenylpyrazolyl derivatives *49* and *50* were however much less active, with $IC_{50}$ values in the high μM range [100], as was the steroidal D-ring fused oxazolidine *52* (Fig. 4, Table 2, entries 15-16, and 18) [99].

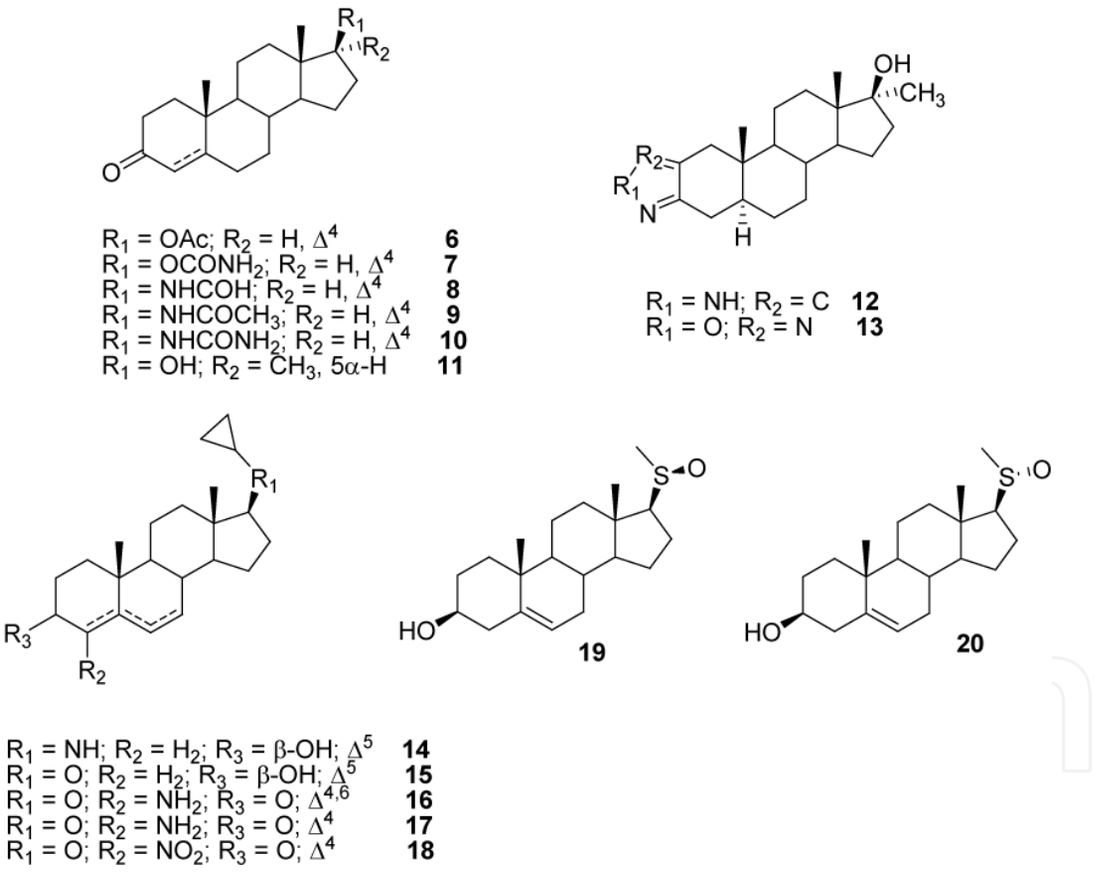


**Figure 3.** Androstane based CYP17 inhibitors.

In 1996, Njar et al. reported the first steroidal inhibitors of CYP17 bearing a heterocyclic moiety bound to C17 by a nitrogen atom [101], which included compounds *53-55* (Fig. 5, Table 2, entries 19-21), among which the imidazolyl derivative *53* was found to be the most promising [101-104]. Later, in 2005, the same group reported the synthesis of galeterone *4* and its $\Delta^4$-3-keto derivative *56* (Fig. 5, Table 2, entries 22-23) [104-106].

