Inhibitors of Steroidal Cytochrome P450 Enzymes as Targets for Drug Development

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Abstract: Cytochrome P450's are enzymes which catalyze a large number of biological reactions, for example hydroxylation, *N*-, *O*-, *S*- dealkylation, epoxidation or desamination. Their substrates include fatty acids, steroids or prostaglandins. In addition, a high number of various xenobiotics are metabolized by these enzymes.

The enzyme 17α-hydroxylase-C17,20-lyase ($P450_{17}$, CYP 17, androgen synthase), a cytochrome P450 monooxygenase, is the key enzyme for androgen biosynthesis. It catalyzes the last step of the androgen biosynthesis in the testes and adrenal glands and produces androstenedione and dehydro*epi*androsterone from progesterone and pregnenolone. The microsomal enzyme aromatase (CYP19) transforms these androgens to estrone and estradiol. Estrogens stimulate tumor growth in hormone dependent breast cancer. In addition, about 80 percent of prostate cancers are androgen dependent. Selective inhibitors of these enzymes are thus important alternatives to treatment options like antiandrogens or antiestrogens.

The present article deals with recent patents (focus on publications from 2000 - 2006) concerning P450 inhibitor design where steroidal substrates are involved. In this context a special focus is provided for CYP17 and CYP19. Mechanisms of action will also be discussed. Inhibitors of CYP11B2 (aldosterone synthase) will also be dealt with.

Keywords: Estrogen, androgen, 17α-hydroxylase-C17,20-lyase, CYP 17, CYP 19, CYP11B2, steroidal inhibitors, non-steroidal inhibitors, aromatase, aldosterone synthase, prostate cancer, breast cancer, P450.

1. INTRODUCTION

1.1. General

General strategies to remove the influence of estrogens and androgens on tumor-growth involve the use of anti-estrogens (breast cancer) and anti-androgens (prostate cancer). Newer, and possibly more efficient alternatives, seek to reduce circulating and tissue levels of the relevant hormone by inhibition of a specific target enzyme [1]. The cytochrome P450 monooxygenase enzyme system is involved in the synthesis and/or degradation of a large number of endogenous compounds and in the biotransformation of drugs and other xenobiotics. Inhibitors of aromatase and 17α-hydroxylase-C17,20-lyase became highly important targets in this area. More recently inhibitors of aldosterone synthase (CYP11B2) were found to be potentially useful for the treatment of e.g. congestive heart failure [2-4]. This review discusses the progress made in the field of inhibitors of these three cytochrome P450 enzymes.

1.2. Enzyme Assays

In this section those biological assays are briefly described which were employed for the determination of *in vitro* and *in vivo* activities. For a more detailed description of these tests, original literature should be consulted. Whenever possible, reference compounds were included into the data sets presented. Such standard references are considered to be highly important, since a comparison of inhibitory activities reported by different research groups has to be done with caution. Different enzyme preparations might have been used (e.g. microsomal preparations or whole cells) and mainly IC_{50} values are given for *in vitro* tests which are dependent on substrate concentration, Km value of the substrate and, especially for irreversible inhibitors, also on the enzyme concentration.

The most common procedure for the determination of aromatase inhibition is the one developed by Thompson and Siiteri. The compounds are incubated using [1β2β ^{3}H] Testosterone as a substrate with human placental microsomes as enzyme preparation. The activity of the compounds should be compared with the one of known references Letrozole, YM511, Anastrozole or Aminoglutethimide.

Inhibition of aromatase *in vivo*: In juvenile female rats androstenedione treatment stimulates uterine weight significantly. This effect is caused by ovarian aromatization of the androgen and can be antagonized dose-dependently by aromatase inhibitors.

The anti-tumor activity of compounds can be determined using the dimethylbenzanthracene (DMBA) induced mammary carcinoma of the ovariectomized testosterone-propionate treated Sprague-Dawley rat. The effects of the compounds are described as percent of complete / partial remission, no change and progression in comparison to an ovariectomized control.

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The effect of an inhibitor on the plasma estradiol (E2) level can be determined using pregnant mares serum gonadotropin (PMSG) stimulated female rats. The E2 lowering effect of the compounds is determined after subcutaneous application of a single dose of inhibitor using conventional radioimmunoassay techniques [5].

Steroid sulfatase (STS) inhibitory potency is assessed using [1β ^{3}H] androstenedione as a substrate and intact monolayers of JEG-3 cells [6,7].

The combined activity *versus* the enzyme complex 17β-hydroxylase-C17,20-lyase is determined using microsomal enzyme fractions derived from rat or human testes using progesterone as substrate. In order to assess the more preferred second step of this enzymatic reaction recombinant C17,20-lyase can be expressed in SF9 cells from which enriched micorsomes are prepared with ^{3}H-dihydroepiandrosterone as substrate [8,9].

Activity for CYP11B2 (aldosterone synthase) and selectivity versus CYP11B1 (steroid 11 β-hydroxylase) is determined using V79MZh 11B1 and V79MZh 11B2 cells with 11-desoxycorticosterone as a substrate [10].

2. INHIBITORS OF AROMATASE

2.1. Mechanism and Types of Inhibition

Estrogens have crucial roles in various physiological processes which include the development of the female sexual organs, the reproductive cycle and other neuroendocrine functions. These hormones are also involved in mammary and endometrial carcinomas. Approximately two thirds of postmenopausal breast cancer patients have an estrogen-dependent tumor, the growth of which requires estrogen due to the presence of estrogen receptors. Estradiol, the most potent endogenous estrogen is biosynthesized from testosterone by the cytochrome P450 enzyme aromatase as shown in Fig. (**1**).

The enzyme aromatase is a monooxygenase, and represents a complex of a cytochrome P450 heme protein and a NADPH dependent cytochrome P450 reductase which pro-

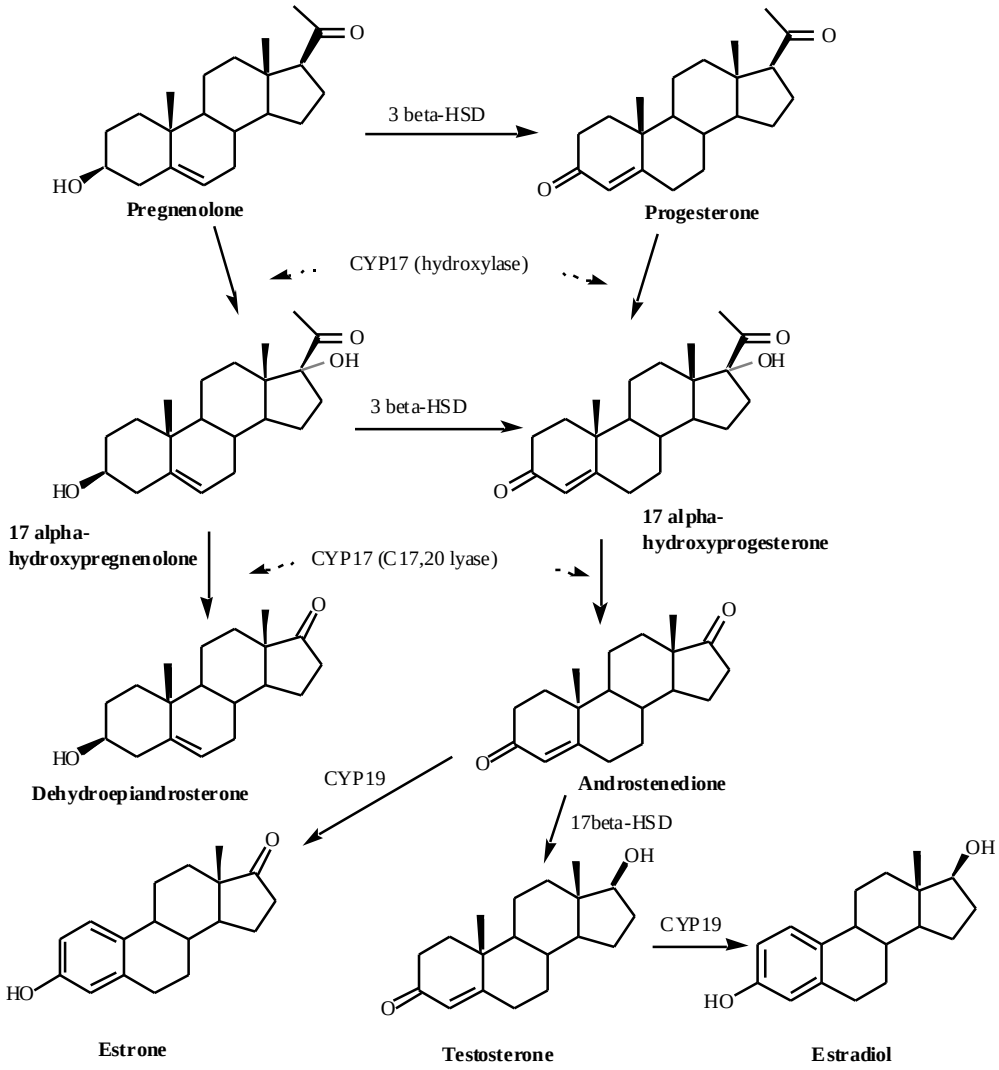


Fig. (1). Biosynthesis of Estradiol.

vides the heme protein with electrons. The catalytic process requires 3 moles NADPH and 3 moles of molecular oxygen per mole substrate. The methyl group in position 10 is cleaved as formic acid and the steroidal A ring is aromatized. This aromatization process consists of three reaction steps. The two first ones are hydroxylation processes on the methyl group in position 10 resulting in the formation of a geminal diol which, after cleavage of water, results in the formation of an aldehyde. The final hydroxylation occurs most probably on the same carbon atom with a nucleophilic attack of an iron (III) peroxy species on the carbonyl carbon. Cleavage of formic acid results in the aromatization and gives the estrogenic products [11].

The highest concentrations of this enzyme are found in the ovaries of premenopausal women, in the placenta, and in the peripheral adipose tissue of postmenopausal women. Consequently numerous groups developed inhibitors as depicted in Fig. (**2**), of the enzyme aromatase in order to suppress estrogenic effects [11].

Various steroidal structures were prepared, amongst them competitive inhibitors and mechanism based inhibitors like Formestane and Exemestane [12]. In view of undesired side effects due to the steroidal nature of the compounds and because of extensive first-pass metabolism (Formestane), non-steroidal compounds became more attractive as drug targets.