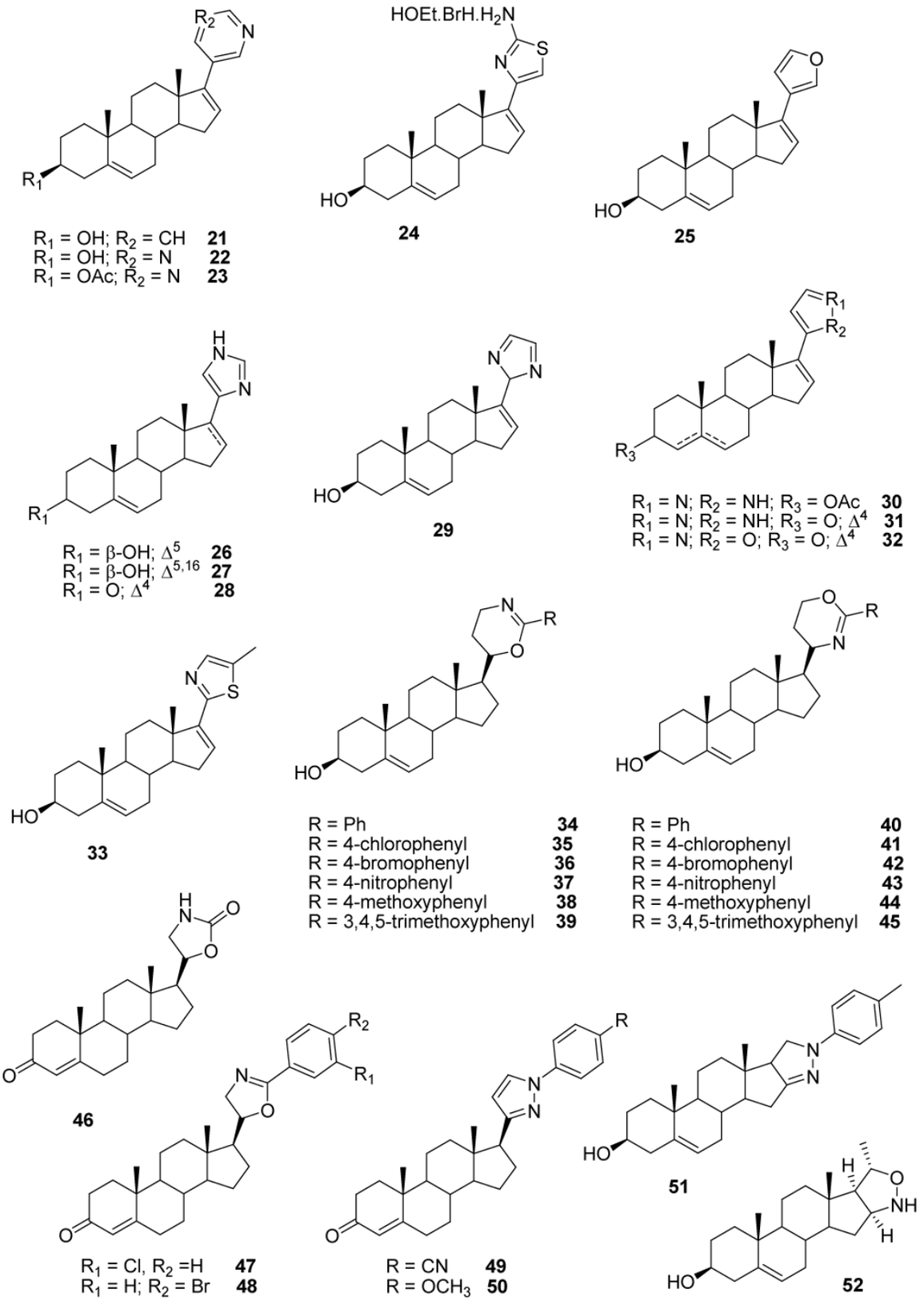


**Figure 4.** Androstane based CYP17 inhibitors.

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| Entry | Compound | CYP17 inhibition (nM) | Ref. |
|---|---|---|---|
| 1 | 21 | Human (OHase): 4<br>Human (lyase): 2.9 | [86,107] |
| 2 | 3 | Human (OHase): 18<br>Human (lyase): 17 | |
| 3 | 22 | Rat: 220<br>Human: 24<br>*E.coli* [a]: 30 | [87, 88] |
| 4 | 23 | Rat: 1460<br>Human: 38<br>*E.coli* [a]: 2500 | |
| 5 | 26 | Rat: 91<br>Human: 66 | [89, 90] |
| 6 | 27 | Rat: 49<br>Human: 24 | |
| 7 | 28 | Rat: 79<br>Human: 58 | |
| 8 | 29 | ND[b]<br>Human: 21 | |
| 9 | 30 | Rat: 28<br>Human: 42 | |
| 10 | 31 | Rat: 76<br>Human: 59 | |
| 11 | 32 | Rat: 32<br>Human: 39 | |
| 12 | 46 | Rat: 3000 | [97] |
| 13 | 47 | Rat: 4800 | [98] |
| 14 | 48 | Rat: 5000 | |
| 15 | 49 | Rat: 22000 | [100] |
| 16 | 50 | Rat: 59000 | |
| 17 | 51 | Rat: 5800 | [99] |
| 18 | 52 | Rat: 26000 | |
| 19 | 53 | Rat: 9<br>Human: 8<br>LNCaP-CYP17 cells[c]: 1.25 | [102, 103] |
| 20 | 54 | Rat: 8<br>Human: 7<br>LNCaP-CYP17 cells[c]: 2.96 | |
| 21 | 55 | Rat: 10<br>Human: 13 | |

| Entry | Compound | CYP17 inhibition (nM) | Ref. |
|---|---|---|---|
| | | LNCaP-CYP17 cells[c]: 7.97 | |
| 22 | 4 | *E.coli*[a]: 300 | [105, 106] |
| 23 | 56 | *E.coli*[a]: 915 | |
| 24 | 61 | LNCaP-CYP17 cells[c]: 11500 | [4] |
| 25 | 62 | LNCaP-CYP17 cells[c]: 17100 | |

**Table 2.** $IC_{50}$ values for androstane CYP17 inhibitors. [a]Recombinant human CYP17 expressed in *E.coli*; [b]ND = Not Determined; [c]Recombinant human CYP17 expressed in LNCaP cells.

Thus, *in vitro* results with compounds *53-55* revealed a high inhibitory potential of the human enzyme expressed in LNCaP cells. In addition, compounds *53* and *55* completely suppressed T and DHT stimulated growth of LNCaP cells below 5 μM, and displayed antiandrogenic activity [102, 108]. *In vivo* experiments confirmed these results and showed that the compounds were however less effective than castration [109]. The C17-benzimidazole derivative *4* became the first example of a CYP17 inhibitor and antiandrogen that could effectively suppress androgen-dependent tumor growth better than castration [105]. In 2007, our group reported the synthesis of the 1*H*- and 2*H*-indazole androstanes *57-60* which despite being poor inhibitors of human CYP17 displayed selective inhibition of PC-3 cells suggesting that mechanisms other than interference with the AR could be involved in their cytotoxicity [5]. We also synthesized a series of steroidal carbamates out of which compounds *61* and *62* (Fig. 5, Table 2, entries 24-25) were inhibitors of human CYP17 with $IC_{50}$ values of 11.5 and 17.1 μM, respectively [4].