A common structural feature of these non-steroidal inhibitors is the presence of a sterically non-hindered hetero-atom which coordinates the heme iron of the cytochrome

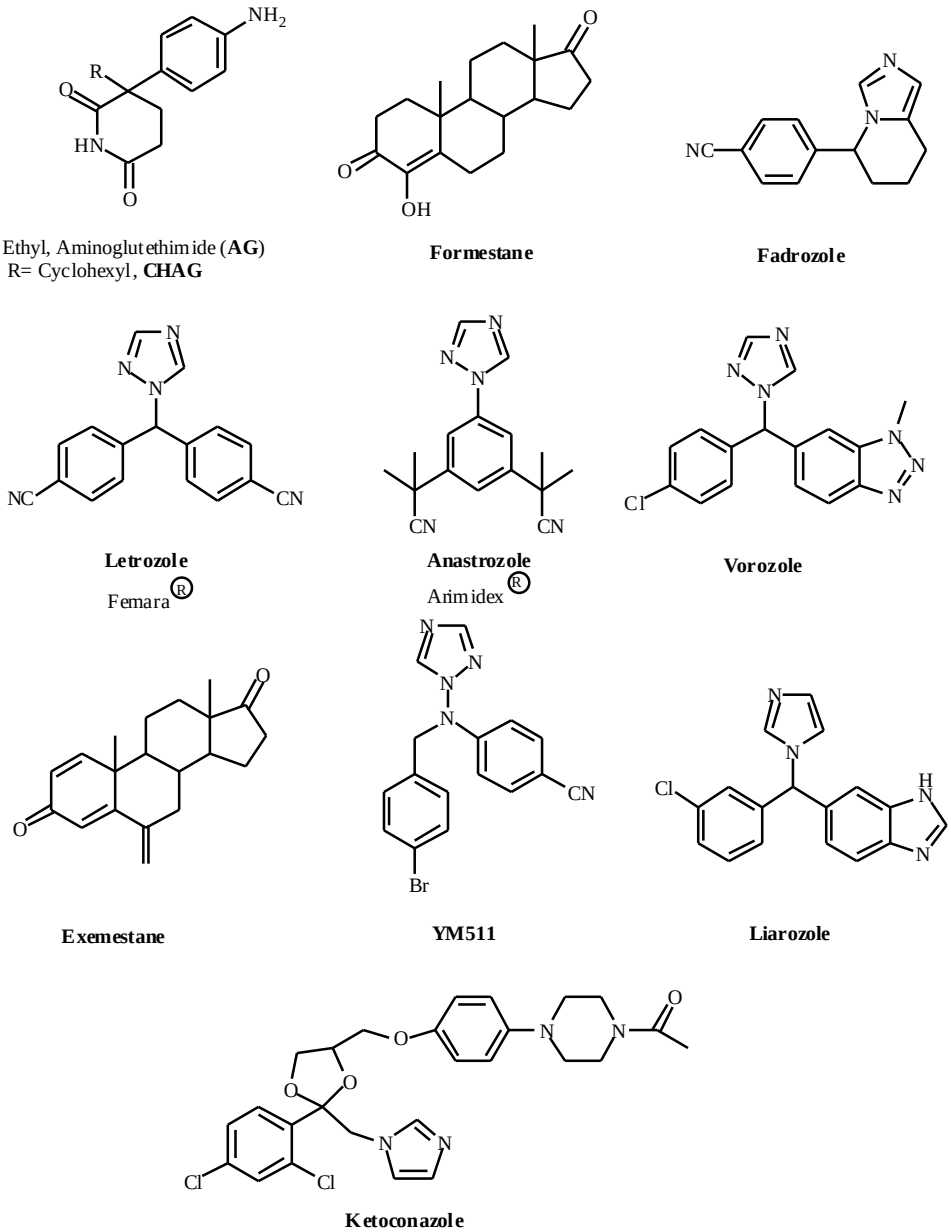


Fig. (2). Aromatase inhibitors which suppress estrogenic effects.

P450 [1,13-18]. Aminglutethimide (AG), a rather weakly potent "first generation inhibitor", was used with success in the treatment of patients with advanced breast cancer, but suffered from a poor selectivity with respect to other P450 enzymes. Fadrozole, Fig. (**2**), a "second generation inhibitor" is more potent and more selective but still influences e.g. aldosterone and corticosterone biosynthesis, Fig. (**3**). The two most advanced "third generation inhibitors" are represented by Anastrozole [19] and Letrozole [20]. Others like **YM511** [14,15] or **CHAG**[16] are highly active as well, but were abandoned because of market competitive reasons.

2.2. Novel Inhibitors of Aromatase

Highly active inhibitors of aromatase have been reported by Park *et al.* [21]. Formally the compounds of Table **1** can be considered analogues of Vorozole where the benzotriazole moiety has been replaced by more polar benzothiazole / benzoxazole groups. Furthermore, an imidazole heterocycle was used instead of a 1,2,4-triazole ring to coordinate the heme iron. In the benzoxazole series the most active compounds were found to be analogues with a nitrile substituent on the phenyl ring. Methylation of the benzoxazole nitrogen leads to a decrease of activity for the 4-CN derivatives (**PCH113** / **PCH27**); activity was not affected significantly for the 3-CN phenyl derivatives (**PCH119** / **PCH122**). The replacement of the oxygen by a sulfur atom resulted in a pronounced increase of inhibitory potency (**PCH215** / **PCH113**). This improvement of *in vitro* activity leads to a better activity *in vivo* as well. At a dose of 10 µg/kg compound **PCH215** reveals an inhibition of 56% in the antiuterotrophic test on juvenile SD rats (example **PCH113** only 19%).

In the next series of compounds (Table **2**), the imidazole heterocycle is replaced by a 1,2,4-triazole ring, a modi-

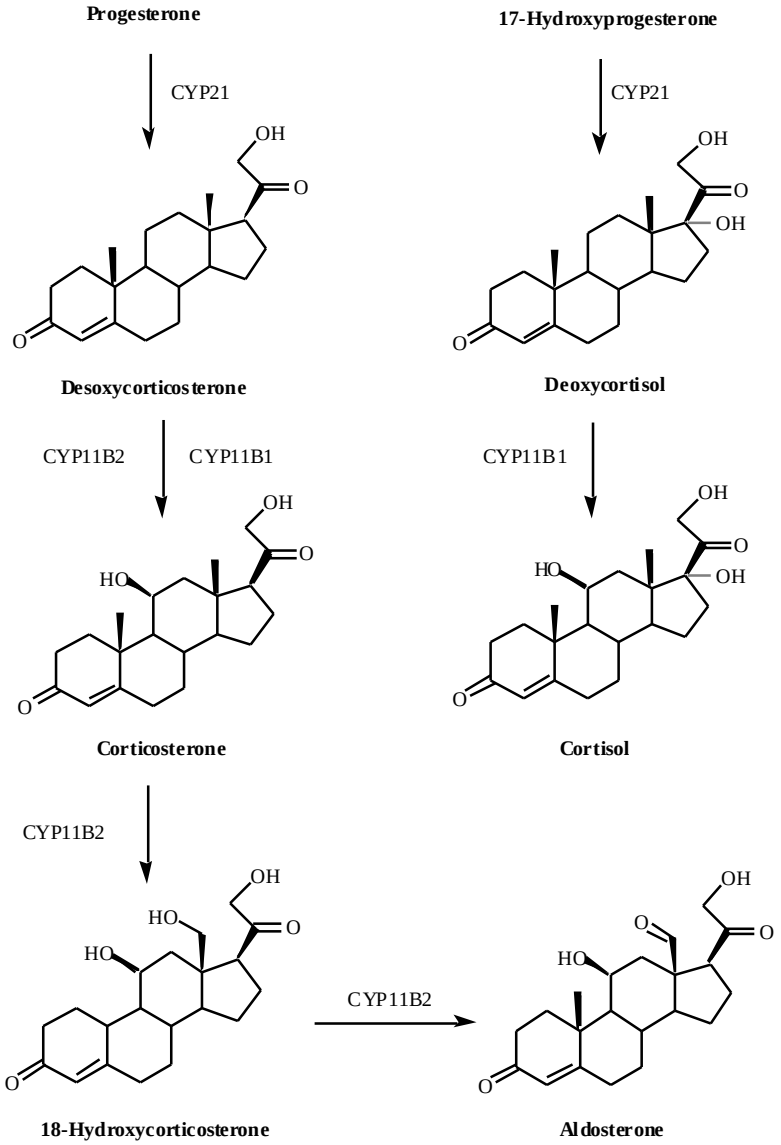
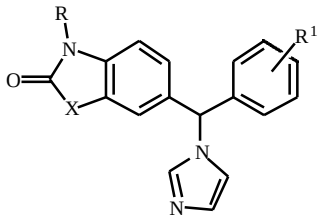


Fig. (3). Aldosterone and Corticosterone Biosynthesis.

Table 1. Inhibition of Human Placental Aromatase by Imidazole Substituted Benzoxazoles and Benzothiazoles



Compound[a]	X	R	R^1	IC_{50} [nM][b]	*in vivo* (10 µg/kg), % inhibition[c]
PCH10	O	H	H	85	-
AL22	O	CH_3	H	320	-
PCH15	O	CH_3	4-Cl	>2000	-
PCH113	O	H	4-CN	13	19
PCH27	O	CH_3	4-CN	46	-
PCH119	O	H	3-CN	25	-
PCH122	O	CH_3	3-CN	19	39
PCH100	S	H	H	34	-
PCH28	S	CH_3	H	12	13
PCH215	S	H	4-CN	4	56
PCH165	S	CH_3	4-CN	5	23
PCH241	S	CH_2CH_3	4-CN	4	16
Letrozole				4	90

[a]The numbering of the compounds is identical to that of the corresponding patent citation. [b]Enzyme inhibition was determined according to Purba et al. using human placenta microsomes. [c]*in vivo* Activity was determined according to Bhatnangar et al. [22] measuring the inhibition of androgen stimulated uterine growth (juvenile Sprague-Dawley rats).

fication which represents a closer representation of the "Vorozole" motif. Surprisingly compound **PCH20** is only a weak inhibitor (IC_{50} > 3000 nM). The substitution of the oxygen for a sulfur atom resulted in highly potent inhibitors which are furthermore characterized by a remarkable *in vivo* activity (**PCH158**, 56 %). However, a significant improvement over the sulfur series of Table **1** could not be achieved, since compound **PCH215** has a similar *in vivo* activity. When the sulfur was replaced by a selenium atom, a higher *in vivo* activity was observed (**PCH300**, 86 %).

It can be noted that *N*-alkylation does not affect *in vitro* activity (**PCH216 / PCH260** and **PCH302 / PCH 303**), whereas *in vivo* activity is influenced significantly.

The relocation of the imidazole-methyl group from the 6- to the 5 position of the benzoxazole / benzothiazole was studied as well (Tables **3** and **4**). In the benzoxazole series, very potent inhibitors were found and again the *para* cyanophenyl group proved to be very efficient. With analogue **PCH128** (IC_{50} = 6 nM) a maximum of activity was observed.

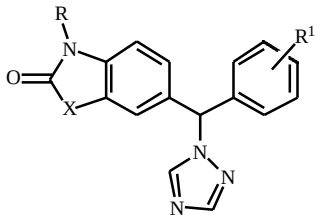
Analogous to the series of structures presented in Table **2**, the replacement of the imidazole-ring by a 1,2,4-triazole group results in a significant improvement of both *in vitro* and *in vivo* activity (Table **4**). The benzothiazoles gave the most potent inhibitors and with compound **PCH163** a highly active congener *in vivo* could be identified (83 % inhibition at 10 µg/kg).

Also at a lower dose (1 µg/kg) this compound reveals a very good *in vivo* activity (57 %, Table **5**). From the other structures tested, compounds **PCH158** and **PCH300** showed a high activity at this low dose as well.

It can be concluded that benzothiazoles are superior to benzoxazoles and a 1,2,4-triazole is preferred over an imidazole heterocycle as heme complexing agent. However, selenium appears to be equivalent to sulfur as hetero atom. One might argue that these selenium-type compounds are no real drug candidates in view of the possible formation of toxic metabolites. The structural resemblance to Ebselen (2-phenyl-1,2-benzisoselenazol-3(2*H*)-one) could however allow the assumption that only metabolites of low toxicity are formed. This highly interesting structure-activity study resulted in active structures *in vitro* and *in vivo*. Furthermore the rather uncommon use of a selenium atom as a replacement option for oxygen gave active congeners as well.

There is evidence that a considerable amount of estrone formed from androstenedione is converted to estrone sulfate.