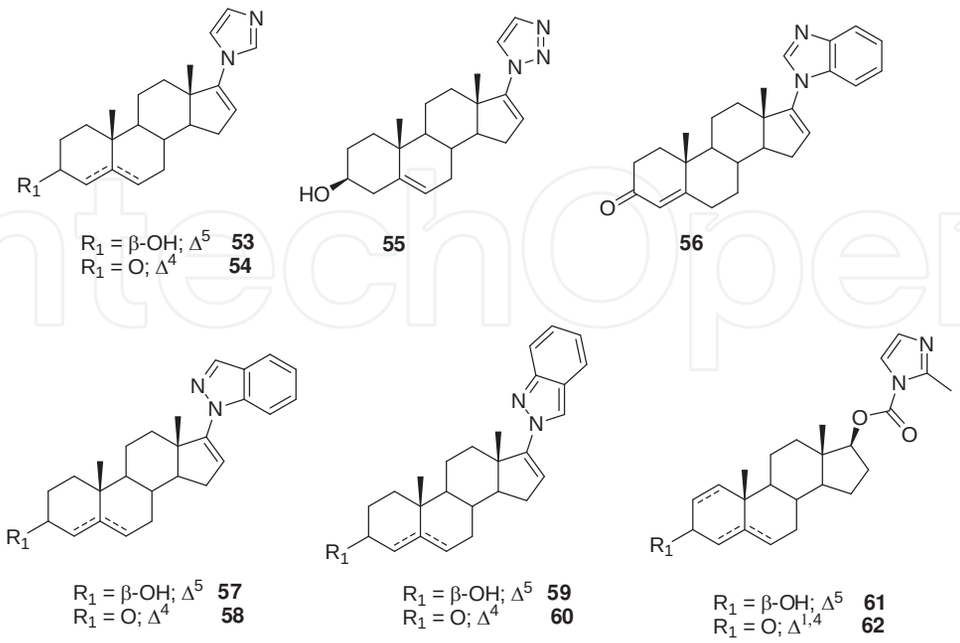


**Figure 5.** Androstane based CYP17 inhibitors.

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### 3.2. Pregnanes

Among the pregnane CYP17 inhibitors, compounds *63-65* (Fig. 6, Table 3, entries 1-3) bearing 20-substituents with moderate to strong dipole properties were more active than ketoconazole in inhibiting human CYP17, displaying $IC_{50}$ values of 16 to 230 nM and 16 to 190 nM for the hydroxylase and lyase activities, respectively [90, 110, 111]. In 2000, Hartman et al. tested several pregneneoximes *66-76* among which some were potent inhibitors of both rat and human CYP17 (Fig. 6, Table 3, entries 4-11) [112]. Compound *66* was effective *in vivo* and suppressed plasma T concentrations more potently than ketoconazole. The hydroxamic acid derivative *77* (Fig. 6) was not a CYP17 inhibitor [113].