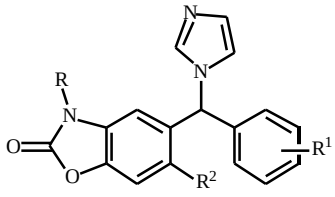
Table 2. Inhibition of Human Placental Aromatase by 1,2,4 Triazole Substituted Benzoxazoles, Benzothiazoles and Benzoselenothiazoles



Compound[a]	X	R	R^1	IC_{50} [nM][b]	*in vivo* (10 µg/kg), % inhibition[c]
PCH20	O	CH_3	H	> 3000	-
PCH216	S	H	4-CN	7,5	32
PCH158	S	CH_3	4-CN	9	56
PCH260	S	CH_2CH_3	4-CN	4,5	50
PCH258	S	CH_3	3-CN	32	-
PCH259	S	CH_3	4-NO_2	3	63
PCH243	S	CH_2CH_3	4-NO_2	4	-
PCH302	Se	H	4-CN	6,5	20
PCH300	Se	CH_3	4-CN	5	86
PCH303	Se	CH_2CH_3	4-CN	4	60
PCH304	Se	CH_3	4-NO_2	4	-
PCH305	Se	CH_2CH_3	4-NO_2	4	-

[a-c] see table 1

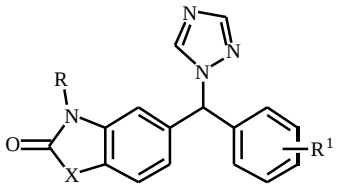
Table 3. Inhibition of Human Placental Aromatase by Imidazole Substituted Benzoxazoles



Compound[a]	R^2	R	R^1	IC_{50} [nM][b]	*in vivo* (10 µg/kg), % inhibition[c]
PCH124	H	H	H	15	34
PCH31	H	CH_3	H	47	-
PCH128	H	H	4-CN	6	29
GCA36	OCH_3	H	4-CN	20	-

[a-c] see table 1

Table 4. Inhibition of Human Placental Aromatase by 1,2,4 Triazole Substituted Benzoxazoles, and Benzothiazoles



Compound[a]	X	R	R^1	IC_{50} [nM][b]	*in vivo* (10 μg/kg), % inhibition[c]
PCH183	O	CH_3	H	1800	-
PCH160	O	CH_3	4-Cl	19	-
PCH105	O	H	4-CN	17	-
PCH196	O	CH_3	4-CN	25	-
PCH132	S	H	H	180	-
PCH134	S	CH_3	H	180	-
PCH163	S	CH_3	4-CN	6	83
PCH246	S	CH_2CH_3	4-CN	5,5	45

[a-c] see table 1

Table 5. Effect of Select Derivatives on the Androgen Stimulated Uterine Growth at Doses of 1 and 10 μg/kg

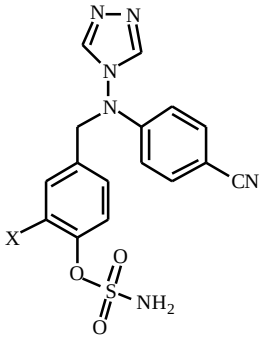
Compound[a]	% inhibition (10 μg/kg)	% inhibition (1 μg/kg)[b]
PCH215	56	0
PCH158	56	54
PCH260	50	32
PCH259	63	31
PCH300	86	49
PCH303	60	38
PCH163	83	57
PCH246	45	22

[a] See Table 1[b] *in vivo* activity was determined according to Bhatnangar [22] *et al.* measuring the inhibition of androgen stimulated uterine growth (juvenile Sprague-Dawley rats).

The hydrolysis of estrone sulfate to estrone represents an important source of estrogen in breast tumors. Consequently, the combined inhibition of both aromatase and steroid sulfatase could result in an improvement over pure aromatase inhibition [16].

The compounds presented by Potter *et al.* [23] are analogues of the very potent aromatase inhibitor **YM511**, which itself is derived from Letrozole (Table **6**). Replacement of the bromine atom in **YM511** by a sulfamic acid group resulted in a strong inhibitor of aromatase (**STX258**). Steroid

Table 6. Inhibition of Aromatase and Steroid Sulfatase by Sulfamide Derivatives



Compound[a]	X	Aromatase IC_{50} (nM)[b]	Sulfatase IC_{50} (nM)[c]
STX258	H	100	227
STX700	F	12	40
STX694	Cl	2.3	20
STX681	Br	0.8	39
YM511		0.5	

[a] See Table 1 [b,c]Aromatase and sulfatase inhibition was determined using JEG cells with androstenedione and estrone sulphate as substrates.

sulfatase was inhibited as well. The introduction of a halogen atom in position *ortho* of the sulfamic acid group leads to a strong increase of aromatase inhibitory potency with a maximum for the bromo analogue. Steroid sulfatase inhibition is also improved when the hydrogen is replaced by a halogen

atom. However, the maximum activity here is to be found with a chlorine substitution (**STX694**). As could have been expected, the maximum activity for both enzymes does not fall together, but the activity of compounds **STX694** and **STX681** as such is remarkable.

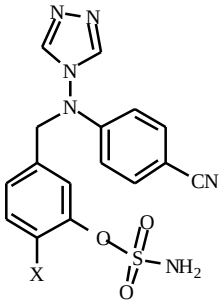
Other structures prepared in this series did not result in active dual inhibitors. Some examples are shown in Table **7**.

More recent studies by the same group [24] aimed at exploring the influence of a relocation of the sulfamide group from the *para* to the *meta* position. This resulted in a significant increase in aromatase inhibitory potency compared to the "*para*-series" (IC_{50} = 39 nM for **STX334** (Table **8**) and IC_{50} = 100 nM for **STX258** (Table **6**).

However, a drastic loss of sulfatase activity accompanied this modification. In analogy to the *para* congeners the introduction of a fluorine atom in position *alpha* of the sulfamide group gives an enhancement of both aromatase and sulfatase inhibition (**STX1122**). Surprisingly a shift to heavier halogens does not increase sulfatase activity but a complete loss of inhibition is observed. The bromine analogue also does not show any sulfatase inhibition but still is an excellent aromatase inhibitor. The introduction of a donor substituent (OCH_3) instead of a halogen atom does not improve sulfatase inhibition significantly which underlines the importance of both electronic and steric effects.

Very close analogues of **YM511** [14,15] were prepared in the series of compounds according to Fig. (**4**), where the 1,2,4-triazole moiety is replaced by an imidazole ring [25]. In the 4-position of the benzyl group substituents like CF_3, CN or halogens were introduced (Table **9**). In the test for reduction of plasma estradiol levels the compound shown in entry 1 (Table **9**) proved to be nearly twice as active as **YM511**. This compound is characterized by the simultaneous presence of fluorine atoms in positions *meta* and *para*.

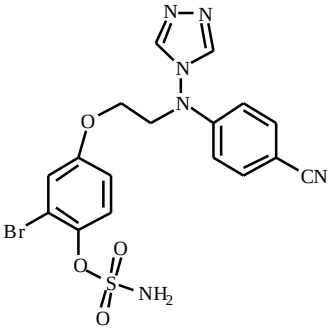
Table 8. Inhibition of Aromatase and Steroid Sulfatase by 1,2,4-Triazoles



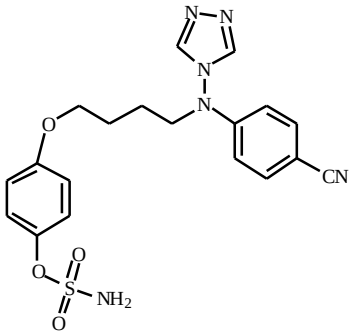
Compound[a]	X	Aromatase IC_{50} (nM)[b]	Sulfatase IC_{50} (nM)[c]
STX334	H	39	5133
STX1122	F	0.77	590
STX559	Cl	0.92	> 10000
STX1217	Br	3.9	> 10000
STX661	OMe	12	12 % at 10 μM

[a-c] See Table 6

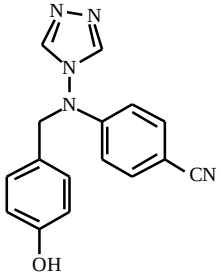
Table 7. Inhibition of Aromatase and Steroid Sulfatase by Different 1,2,4-Triazoles



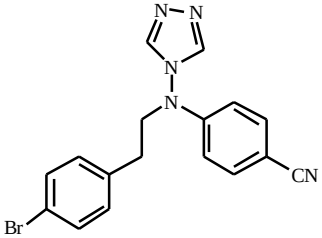
STX301



STX288



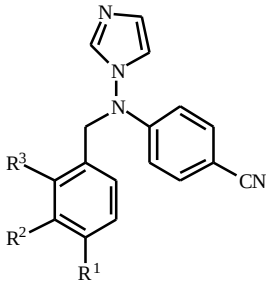
STX265



STX290

Compound[a]	Aromatase IC_{50} (nM)[b]	Sulfatase IC_{50} (nM)[c]
STX301	119	n.d.
STX288	31	> 10 μM
STX265	23	n.d.
STX290	1.3	n.d.

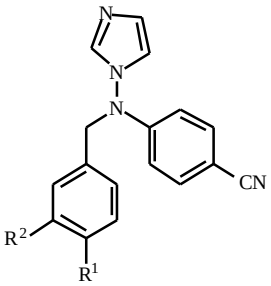
[a-c] See Table 6



R^1 = CF_3, Hal, OMe, Me, CN, SMe, SO_2Me

Fig. (4). Imidazole analogues of YM511.

Table 9. Reduction of the Plasma Estradiol Level *in vivo* Activity of Some Select Imidazole Derivatives



Entry	R^1	R^2	% inhibition[a]
1	F	F	60
2	CF_3	H	57
3	Cl	H	47
4	CN	H	57-59
5	F	H	49-57
YM511	-	-	35

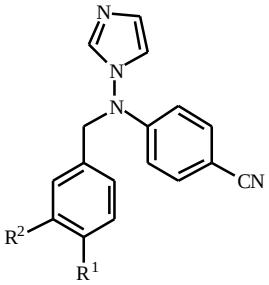
[a]*in vivo* Activity at 10 μg/kg; reduction of plasma estradiol levels determined by radioimmunoassay

All compounds prepared in this series proved to be highly active inhibitors of aromatase *in vitro* with IC_{50} values in the range of 0.14-1.6 nM (**YM511**, IC_{50} = 0.3 nM).

Further structural modifications in this series of compounds lead to analogues depicted in Tables **10** and **11** [26]. In the first series, structures were prepared which have a *para*-hydroxy phenyl group. The derivative shown in entry 1 of Table **10** is characterized by a very good aromatase inhibitory potency (IC_{50} = 0.17 nM) which is superior to the one of Anastrozole and also Letrozole.

The introduction of substituents in position *ortho* of the hydroxyl group does not affect activity to a significant extent. None of the compounds in this series inhibits the enzyme steroid sulfatase. However, when the OH-group

Table 10. Inhibition of Aromatase and Steroid Sulfatase by Imidazole Derivatives

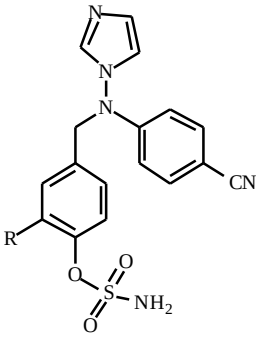


Entry	R^1	R^2	Aromatase IC_{50} (nM)[a]	Sulfatase IC_{50} (nM)[b]
1	OH	H	0.17	n.d.
2	OH	Br	0.17	n.d.
3	OH	OMe	0.21	n.d.
4	OH	NO_2	0.3	n.d.
5	OH	NH_2	2.2	n.d.
Anastrozole			6.93	n.d.
Letrozole			0.47	n.d.

[a,b] Determined using the JEG-3 cell line with androstenedione and estrone sulfate as substrates.

(Table **10**, entry 1) is replaced by a sulfamate group a strong dual inhibitor of aromatase and steroid sulfatase could be identified (Table **11**, entry 1). Also here the introduction of a bromine atom in position *ortho* leads to a better inhibition for both enzymes.

Table 11. Inhibition of Aromatase and Steroid Sulfatase by Imidazole Derivatives in the Presence of a Sulfamate



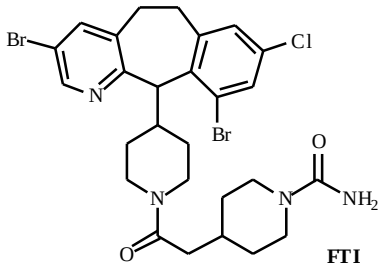
Entry	R	Aromatase IC_{50} (nM)[a]	Sulfatase IC_{50} (nM)[b]
1	H	0.57	5.5
2	Br	0.13	3.4
3	OMe	0.79	13.8

[a,b] See table 9.