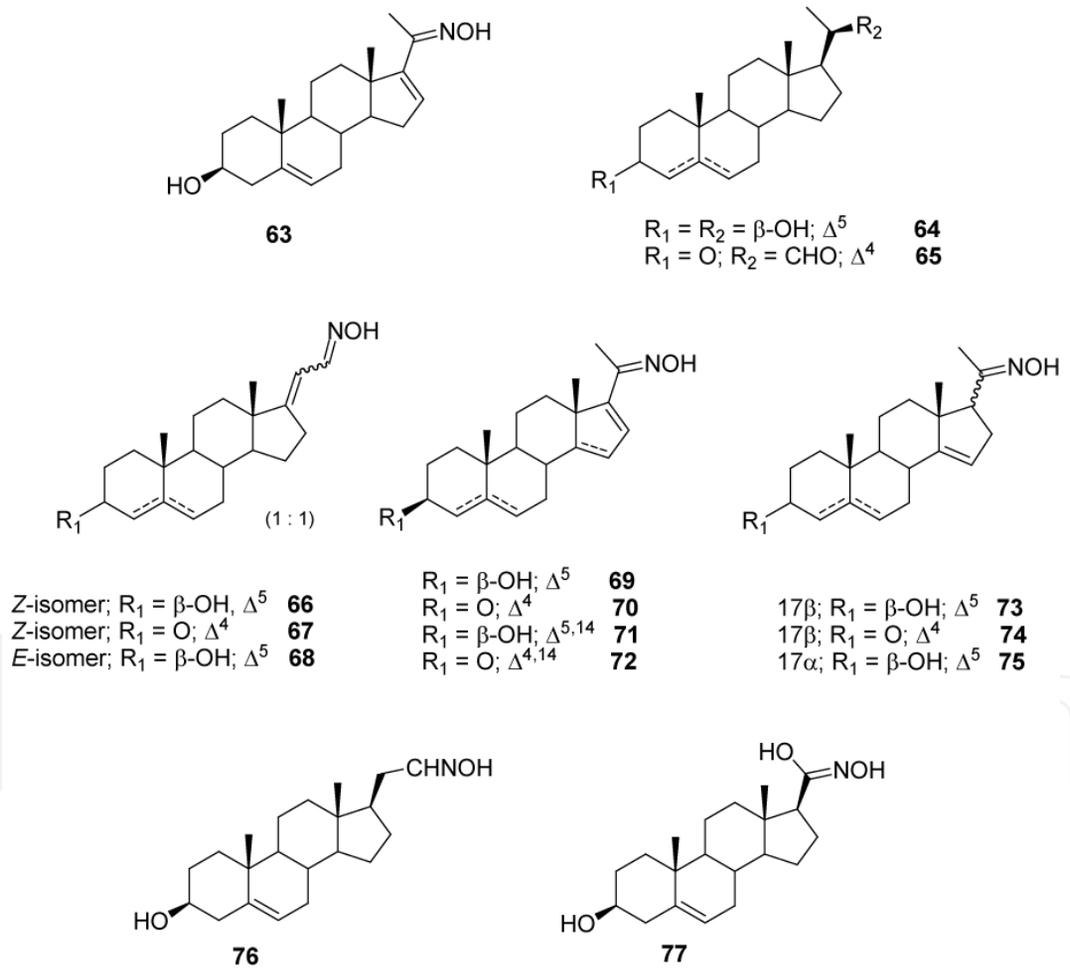


**Figure 6.** Pregnane based CYP17 inhibitors.

| Entry | Compound | CYP17 inhibition (nM) | Ref. |
|---|---|---|---|
| 1 | 63 | Human (OHase): 16<br>Human (lyase): 16 | [90, 110, 111] |
| 2 | 64 | Human (OHase): 180<br>Human (lyase): 190 | |
| 3 | 65 | Human (OHase): 230<br>Human (lyase): 160 | [90, 110, 111, 114] |
| 4 | 66 | Rat: 520<br>Human: 77<br>*E. coli* [b]: 230 | [112] |
| 5 | 67 | Rat: 140<br>Human: 180 | |
| 6 | 69 | Rat: [a]<br>Human: 170<br>*E. coli* [b]: 520 | |
| 7 | 70 | Rat: [a]<br>Human: 100 | |
| 8 | 71 | Rat: [a]<br>Human: 200<br>*E. coli* [b]: 420 | |
| 9 | 72 | Rat: [a]<br>Human: 200 | |
| 10 | 74 | Rat: 300<br>Human: 300 | |
| 11 | 76 | Rat: 2760<br>Human: 270 | |
| 12 | 78 | Rat: 210<br>Human: 540 | [115, 116] |
| 13 | 79 | Rat: 34000<br>Human: 1520 | |
| 14 | 80 | Rat: 1200 | [115] |
| 15 | 81 | Rat: 36000 | |
| 16 | 82 | Rat: 9670<br>Human: 970 | [116] |
| 17 | 83 | Rat: 430<br>Human: 290 | |
| 18 | 84 | Rat: 530<br>Human: 400 | |

| Entry | Compound | CYP17 inhibition (nM) | Ref. |
|---|---|---|---|
| 19 | 85 | Rat (OHase): 75.8<br>Rat (lyase): 55.8 | [117] |
| 20 | 86 | Rat: 600 | [118] |

**Table 3.** $IC_{50}$ values for pregnane CYP17 inhibitors. [a]≥ 125 μM; [b]*E. Coli* cells coexpressing human CYP17 and NADPH reductase

A difference in the inhibitory potential of rat CYP17 of the aziridinylpregnanes *78-81* was observed between the *S*- and *R*-isomers, the *S*-isomers *78* and *80* being 162 and 30-fold more potent than the *R*-isomers, respectively (Fig. 7, Table 3, entries 12-15) [115]. However, this finding was not corroborated by later studies that used the human enzyme [116]. The activity of compounds *82-85* (Fig. 7, Table 2, entries 16-19) was also reported [116, 117]. Several fluorinated pregnanes *86–91*and *93* were synthesized in search of greater metabolic stability (Fig. 7, Table 3, entry 20, Table 4). Inhibition of the cynomolgous monkey enzyme at 1 μM, following preincubation with the enzyme with compounds *87-93*, is depicted on Table 4[118-122].