The bromo derivative (Table **11**, entry 2) was furthermore assessed for its inhibitory potency *versus* human carbonic anhydrase II. This enzyme catalyses the conversion between carbon dioxide and bicarbonate and is considered to be implicated in hormone-dependent and non-hormone dependent cancer genesis. EMATE (estrone-3-sulfomate) a known steroid sulfatase inhibitor was also shown to be an inhibitor of carbonic anhydrase. It proved to be highly active as an inhibitor of carbonic anhydrase with an IC_{50} value of 16.2 nM (Acetazolamide IC_{50} = 11,3 nM). Various *in vivo* tests were performed as well. Most importantly it could be shown that this compound resulted in a 40% decrease in tumor volume (estrogen receptor positive human breast tumor tissue xenografted into ovariectomized nude rats under administration of estrone sulfate).

2.3. Combination Therapies

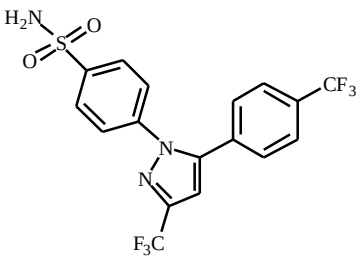
The combination of a farnesyltransferase inhibitor (Fig. (**5**), **FTI**) with the aromatase inhibitor Letrozole led to unexpected results [27]. Proliferation studies were undertaken with MCF7 human breast tumor xenografts *in vivo*. Letrozole alone resulted in a tumor regression of 25% (inhibition of tumor growth 127%) in this model, whereas **FTI** alone only inhibited very weakly (16%). However, the combination of both compounds resulted in a tumor regression of 50% (inhibition of tumor growth 152%), thus revealing synergistic effects of such a "mixed" treatment.



FTI

Fig (5). Farnesyltransferase inhibitor (**FTI**).

Synergistic effects could also be observed based on a combination of a COX2 inhibitor with an aromatase inhibitor [28]. The results of a combined treatment using Exemestane and Celecoxib, as shown in Fig. (**6**), on the DMBA-induced mammary carcinoma in rats are shown in Table **12**. The combination of these drugs causes significantly improved regression rates of the tumor (48%).



Celecoxib

Fig. (6). Celecoxib in a combination treatment.

Exemestane and Celecoxib alone do not give any significant remission, but a partly ceased progression. This effect is more pronounced for Exemestane (78%) than for Celecoxib (30%). Although the combined treatment does not reach the effect of an ovariectomy, the synergistic potential of this treatment is remarkable.

Table 12. Effect of Exemestane and Celecoxib Alone or in Combination on the DMBA Induced Mammary Carcinoma in Rats

Treatment	CR+PR %[a]	NC %[b]	P %[c]	Rats with NT %[d]
Vehicle	0	5	95	73
CXB	0	30	70	67
EXE	5	78	17	67
CXB + EXE	48	47	5	47
Ovariectomy	96	4	0	0

[a]CR: Complete remission, PR: Partial remission; [b]NC: No change; [c]P: Progression; [d]NT: New tumors appearing during the 4-week study period. EXE (Exemestane): 50 mg/kg/wk i.m.; CXB (Celecoxib): 500 mg/kg of diet

3. INHIBITORS OF 17α-HYDROXYLASE-C17,20-LYASE ($P450_{17}$,CYP17)

An estimated three-quarter of all prostate cancers are androgen dependent. This cancer is the second leading cause of cancer related death and is the most prevalent cancer amongst men in the western world. The American Cancer Society has reported 179,300 new cases and 37,000 deaths from prostate cancer in the United States in 1999. American men have a one-in-six chance of developing the disease [29]. Prostate cancer tends to be a disease of older men with more than 75% of the diagnoses being in men over 65 years of age.

Several approaches exist to decrease androgen influence on the development of prostate cancer. Antiandrogens (like Flutamide, Cyproteroneacetate) [30,31] interact with the androgen receptor, preventing the androgens from unfolding their tumor stimulating activity. Besides, antiandrogens, different compounds have been used to suppress androgen formation. Estrogens unfold their activity on the hypothalamic level, reducing the release of gonadorelin (GnRH). GnRH causes the pituitary gland to release other hormones like the luteinizing hormones [LH] and follicle-stimulating hormones [FSH] (LH and FSH control development in children and fertility in adults). Consequently, the reduced pituitary LH/FSH formation results in a decrease of the testicular androgen production. The use of gonadorelin analogs (like Busereline) [32] also inhibits testicular androgen formation [33]. However, neither this strategy nor orchiectomy, the surgical removal of the main organs involved in androgen formation, reduces the production of adrenal androgens. Since androgens are also synthesized in the adrenal glands, tumor growth cannot be completely blocked. Presently, chemical or surgical castration has been

combined with antiandrogen (Flutamide, Bicalutamide) treatment to reduce the stimulatory effects of the remaining androgens. The selective suppression of androgen biosynthesis became therefore an important therapeutic strategy in order to inhibit tumor growth [34].

3.1. Mechanism and Current Treatment

As shown in Fig. (**1**), the steroid biosynthesis begins in cells of the adrenal gland where the initial product in sterol biosynthesis, cholesterol, is converted into the adrenal steroid hormones aldosterone, hydrocortisone, and corticosterone by a series of P450-mediated hydroxylation steps. The cholesterol side-chain cleavage activity that represents the first step in steroid hormone biosynthesis is a P450-mediated oxidation and cleavage of a pair of adjacent methylene groups to two carbonyl fragments, pregnenolone and isocaprylaldehyde. Another critical set of enzymatic conversions in steroid metabolism is facilitated by 17α-hydroxylase-C17, 20-lyase (CYP17, $P450_{17}$). CYP17 is a bifunctional enzyme which possesses both a C17, 20-lyase activity and a Cl7-hydroxylase activity. Significantly, these two alternative enzymatic activities of CYP17 result in the formation of critically different intermediates in steroid biosynthesis and each activity appear to be differentially and developmentally regulated.

The C17, 20-lyase activity of CYP17 catalyzes the conversion of 17α-hydroxy-pregnenolone and 17α-hydroxy-progesterone to dehydroepiandrosterone (DHEA) and androstenedione, respectively. Both DHEA and androstenedione lyase products are key intermediates in the synthesis of not only the androgens testosterone and dihydrotestosterone (DHT), but also the estrogens 17β-estradiol and estrone. Indeed, adrenal and ovarian estrogens are the main sources of estrogens in postmenopausal women. In contrast, the Cl7-hydroxylase activity of CYP17 catalyzes the conversion of the common intermediate progesterone to 17-hydroxy-progesterone, a precursor of cortisol. Therefore the first activity of CYP17, the C17-hydroxylase activity, promotes the formation of glucocorticoids while the second activity of CYP17, the C17,20-lyase activity, promotes the formation of sex hormones - particularly androgens including testosterone as well as estrogens. Further enzymatic transformations finally lead to testosterone and dihydrotestosterone, steroids with even higher androgenic potency.

In order to avoid unwanted side effects, androgen biosynthesis inhibitors have to be specific enough not to influence corticoid biosynthesis. A promising novel strategy for the treatment of prostate cancer is the development of strong and selective inhibitors of $P450_{17}$ which would result in a complete and exclusive elimination of androgen biosynthesis. For that reason $P450_{17}$ attracted attention as a therapeutic target and attempts were made to obtain specific steroidal as well as non-steroidal inhibitors [34,35].

Pharmacological inhibition of CYP17 may be a promising alternative treatment to antiandrogens and LHRH agonists. Testicular, adrenal, and peripheral androgen biosynthesis would all be reduced instead of the simple reduction of testicular androgen production, which was previously used for prostate cancer treatment.

The first compound to be identified as a $P450_{17}$ inhibitor was the well known antimycotic Ketoconazole (IC_{50} = 740 μM). Ketoconazole, shown in Fig. (**2**), was demonstrated to be active against prostate cancer in a clinical study [36,37]. However, this drug is a relatively non-selective inhibitor of cytochrome P450 enzymes, has weak CYP17 activity, and has a number of notable side effects associated with it including liver damage.

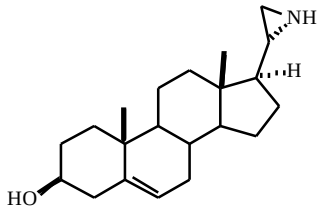
In addition to the use of CYP17 inhibitors in the treatment of prostate cancer, a second potential indication would be for estrogen-dependent breast cancer. In postmenopausal patients with advanced breast cancer, treatment with high doses of Ketoconazole resulted in suppression of both testosterone and estradiol levels, implicating CYP17 as a potential target for hormone therapy. Chemotherapy is usually not highly effective, and is not a practical option for most patients with prostate cancer because of the adverse side effects which are particularly detrimental in older patients. However, the majority of patients initially respond to hormone ablative therapy although they eventually relapse, as is typical with all cancer treatments.

New agents who act by different mechanisms could produce second responses in a portion of relapsed patients. Although the percentage of patients who respond to second-line hormonal therapy may be relatively low, a substantial number of patients may benefit because of the high incidence of prostate cancer. Furthermore, there is the potential for developing more potent agents than current therapies, none of which are completely effective in blocking androgen effects.

Therefore, the need exists for C17, 20-lyase inhibitors that overcome the above mentioned deficiencies.

3.2. Steroidal Inhibitors

$P450_{17}$ consists of a heme moiety as prosthetic group, i.e. a porphyrin ring with a central iron. The function of the iron ion is to activate molecular oxygen for the subsequent conversion of the substrate. As a starting point, steroidal inhibitors were synthesized by modifying the steroids pregnenolone and progesterone. The steroid backbone was altered by attaching a heme iron complexing functional group into the 17-position. This modification should prevent $P450_{17}$ to catalyze the hydroxylation step. For example Hartmann *et al.* prepared various nitrogen bearing derivatives like the aziridine **1**, depicted in Fig. (**7**) [8,38-40]. Despite its high *in vivo* activity, **1** became not an appropriate



1

Fig. (7). The steroidal lead compound **1**.

drug candidate due to its acid instability, which results in the hydrolysis of the aziridine ring. But this compound was used as a template in the design of other, new structures.

In the last years, several steroidal inhibitors were developed. Compounds like those Fig. (**8**), having a 3-pyridyl group in the 17-position, were the most active ones [41-43]. For example Abiraterone is the only steroidal compound in clinical trial.

Recently, Hartmann *et al.* reported on the potent inhibitory activity of the pyrimidyl derivatives **2**, depicted in Fig. (**8**). These compounds could be promising candidates for clinical evaluation [44]. The compounds **2a** (R = OAc, IC_{50} = 38 nM) and **2b** (R = OH, IC_{50} = 24 nM) revealed to be two to three times more active than the steroidal inhibitor Abiraterone (IC_{50} = 73 nM) [45]. The progesterone derivative **3** also showed activity towards two other androgen biosynthesis enzymes, 5α-reductase type 1 and 2 (which convert testosterone into dihydrotestosterone, IC_{50} type 1 = 2.4 μM and type 2 IC_{50} = 0.49 μM). Being a dual inhibitor toward $P450_{17}$ and 5α-reductase, derivative **3** might be more advantageous clinically. It seems, that a *meta* arrangement of the ring nitrogen [46] as well as the C16,17-double bond proved to be essential for strong binding to the heme iron [47]. Various 17-azolyl steroids like **4** proved also to be potent inhibitors of $P450_{17}$ *in vitro* and *in vivo* [48,49]. Cyclopropyl ether and amine derivatives (**5** and **6**, respectively) [50,51] as well as the compounds **7**, [52] **8**, [53] and **9** [54] are strong inhibitors of $P450_{17}$ as well. Due to their progesterone-like A-ring structure, these compounds inhibit also 5α-reductase.

Brodie *et al.* disclosed various 17-azolylsteroids containing 1-pyrazolyl, 1-imidazolyl, 1,2,3-triazol-1-yl, 1,2,3-triazol-2-yl, 1,2,4-triazol-1-yl androgen synthesis inhibitors (Table **13**) in US 6,200,965 [55], US 5,994,335 [55], US 6,44,683 [55] and WO98/33506 [56].

The percentage inhibition data for the initial target compounds of this study are depicted in Table **13**. 2*H*-1,2,3-Triazole and tetrazoles were non-inhibitory, while 1*H*-pyrazoles were moderate inhibitors. By contrast 1*H*-1,2,4-triazole (entry 1, **VN/63-1**), 1*H*-imidazole (entry 4, **VN/85-1**) and 1*H*-l,2,3-triazole (entry 8, **VN/87-1**) were potent inhibitors of the enzyme. Ketoconazole (entry 10) also showed strong inhibition. Given that these Δ^{16}-17-azole compounds of Table **13** are structurally similar, (i.e., they all possess the Δ^5-3β-ol functionality) the striking difference in the inhibitory properties observed may be due to the

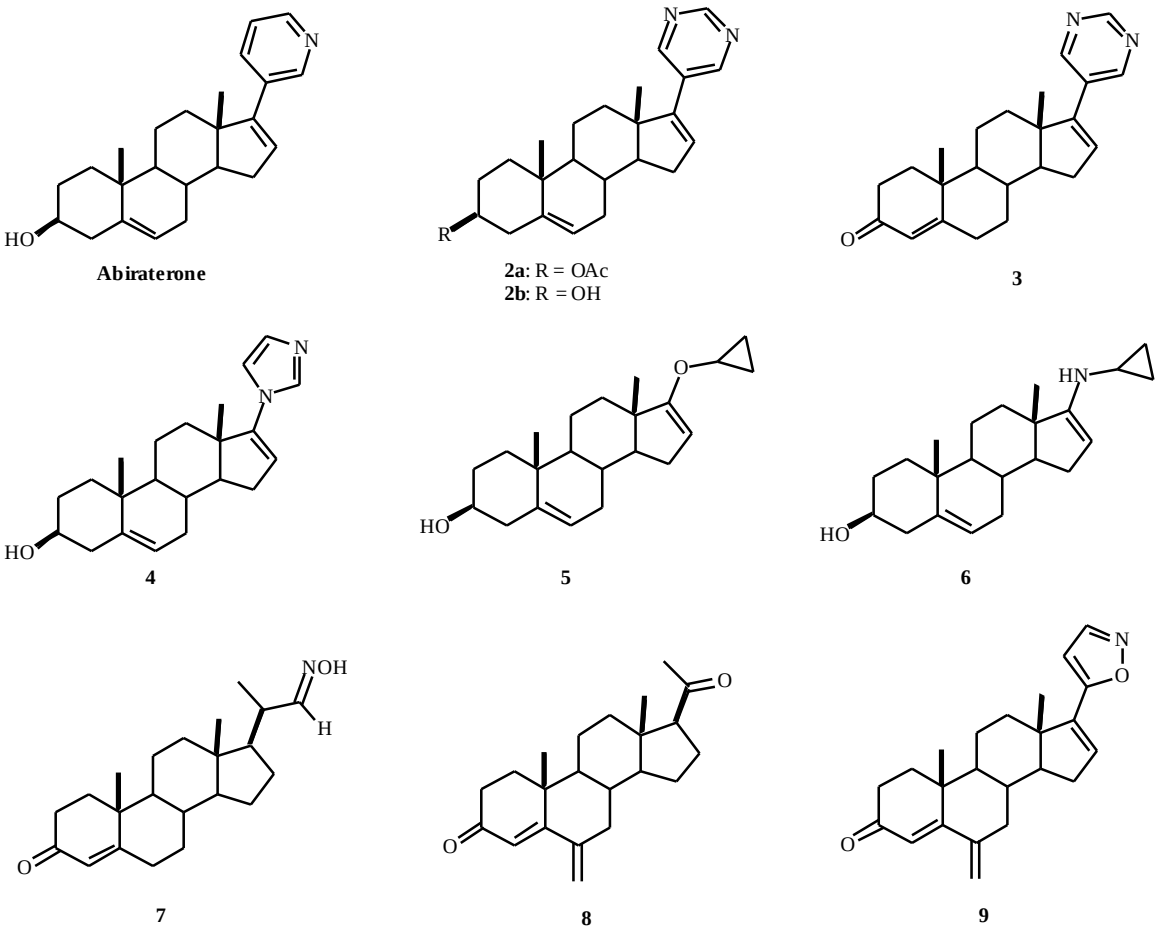
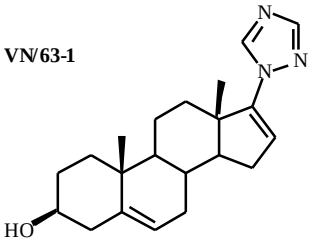
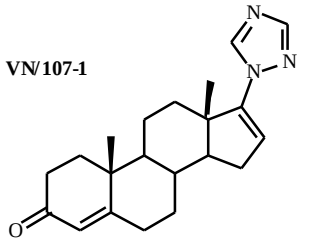
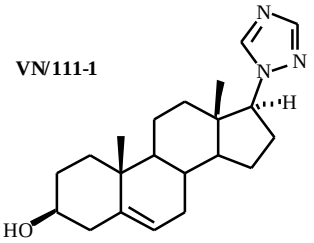
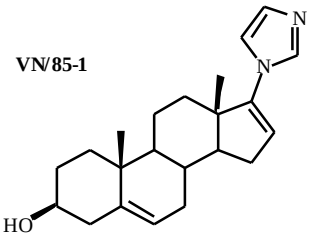
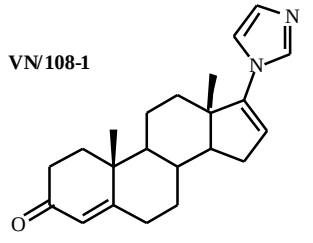
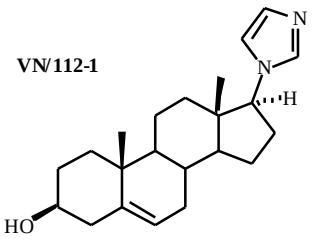
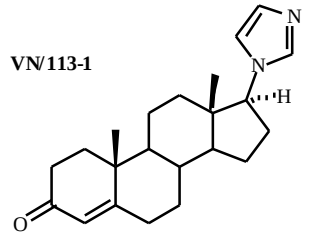
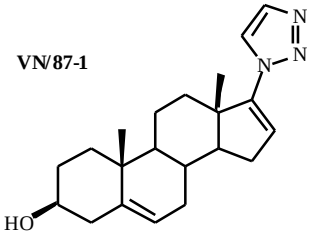
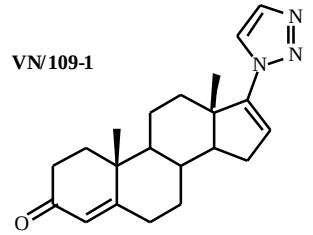


Fig. (8). Potent inhibitors of $P450_{17}$.

Table 13. Inhibitory Potency of Δ^{16}-17 Azolyl Steroids Towards Human and Rat $P450_{17\alpha}$ and Human Steroid 5α-Reductase

Entry	Compound[a]	IC_{50} (nM)[b] Human $P450_{17}$	IC_{50} (nM)[b] Rat $P450_{17}$	IC_{50} (nM)[b] 5α-Reductase
1	**VN/63-1** 	90 ± 14	26 ± 13	160,000
2	**VN/107-1** 	55 ± 11	11 ± 3	152 ± 10
3	**VN/111-1** 	219 ± 21	-	-
4	**VN/85-1** 	8 ± 1	9 ± 2	400,000
5	**VN/108-1** 	7 ± 1	8 ± 0.7	142 ± 5

(Table 13) Contd….

Entry	Compound[a]	IC_{50} (nM)[b] Human $P450_{17}$	IC_{50} (nM)[b] Rat $P450_{17}$	IC_{50} (nM)[b] 5α-Reductase
6	**VN/112-1** 	62 ± 2	-	-
7	**VN/113-1** 	36 ± 9	-	765 ± 100
8	**VN/87-1** 	13 ±1	10 ± 0.4	10,000
9	**VN/109-1** 	19 ± 1	9 ± 2	198 ± 33
10	**Ketoconazole**	78 ± 3	209 ± 17	-
11	**Finasteride**	-		33 ± 2

[a] The numbering of the compounds is identical to that of the corresponding patent citation. [b] Mean ± SDM of at least two experiments

differences in their basicities, a property imposed by the inherent different electronic character of each of the azole heterocycles. In addition, the presence of a nitrogen atom at either the 3' or 4' position seems important for potent inhibition of the enzyme

There was no marked difference between the inhibitory potencies of the Δ^5-3β-ol azoles (entries 1, 4 and 8) with those of the corresponding Δ^4-3-ones (entries 2, 5 and 9). Three of the compounds, i.e., **VN/107-1** (entry 2), **VN/108-1** (entry 5) and **VN/87-1** (entry 8) with K_i values of 1.2, 1.8 and 1.4 nM, respectively, (K_m of the substrate, 17α-hydroxypregnenolone was 530 nM), were the most potent inhibitors, and they are indeed the most potent inhibitors of human testicular microsomal $P450_{17}$ described to date. These compounds were 20-32 times more potent as $P450_{17}$ inhibitors when compared in the same assay with Ketoconazole (K_i*32* = 38 nM). Some Δ^{16}-17-(3-pyridyl) compounds were recently classified as the most potent inhibitors of this enzyme [46]. However, three of their most potent inhibitors were 9 to 12 times more potent as $P450_{17}$ (lyase activity) inhibitors when compared in the same assay with Ketoconazole.[46] The requirement of the 16,17-double bond was also observed with these $P450_{17}$ inhibitors: 17β-(1*H*-1,2,4-triazolyl)- and 17β-(1*H*-imidazolyl)- compounds, com-

pounds **VN/111-1** (entry 3) and **VN/112-1** (entry 6) both exhibited diminished potency compared to the corresponding parent Δ^{16} compounds (**VN/63-1** (entry 1) → **VN/111-1** (entry 3), IC_{50} 90 → 219 nM, and **VN/85-1** (entry 4) → **VN/112-1** (entry 6), IC_{50} 8 → 62 nM). A similar observation has been previously reported [46,54,57] for a number of Δ^{16}-17-heteroaryl $P450_{17}$ inhibitors. Conversion of **VN/112-1** (entry 6) to the Δ^4-3-one compound, **VN/113-1** (entry 7) resulted in a modest increase in inhibitory activity (62 → 36 nM). When the lyase reaction was monitored in the presence of various concentrations of the imidazole, **VN/85-1** (entry 4), a family of non-linear progress curves were obtained in which the extent of inhibition increased with time. This suggest that **VN/85-1** (entry 4) may be a slow-binding inhibitor [58]. Although the other potent inhibitors were not examined in this assay, it is likely that they may also behave in a similar fashion. **VN/85-1** (entry 4) appears to be the first example of a slow-binding inhibitor of cytochrome $P450_{17}$.

The inhibitory potency of (20*R*)- and (20*S*)-aziridinyl steroids have recently been reported, and this stems in part from the additional stabilization due to coordination of the heteroatom of their aziridinyl ring to the heme of rat $P450_{17}$ [39]. The spectroscopic data described above suggest that this may also be the case for the Δ^{16}-17-azole steroids of the present invention. The ability of the steroidal azole nitrogen atom to coordinate with the heme of $P450_{17}$ indicates that C-17 and C-20 (the sites of enzymatic hydroxylations) can be positioned in close proximity to the heme center when these substrate-like inhibitors are bound to the enzyme. Although it is not certain that these compounds bind in exactly the same manner as the natural substrates, their high binding affinities make a significantly different mode of binding unlikely.