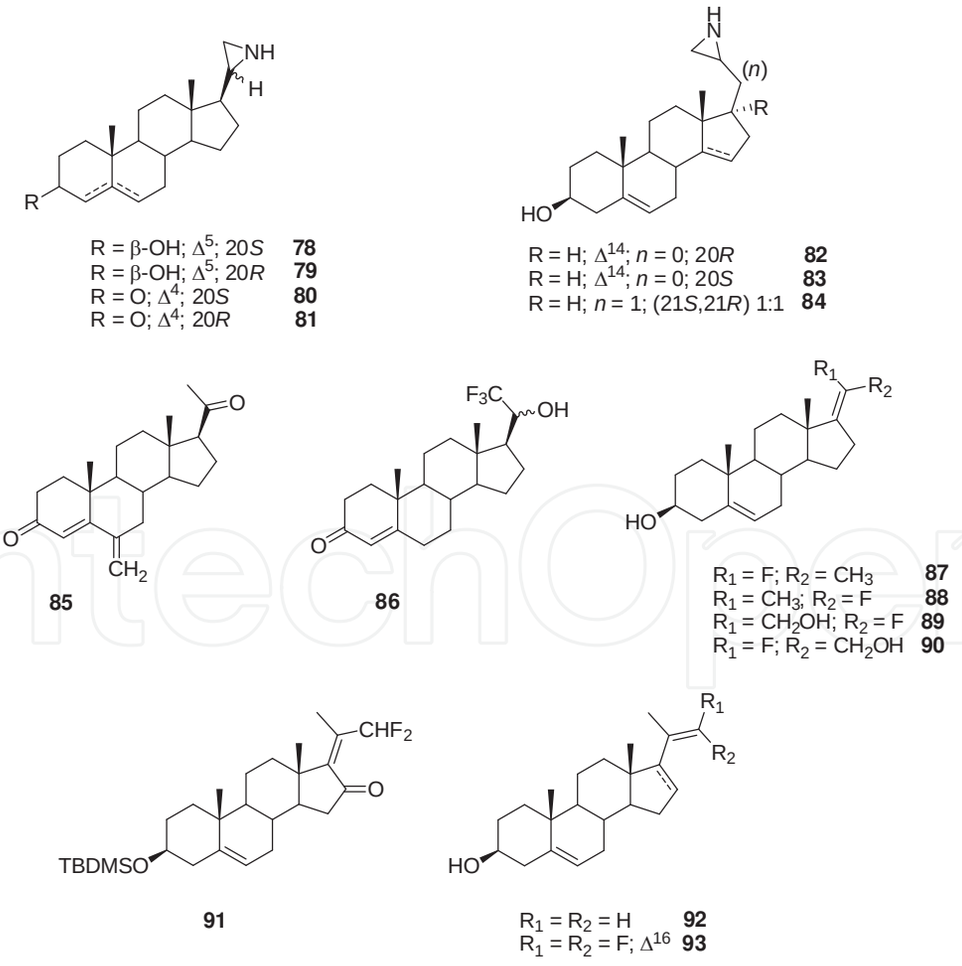


**Figure 7.** Pregnane based CYP17 inhibitors.

| Entry | Compound | % Inhibition | Ref. |
|---|---|---|---|
| 1 | 87 | 61 | [119-121] |
| 2 | 88 | 60 | |
| 3 | 89 | 61 | |
| 4 | 90 | 94 | |
| 5 | 91 | 85 | [122] |
| 6 | 92 | 60 | |
| 7 | 93 | 62 | |

**Table 4.** Inhibition of cynomolgous monkey testicular CYP17 by pregnane derivatives, at 1 μM, following preincubation with enzyme.

### 3.3. Other steroidal inhibitors

Other reported steroidal inhibitors of CYP17 are depicted on figure 8. The 17-aza derivative *94* inhibited human CYP17 with an $IC_{50}$ value of 4.9 μM [123]. Compound *95* inhibited both 5*α*-reductase and CYP17 with $k_i$ values of 27 and 14 nM, respectively [124]. The oxime *96* was also a dual inhibitor with the ability to reduce serum and prostatic T and DHT concentrations *in vivo* [125].

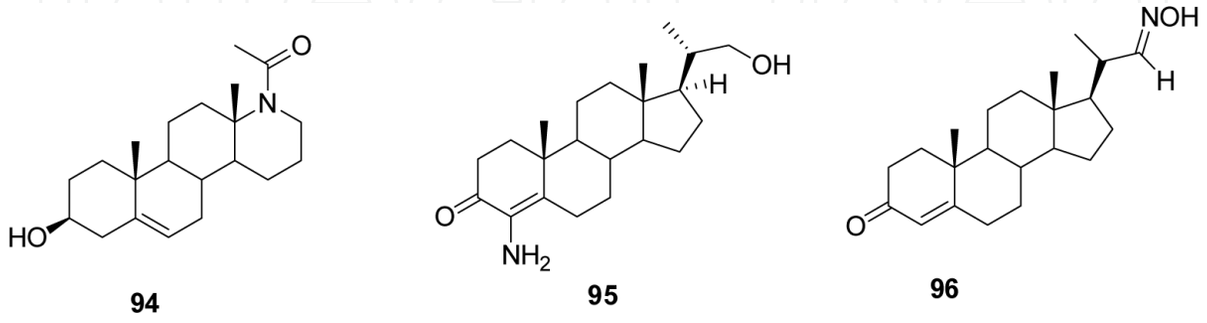


**Figure 8.** Other steroidal inhibitors of CYP17.

## 4. Abiraterone and galeterone

As previously mentioned, abiraterone acetate *3* (Fig. 1) constitutes the first and still the only steroidal CYP17 inhibitor approved by the FDA in 2011, being indicated for the treatment of mCRPC after chemotherapy [14].