Before evaluating these potent inhibitors *in vivo* in rodent models as potential therapeutic agents for the treatment of prostate cancer, the potency of these inhibitors was also accessed towards the rat testicular microsomal $P470_{17}$. A comparison was made between the inhibitory activity, expressed as IC_{50} values, displayed by the Δ^5-3β-ols, compounds **VN/63-1** (entry 1), **VN/85-1** (entry 4) and **VN/87-1** (entry 8), the Δ^4-3-one compounds, compounds **VN/107-1** (entry 2), **VN/112-1** (entry 6) and **VN/109-1** (entry 9), and Ketoconazole (entry 10) towards $P470_{17}$ located in human and rat testicular microsomes. The results are presented in Table **13** and show that whereas the potencies of compounds **VN/85-1** (entry 4), **VN/107-1** (entry 2) and **VN/109-1** (entry 9) all increased towards the rat enzyme by 3.5-, 5- and 2-fold respectively, the potencies of compounds **VN/85-1** (entry 4), **VN/112-1** (entry 6) and **VN/87-1** (entry 8) remained unchanged, whereas that for Ketoconazole (entry 10) decreased approximately 3-fold. The most potent inhibitors, compounds **VN/85-1** (entry 4), **VN/112-1** (entry 6) and **VN/87-1** (entry 8) appear to be the first examples of inhibitors that are equipotent towards the human as well as the rat $P450_{17}$ enzymes. This finding indicates that results from pre-clinical *in vivo* studies with rats are likely to reflect the clinical situation.

About 70 novel steroidal androgen synthesis inhibitors are described in WO9833506 [56]. The most active inhibitors toward human testicular 17α-hydroxylase-C17,20-lyase as well as 5α-reductase are shown in Table **14**.

Compound **I-47** (entry 1) containing a 17β-(4'-imidazolyl ring) demonstrated potent inhibition of 17α-hydroxylase-C17, 20-lyase (IC_{50} = 25 / 23 nM). This suggests that the imidazolyl nitrogen lone pair at this position could coordinate to the iron atom of the heme cofactor in the active site of the enzyme. The introduction of a 16,17 double bond in **I-49** (entry 2, IC_{50} = 9 / 9.5 nM) increased the inhibition 2-fold. Potter *et al.* also found that 17-(3'-pyridyl) substituents together with a 16,17-double bond showed potent activity.[46] The 3-acetoxy derivative **L-12** (entry 3) had lower potency than **I-49**, which may reflect a limited bulk tolerance at the 3-position. However, **L-12** still retained reasonable activity (IC_{50} 75 / 25 nM) and might be useful as a pro-drug of **I-49** *in vivo*. These derivatives are based on a 5-ene-3β-ol structure and are similar to the natural pregnenolone. In contrast, the substrate for 5α-reductase (testosterone) contains a 4-ene-3-one moiety. As expected, **I-47** and **I-49** did not inhibit 5α-reductase. On the other hand, **L-41** (entry 4) and **L-6** (entry 5), which are the 4-ene-3-one derivatives of **I-47** and **I-49**, respectively, showed activity against 5α-reductase (IC_{50} = 122 nM and 522 nM, respectively) while still retaining their strong potency against 17α-hydroxylase-C17, 20-lyase (IC_{50} = 59 / 5.5 nM and 16 / 2 nM, respectively).

L-10 (entry 7), 20β-hydroxy-4,16-pregnadiene-3-ol is a potent 5α-reductase inhibitor (IC_{50} = 20 nM) and comparable to Finasteride (IC_{50} = 14 nM). As the 20β-ol of **L-10** might be metabolized to 20-one *in vivo*, the 16-dehydroprogesterone (**L-13**, entry 8) was also tested and found to be a potent inhibitor of both 17α-hydroxylase (IC_{50} = 73 / 24 nM) and 5α-reductase (IC_{50} = 22 nM). Progesterone is also known to be a potent 5α-reductase inhibitor, but its rapid metabolism in the body and lack of oral activity, detracts from its value as a therapeutic agent. However, as **L-10** and **L-13** both have a 16,17-double bond, their 17β-acetyl side chain should be difficult to degrade *in vivo*. The introduction of a 20-oxime group generally enhances inhibition of 17α-hydroxylase-C17,20-lyase. The 4-ene-3-one derivative **L-2** (entry 6) not only showed high activity toward 17α-hydroxylase-C17,20-lyase (IC_{50} = 6 / 5 nM), but also showed potent activity for 5α-reductase (IC_{50} = 52.5 nM).

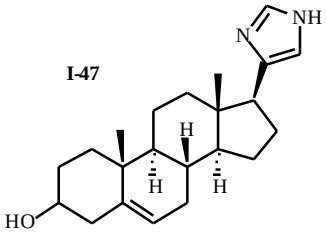
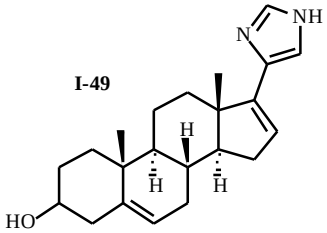
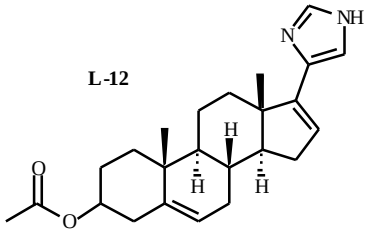
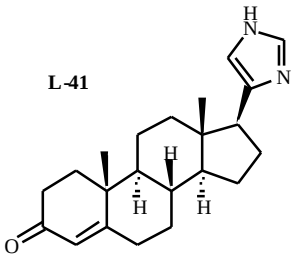
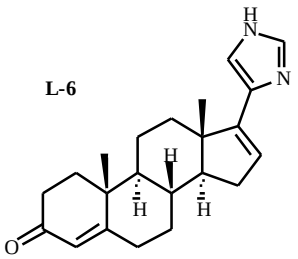
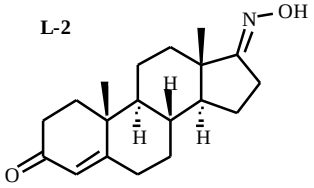
Pribish *et al.* disclosed cyclopropyl(amino/oxo) aza-steroids [59] (Table **15**). Entries 2 and 3 of the compounds prepared in this series are characterized by a dual inhibition of both C17,20 lyase and 5α-reductase. These dual acting structures have a steroidal A-ring which is similar to the one of Finasteride or 4-MA for example, both of which are known inhibitors of 5α-reductase. The presence of a cyclopropyl ring in position 17 could be responsible for a mechanism-based inhibition of the C17, 20 lyase reaction.

Peet *et al.* studied 20-fluoro-pregnadiene derivatives as inhibitors of C17, 20-lyase and 5α-reductase. The *in vitro* results are shown in Table **16** [60].

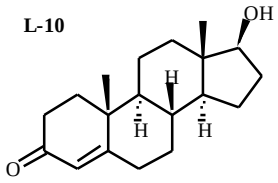
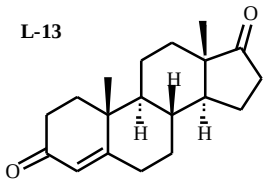
3.3. Non-Steroidal Inhibitors

Although highly potent steroidal inhibitors are nowadays accessible, there are good reasons to replace steroidal drugs

Table 14. Most potent inhibitors of Human $P450_{17\alpha}$ and Human Steroid 5α-Reductase

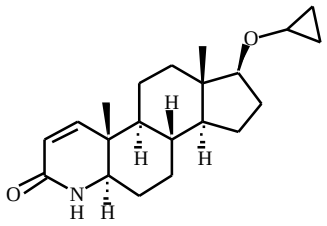
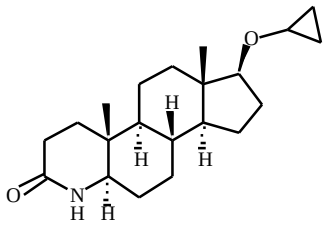
Entry	Compound[a]	IC_{50} (nM)[b] Human 17α-OHase / C17,20-lyase	IC_{50} (nM)[c] 5α-Reductase
1	**I-47** 	25 / 23	NI
2	**I-49** 	9 / 9.5	NI
3	**L-12** 	75 / 25	NT
4	**L-41** 	59 / 5.5	122.0
5	**L-6** 	16/ 2	522.0
6	**L-2** 	8.5 / CI	53

(Table 14) Contd….

Entry	Compound[a]	IC_{50} (nM)[b] Human 17α-OHase / C17,20-lyase	IC_{50} (nM)[c] 5α-Reductase
7	**L-10** 	NI	21
8	**L-13** 	73 / 24	22
8	**Finasteride**	NI	14.4
9	**Ketoconazole**	437 / 150	NI

NI = No inhibition; CI = Complete Inhibition; NT = Not Tested; [a] The numbering of the compounds is identical to the one used in the corresponding patent citation. [b] Measuring of the conversion of radiolabeled pregnenolone to 17α-hydroxypregnenolone and DHEA; [c] Incubation of human prostatic microsomes (0.6 mg proteine in 0.5 mL phosphate buffer) with [7-^{3}H]testosterone (10 nM, 6 10^{5} dpm), NADP (0.65 mM; glucose-6-phosphate 7.1 mM; glucose-6-phosphate dehydrogenase 2.5 IU in 100 µL phosphate buffer), inhibitor (10 nM and 100 nM) under oxygen for 30 min at 37°C.

Table 15. Aza-Steroid Inhibitors of Cynomolgus Monkey Testicular C17,20 Lyase and Prostatic 5α-Reductase

Entry	Compound[a]	C (µM)	Preincubation time (min)	% Inhibition C17,20 Lyase	% Inhibition 5α-Reductase
1	 **MDL 103,129**	10	0	77.3	-
			40	84.3	-
		1	0	40.7	-
			40	46.9	-
		0.1	0	6.4	-
			40	18.3	-
2	 **MDL 103,432**	10	0	68.3	99.4
			40	80.2	99.4
		1	0	33.0	98.7
			40	54.2	99.3
		0.1	0	23.4	78.7
			40	20.2	80.0

(Table 15) Contd….

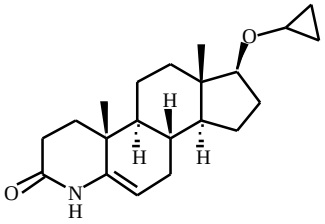
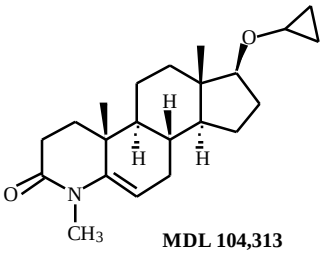
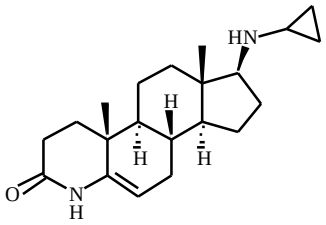
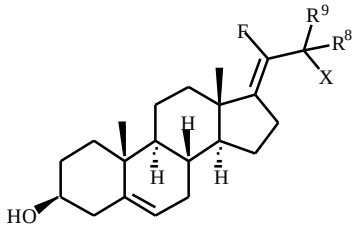
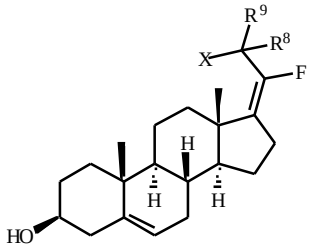
Entry	Compound[a]	C (μM)	Preincubation time (min)	% Inhibition C17,20 Lyase	% Inhibition 5α-Reductase
3	 **MDL 103,496**	10	0	39.2	99.6
			40	87.7	99.4
		1	0	29.0	86.2
			40	62.2	86.7
		0.1	0	5.0	24.6
			40	26.6	29.5
4	 **MDL 104,313**	10	0	63.6	-
			40	70.2	-
		1	0	23.8	-
			40	39.9	-
		0.1	0	-18.4	-
			40	-8.7	-
5	 **MDL 105,831**	10	0	69.6	-
			40	100	-
		1	0	26.0	-
			40	53.8	-
		0.1	0	11.4	-
			40	24.6	-

Table 16. Aza-Steroid Inhibitors of Cynomolgus Monkey Testicular C17,20 Lyase



Z-isomer



E-isomer

Entry	Isomer	-CR^8R^9-X	Concentration (mM)	Preincubation time (min)	% Inhibition C17,20 Lyase[a]
1	*Z*	CH_2OH	10	0	100
			1	0	96
			10	40	100
			1	40	94

(Table 16) Contd….

Entry	Isomer	-CR^8R^9-X	Concentration (mM)	Preincubation time (min)	% Inhibition C17,20 Lyase[a]
2	*E*	CH_2OH	10	0	85
			1	0	63
			10	40	87
			1	40	61
3	*Z*	CH_3	10	0	78
			1	0	49
			10	40	94
			1	40	72
4	*E*	CH_3	10	0	88
			1	0	54
			10	40	94
			1	40	60

[a] Substrate 0.05M 17-α-hydroxyl pregnenolone.

by steroidomimetics. Independent of their mode of action, steroidal compounds often show some affinity towards one or several steroid receptors (estrogen-, gestagen-, androgen-, and mineralocorticoid- or glucocorticoid-receptors), acting as an agonist or antagonist. However, this insufficient selectivity results in unwanted side effects [33].

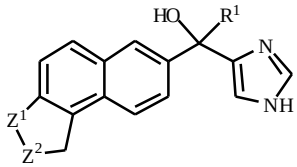
For the development of non-steroidal inhibitors, an iron complexing group, mostly a nitrogen bearing heterocycle, was combined with a lipophilic moiety. Additional substituents could be introduced to mimic the hydrophilic C3 group of the substrate. As a substitute of the steroidal nucleus different strategies were used. Either A-ring [61], B-(D)-ring [62-64] or A-B-ring [35, 65-69] mimics of the steroidal scaffold were developed.

Hartmann *et al.* designed different classes of non-steroidal compounds. [66-68] In the class of dihydro-naphthalenes, two highly active *in vitro* inhibitors of $P450_{17}$ bearing 4-pyridyl [70] or an imidazolyl substituent [69] were found. They revealed not only a high inhibitory activity but also a high selectivity towards $P450_{17}$ (CYP 17) in com-parison with four other enzymes of steroid hormone biosynthesis (CYP 11A1, CYP 11B2, CYP 19 and CYP 5) [33,70].

Imidazole derivatives based on a naphthyl ground structure and characterized by a 5- or 6-membered ring incorporating an amide bond, are described in EP1344777 [71] (Table **17**).

All compounds are active inhibitors and in addition, the derivative of entry 1 revealed a high inhibitory activity towards testosterone synthesis *in vivo* (Sprague-Dawley rats). After oral administration of the test compound (25 mg/kg), the testosterone level relative to the control group dropped down to 6%.

Table 17. Inhibitory Activities of Imidazoles Derivatives Towards Rat C17,20-Lyase

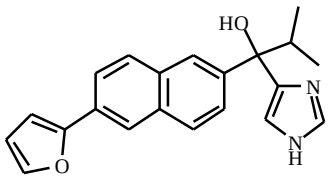
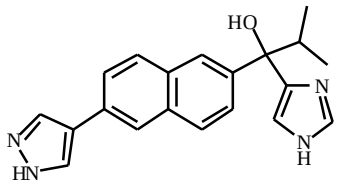
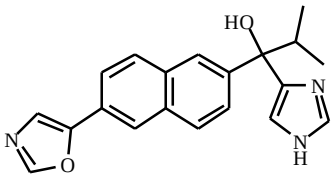
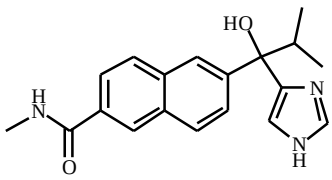
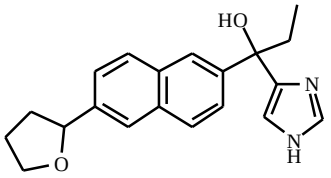
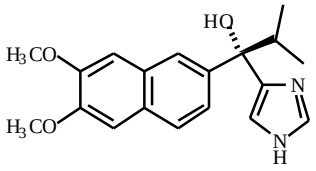
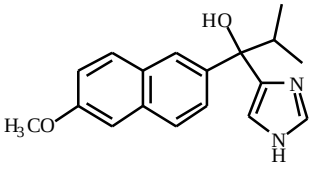
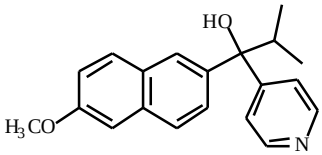
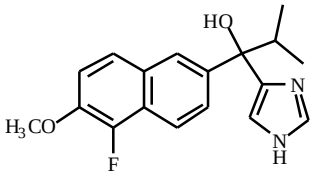
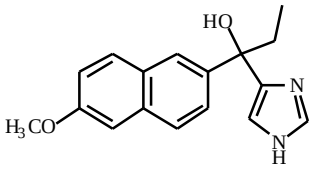
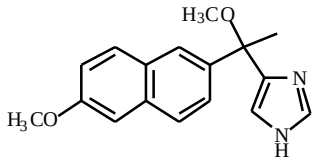


Entry	R^1	Z^1	Z^2	IC_{50} (nM)[a]
1	$CH(CH_3)_2$	C=O	NH	<10
2	$CH(CH_3)_2$	C=O	NCH_3	15
3	$CH(CH_3)_2$	C=O	NCH_2CH_3	23
4	$CH(CH_3)_2$	NH	C=O	42
5	$CH_2CH(CH_3)_2$	C=O	NCH_3	22
6	$CH(CH_3)_2$	C=O	NCH_3	-

[a] [1,2-^{3}H]-17α-hydroxyprogesterone (10 nM).

In analogy, WO0130762 [72] and EP1073640 [73] describe imidazol derivatives bearing a naphthalene moiety where now the condensed amid moiety (see Table **18**) was replaced by various functionalities. The most active

Table 18. Inhibitory Activities of Imidazoles Derivatives Towards Rat C17,20-Lyase

Entry	R^1	IC_{50} (nM)[a]
1[b]		28
2[b]		15
3[b]		14
4[b]		6.1
5[b]		18
6[b]		3.3
7[c]		33
8[c]		32
9[c]		32
10[c]		41
11[c]		35

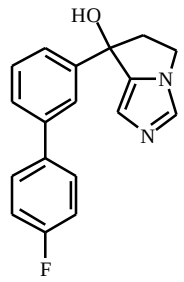
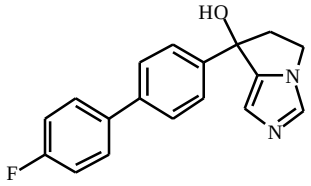
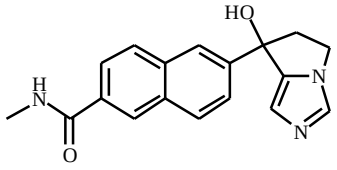
[a] [1,2-^{3}H]-17α-hydroxyprogesterone at 10 nM [b] WO01/30762 [68]; [c] EP1073640 [69]

derivatives showed again a significant reduction of the testosterone level *in vivo* (entry 2 : 10%, entry 4 : 4.5%, entry 5 : 7.4%, entry 7 : 2%, entry 9 : 9% and entry 10 : 41%). In this series of inhibitors, the substitution of a 1*H*-imidazole moiety (entry 7) versus a 3-pyridyl ring (entry 8) has no influence on the inhibitory activity. Increasing polarity of the remote phenyl part (opposite to the heme coordinating heterocycle), increases slightly the inhibitory power (amide (entry 4): IC_{50} = 6.1 nM *versus* dimethoxy (entry 6): IC_{50} = 3.3 nM).

Derivatives in which the naphthyl part was replaced by a biphenyl unit have been additionally described in WO02 40484 (Table **19**) [74]. Substitution of the naphthyl unit by a fluoro-biphenyl moiety, resulted in highly active inhibitors. The substitution pattern seems to be important in order to guarantee a good interaction with the active side of the target enzyme. The 4-substituted derivative (entry 2, Table **19**) is less active than the 3-substituted analogue (entry 1). The latter was again tested towards testosterone synthesis *in vivo*. Oral administration of the test compound at a dose of 25 mg/kg results in a reduction of the testosterone level to 4.4%, compared to the untreated control group.

In analogy to the biphenyl based imidazolyl inhibitors represented in Table **19**, EP1227086 [75] describes biphenyl based imidazolyl alcohols where the imidazole ring is not incorporated in a cyclic 5-membered ring (Table **20**). Again, the inhibitory power depends on the substitution pattern of the biaryl unit. The 4-substituted derivative is less active than the 3-substituted congener (entry 1 *versus* entry 4).

Table 19. Inhibitory Activities of Imidazoles Derivatives Towards Rat C17,20-Lyase

Entry	R^1	IC_{50} (nM)[a]
1		10
2		25
3		54

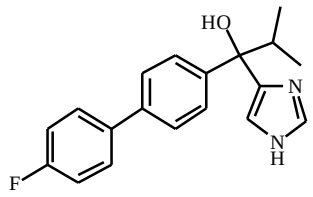
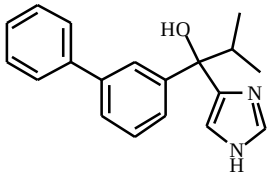
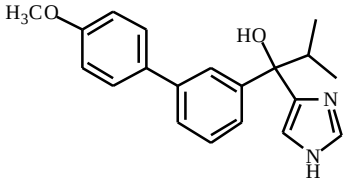
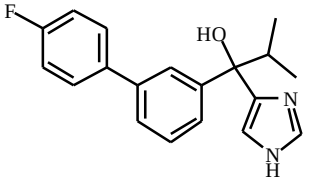
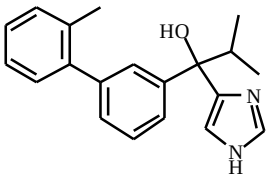
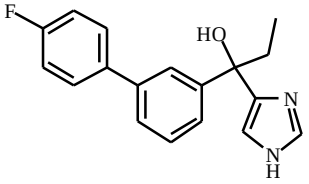
[a] [1,2-^{3}H]-17α-hydroxyprogesterone at 10 nM

Introduction of a polar substituent at the 4'-position increases the activity slightly (entry 2, H : IC_{50} = 15 → entry 3, OCH_3 : IC_{50} = 10 → entry 4, F : IC_{50} = 8.3). These three derivatives were also tested *in vivo* for their ability to block testosterone formation. The compounds revealed an almost identical, excellent inhibition at a dose of 50 mg/kg (oral application) and the testosterone level was reduced to 4.3%, 4.6% and 4.7% of the control group.

Azole derivatives, using instead of an imidazole ring a 3-pyridyl unit for complexation of the prosthetic heme iron are described in EP1348706 [76]. These pyridyl thiazoles revealed very potent inhibitory potency in the *nano*-molar range, as depicted in Table **21**.

Novel thiazoles bearing 3-pyridyl heterocyclic compounds which inhibit the lyase activity of CYP17 are also reported in WO03/027085.[9] The inhibitory activity of 2-[4-methyl-3-pyridyl)-4-(2,4-dichlorophenyl)thiazole (**10**) was compared to that of 2-(3-pyridyl)-4-(2,4-dichlorophenyl) thiazole (**11**) described in EP411,718 [77]. Against C17,20 human lyase, 4-methyl substituted pyridines have consistently been more active than 4-unsubstituted pyridines. A reason for this drastic difference is most likely associated with an increased twist within the thiazole / pyridine moiety which results in an improved coordination of the heme iron by the pyridine nitrogen. In the present case **1** has an IC_{50} value of 15 nM, whereas **2** has an IC_{50} value of 406 nM.