This drug was developed at the Institute of Cancer Research (UK) considering the known efficacy and limitations of ketoconazole in this field and following the observation that non-steroidal 3-pyridyl esters had improved selectivity for the inhibition of CYP17. This led to the preparation of abiraterone *21* (Fig. 4), a $\Delta^{5,16}$-steroid with a 3-pyridyl group bound to C17, which revealed to be a potent and selective irreversible inhibitor of both 17$\alpha$-hydroxylase and $C_{17,20}$-lyase activities of CYP17 [86, 126, 127]. In fact, it was observed that abiraterone *21* is not only a more potent CYP17 inhibitor than ketoconazole but also is a less effective inhibitor of other CYP450 enzymes, responsible for the significant side effects and potential pharmacological interactions of ketoconazole in PC therapy [14, 128]. Accordingly, preclinical studies in mice demonstrated that abiraterone *21* reduced serumT to castrate levels, in spite of a compensatory significant increase in luteinizing hormone (LH) [126]. However, when abiraterone acetate *3*was tested in human PC patients for the first time as a substitute to gonadotropin-releasing hormone (GnRH) analogues, sustained suppression of T production was not observed due to an increase in LH levels [129]. For this reason, abiraterone *21*was developed to be concomitantly used with GnRH analogues in mCRPC [130]. Studies in xenograft models devoid of testicular and adrenal androgens further evidenced that abiraterone *21* inhibited CRPC growth and thus also seem to suppress androgen production in PC tumors [128].

Several Phase I clinical studies [131, 132] revealed that abiraterone acetate *3* is safe and effective on lowering serum androgen levels in both ketoconazole naïve and exposed patients. In addition, its antitumor activity was nearly equivalent in both groups. However, a significant increase in adrenocorticotrophic hormone (ACTH) was developed leading to hypokalemia and hypertension as the predominant toxicities. In order to reduce these side effects eplerenone, a mineralocorticoid antagonist, was introduced. As the highest studied dosage of abiraterone acetate *3* (1000mg) did not lead to limiting toxicities, the useof 1000mg daily was chosen in additional trials [8, 131, 133 135].

The concomitant use of the corticosteroids dexamethasone or prednisone in the efficacy of abiraterone acetate *3*in several conditions was studied in Phase II trials [133-135]. A significant decrease in hyperaldosteronism-related symptoms was observed and therefore prednisone 5mg b.i.d. was included in all subsequent studies, as well as in the FDA label indication. Other Phase II studies evaluated the efficacy of abiraterone in docetaxel-treated CRPC patients, and continued to evidence the importance of this steroidal drug in this stage of the pathology [135].

A Phase III study compared the use of abiraterone acetate *3*and prednisone versus prednisone alone in 1195 ketoconazole-naïve men with mCRPCshowing disease progression dur-

ing or after therapy withdocetaxel. The primary endpoint was overall survival and the secondary endpoints were PSA decline, time to PSA progression and progression-free survival. In this study an increased median overall survival in the abiraterone acetate *3*+ predisone group was observed when compared to that of patients treated with prednisone alone (14.8 vs 10.9 months; hazard ratio of 0.65). In addition, all the other endpoints were met and as expected the toxicities caused by CYP17 blockage occurred mostly in the abiraterone acetate *3*+ prednisone group. Another Phase III study set to be completed in 2014 is evaluating the use of abiraterone acetate *3* and prednisone versus prednisone alone in CRPC prior to chemotherapy [136].

Due to all these beneficial results and after the first Phase III studies, in April 2011, abiraterone acetate *3*was approved by the FDA for the treatment of mCRPC after chemotherapy [14].

Abiraterone *3* is being used in the form of its 3β-acetyl prodrug in order to increase its oral bioavailability, and is quickly deacetylated to the active drug once absorbed. In spite of the fact that high-fat meals increase its oral absorption, it is recommended that this drug should be taken on an empty stomach. Other pharmacokinetic studies revealed that this drug is highly bound to plasma proteins and has a plasma half-life of 10-14h [131, 132]. At present, several other clinical trials are ongoing, mainly for the study of the combination of abiraterone acetate *3* with other relevant drugs in PC treatment [137].

Galeterone *4* (Fig. 1) is structurally similar to abiraterone *21* and was rationally designed as an androgen biosynthesis inhibitor via CYP17 inhibition [8]. In fact, as previously mentioned, several research works evidenced that modification of the C17 substituent of $\Delta^{16}$-steroids, particularly by attachment of nitrogen heterocycles, was a relevant strategy to produce potent inhibitors of the enzyme. Following these considerations, Handratta et al. designed and prepared several $\Delta^{16}$-steroidal C17 benzoazoles and pyrazines and evaluated their CYP17 and 5α-reductase inhibitory activities, binding to and transactivation of the AR, as well as their antiproliferative effects against two human PC cell lines (LNCaP and LAPC4). Some of the compounds including *4* and its $\Delta^4$-3-ketone derivative *56* (Fig. 5) were potent CYP17 inhibitors and antagonists of both wild type and mutant AR. These compounds were the first reported examplesbearing such a dual activity. In addition, these steroids inhibited the growth of DHT-stimulated LNCaP and LACP4 PC cells with $IC_{50}$ values in the low micromolar range. Galeterone *4* and compound *56* were further studied for pharmacokinetic properties and antitumor activities against androgen-dependent LAPC4 human prostate tumor xenografts in severe combined immunodeficient (SCID) mice. Galeterone *4* was more effective than castration in its *in vivo* antitumor activity [104]. Taking this into account, Vasaitis et al. demonstrated by *in vitro* and *in vivo* studies that unlike bicalutamide and castration, galeterone *4* also caused down-regulation of AR protein expression, which appears to contribute to its antitumor efficacy. The authors also evidenced that this compound caused a significant regression of LAPC4 tumors in xenograft models, being more

potent than castration, and that treatment with galeterone *4* was also very effective in preventing the formation of LAPC4 tumors [138].

An *in vitro* study using high-passage LNCaP cells demonstrated that galeterone *4* inhibited the proliferation of these cells that were no longer sensitive to bicalutamide and had increased AR expression. In addition, the combination of galeterone *4*with inhibitors of signal transduction pathways such as gefitinib and everolimus, was proven to be synergistic when compared to either agent alone and superior to their combination with bicalutamide [139]. Later, *in vivo* studies with LNCaP and high-passage LNCaP tumor xenografts in SCID mice indicated that dual inhibition of AR and mammalian target of rapamycin (mTOR) in castration-resistant models can restore the sensitivity of tumours to anti-androgen therapy. The results observed in this study also indicated that the CYP17 and AR inhibitor galeterone *4* combined with the mTOR inhibitor everolimus may be effective in resistant PC [140].

A very recent *in vitro* study with LNCaP and LAPC4 cells demonstrated that both galeterone *4* and abiraterone *21* directly down-regulated the expression and activation of the AR via multiple mechanisms, in addition to their CYP17 inhibitory activities [141].

Due to the impressive biological activities observed, galeterone *4* is currently being evaluated in a phase I/II open label clinical trial (ARMOR1 study) as a potential drug for the treatment of castration resistant prostate cancer. This study began in 2009 and has as primary outcomes the incidence of adverse effects (phase I) and the proportion of patients with 50% or greater decrease in PSA from baseline (phase II) [137].

Recently, in a continuing study of the clinical candidate *4* and analogues as potential agents for PC treatment, putative metabolites of *4* and metabolically stable derivatives were prepared. Putative metabolites included compounds with no double bonds at C16, C5, or both as well as their corresponding 3-oxo derivatives. Metabolically stable analogues of *4*, developed to optimize its potency and to increase its stability and oral bioavailability, included their 3$\alpha$-azido, 3ξ-fluoro, 3β-mesylate and 3β-O-sulfamoyl derivatives. Several *in vitro* studies, including CYP17 inhibitory activity, binding to and transactivation of AR, as well as antiproliferative effects against LNCaP and LAPC4 cell lines, demonstrated that none of the compounds were superior to *4* in the observed effects. The 3ξ-fluoro analogue was, however, nearly 2-fold more efficacious *vs* LAPC4 xenografts than *4*. Nonetheless, the toxicity observed with this halogenated compound was of concern [142].

## 5. Conclusion

PC is one of the most prevalent causes of death in Europe and USA. In spite of important advances in the treatment of localized disease, advanced PC is still incurable. One of the most relevant PC therapeutic strategies involves the inhibition of androgen biosynthesis by

CYP17 inhibition. In fact, starting from the structure of the natural substrates of this enzyme, several steroids, mainly with a heterocyclic ring bound to C17, have been developed over the years as CYP17 inhibitors. All these studies successfully led to the approval of abiraterone acetate *3* by the FDA in 2011 for the treatment of mCRPC after chemotherapy. In addition, other clinical trials involving this drug are being performed in order to expand its clinical usefulness, namely in CRPC prior to chemotherapy and in combination with other drugs. Another steroid that is in Phase I/II clinical trials for CRPC is galeterone *4*, which is structurally similar to abiraterone *21*. However, in addition to bearing a potent and selective CYP17 inhibitory activity, this compound also modulates AR activity. As it is now clear that function of the AR axis remains crucial to a majority of patients with CRPC, its mechanism of action can be of great advantage in PC therapy, either alone or in combination with other AR-modulating agents.In the future it is expected that the invaluable knowledge provided by the use of CYP17 inhibitors in PC treatment will shed more light on the most significant biological pathways involved in this disease. The establishment of a possible role for combination regimens including CYP17 inhibitors in earlier stages of PC as a means to prevent surgery and classical chemotherapy drugs would undoubtedly contribute to improving the quality of life of PC patients.

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