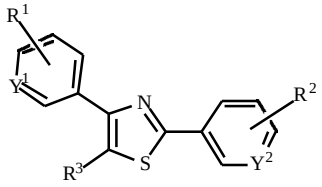
Table 20. Inhibitory Activities of Imidazoles Derivatives Towards Rat C17,20-Lyase

Entry	R^1	IC_{50} (nM)[a]
1		28
2		15
3		10
4		8.3
5		12
6		11

[a] [1,2-^{3}H]-17α-hydroxyprogesterone at 10 nM.

Surprisingly, when both compounds were tested against powdery mildew, a fungal species identified in EP411,718, **2** showed 80% inhibition whereas **1** was devoid activity (Table **22**).

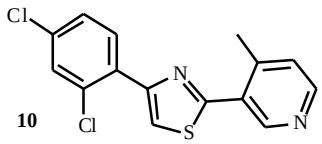
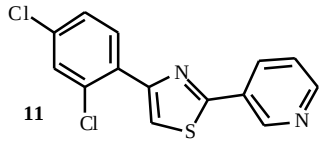
Table 21. Inhibitory Activities of 3-Pyridyl Substituted Thiazoles Towards Rat C17,20-Lyase



Entry	R^1	R^2	R^3	Y^1	Y^2	IC_{50} (nM)[a]
1	3-OCH_3	H	H	C	N	33
2	4-Br	4-CF_3	H	C	N	<10
3	4-OCH_3	4-CF_3	H	C	N	<10
4	4-F	4-CH_3	H	C	N	<10
5	2,4-CH_3	4-OCH_3	H	C	N	30
6	4-CH_3	4-CH_3	H	C	N	<10
7	H	4-F	H	N	C	14

[a] [1,2-^{3}H]-17α-hydroxyprogesterone at 10 nM.

Table 22. Inhibitory Activities of 3-Pyridyl Substituted Thiazoles Towards Human and Mouse C17,20-Lyase

Structure	Human Lyase IC_{50} (nM)[a]	Mouse Lyase IC_{50} (nM)
 **10**	15	39.7
 **11**	406	1400

[a] Recombinant human C17,20 lyase expressed in SF9 cells, Substrate: 17-OH pregnenolone.

Analogously, various 3-pyridyl substituted indoles and indazoles (Type I, WO03027094[78]), tetrazoles (Type 2, WO03027095[79]), imidazoles (Type 3, WO03027096 [80]), pyrimidines (Type 4, WO03027100[81]), pyrroles and pyrazoles (Type 5, WO03027101[82]), thiophenes (Type 6, WO03027105[83]) as well as oxazoles (Type 7, WO03 027107[84]) were prepared and tested towards inhibition of the lyase activity of CYP17 (Fig. (**9**)). All compounds tested in these WO publications had IC_{50} values of less than 10 μM in assays relating to inhibition of the C17, 20 lyase step. Mostly no more detailed data are provided in these documents.

EP1283209 [85] describes novel benzothiophene derivatives revealing potent inhibitory activity of steroid 17α-hydroxylase and/or steroid C17, 20-lyase (Table **23**). They also inhibit aromatase. For example 3-(6-isopropyloxy-benzo[b]thiophen-3-yl)pyridine (entry 5) hydrochloride at 300 nM gave 100% inhibition of 17α-hydroxylase-C17,20-lyase. Apparently, best inhibition is obtained, when the substituent at the 6-position contains an oxygen (entries 1 – 3, 13) or nitrogen (entries 4, 14 and 15) function accompanied with a lipophilic part which can be either an alkyl chain or an (*hetero*)aryl moiety. As it was already previously shown, it seems, that a *meta* arrangement of the ring nitrogen is a prerequisite for high inhibitory activity [46]. In a similar study, the benzothiophene unit was replaced by a benzofuran moiety [86]. In all cases, the benzofuran derivatives are less active than the corresponding benzothiophenes.

WO05005390 and JP2005200317 describe the synthesis of 4-pyridyl-alkyl ester benzoate derivatives (Table **24**) [87,88]. Again as a general trend one can recognize, that the inhibitory potency increases with a remote polar and/or

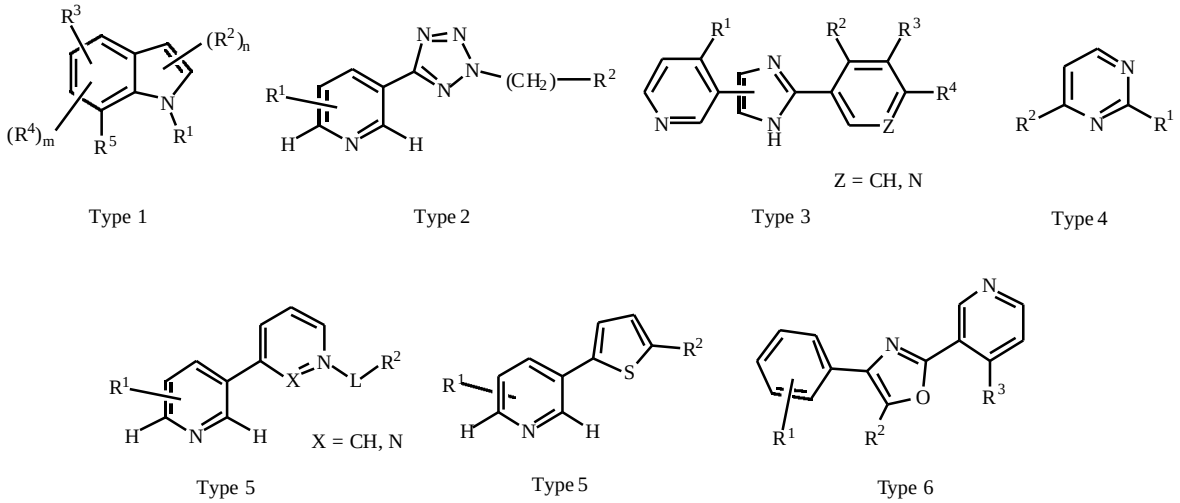
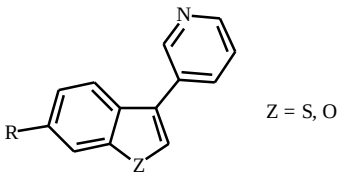


Fig. (9). Different classes of azole inhibitors.

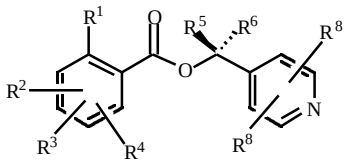
Table 23. Inhibitory Activities of Benzothiophene and -Furan Derivatives Towards Rat C17,20 Lyase



Entry	Substituents R	Z	Inhibitory activity (%)[a]
1	OCH_3	S	89
2	OC_2H_5	S	97
3	$OCH(CH_2)_3$	S	100
4	$NH(CH(CH_2)_3)$	S	98
5	C_3H_7	S	93
6	3-NO_2-C_6H_4	S	86
7	4-F-C_6H_4	S	93
8	"	O	64
9	3-F-C_6H_4	S	91
10	"	O	45
11	C_6H_5	S	93
12	"	O	45
13	3-OH-C_6H_4	S	100
14	3-C_5H_4N	S	100
15	3-NH_2-C_6H_4	S	100

[a] Compounds tested at a concentration of 300 nM; Substrate: 17-α-hydroxyprogesterone: 25 μM

Table 24. Inhibitory Activities of Some Representative 4-Pyridyl-Alkyl Ester Benzoate Derivatives Towards Human C17,20 Lyase



Entry	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	IC_{50} (nM)[a]
1	H	3-OH	4-OH	5-OH	CH_3	H	H	H	13
2	2-OH	4-OCH_3	6-OCH_3	H	C_2H_5	H	H	H	7.3
3	2-OH	4-OCH_3	6-OCH_3	H	CH_3	H	H	H	4.9
4	2-OH	4-OC_2H_5	6-OC_2H_5	H	CH_3	H	H	H	8.8
5	2-OH	4-OCH_3	6-$OCH(CH_3)_2$	H	CH_3	H	H	H	8.1
6	2-OH	4-$OCH(CH_3)_2$	6-OCH_3	H	CH_3	H	H	H	5.3
7	2-Cl	H	H	H	CH_3	H	H	H	7.3
8	2-F	H	H	H	CH_3	H	H	H	20
9	2-Br	H	H	H	CH_3	H	H	H	230
10	2-OCH_3	3-OCH_2O-4		H	CH_3	H	H	H	9.1
11	H	3-OH	4-OH	5-OH	CH_3	CH_3	CH_3	H	8.5

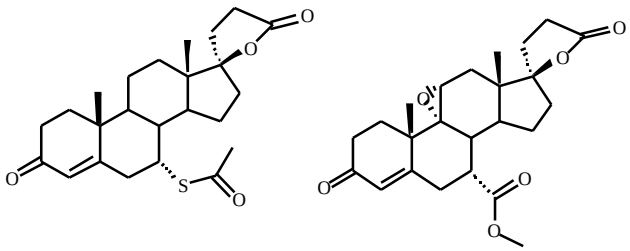
[a] Substrate 17-α-hydroxyl progesterone 25 μM

hydrophilic substituent like OH, OCH_3. If one compares the influence of halogen atoms, the inhibitory activity decreases from chlorine (entry 7) to fluorine (entry 8) and finally, bromine (entry 9).

4. INHIBITION OF ALDOSTERONE SYNTHASE

Already in 1946 Selye [89] observed that deoxycorticosterone causes systemic hypertension, myocardial necrosis and fibrosis when fed to rats. This fact represents the first demonstration of a profibrotic action of mineralocorticoids. However, only recently further studies revealed the essential role of aldosterone for myocardial fibrosis and ventricular hypertrophy [90-93]. Elevated levels of aldosterone which might be caused by an over stimulation of the Renin-Angiotensin-Aldosterone system (RAAS) due to insufficient renal flow are considered crucial for the development of some cardiovascular conditions.

Besides ACE inhibitors, aldosterone receptor antagonists were shown to reduce the cardiovascular damage caused by toxic effects of aldosterone. Spironolactone [94], a rather non-selective receptor blocker is of limited use due to its progestational and antiandrogenic side effects in men (impotence and gynecomastia) and premenopausal women (menstrual distrurbances). These side effects are reduced with the more selective Eplerenone [95]. Although it has a 10-20-fold lower affinity for the aldosterone receptor in comparison to Spironolactone, studies in humans show that Eplerenone has 50-75% of the potency of Spironolactone (Fig. (**10**)) [96].



Spironolactone **Eplerenone**

Fig. (10). Eplerenone and Spironolactone.

However, a main drawback of all aldosterone antagonists is that they cause a dose-dependent increase in serum potassium concentration (hyperkalemia). Therefore the selective blockade of aldosterone synthase could represent a promising alternative.

The enzyme aldosterone synthase (CYP11B2) catalyzes the conversion of 11-deoxycorticosterone to aldosterone *via* the intermediates corticosterone and 18-hydroxycorticosterone. Selective inhibitors of CYP11B2 are considered useful for the prevention, delay of progression, or treatment of hypertension, congestive heart failure, renal failure or atherosclerosis. In order to limit possible side effects glucocorticoid biosynthesis (CYP11B1, 93% sequence homology to CYP11B2) must not be influenced. At present only limited data is available for this field, but studies by Hartmann *et al*. resulted in the development of very active inhibitors (Fig. (**11**), compound **12**, IC_{50} = 6 nM; compound **13**, IC_{50} = 3 nM) [2,10].

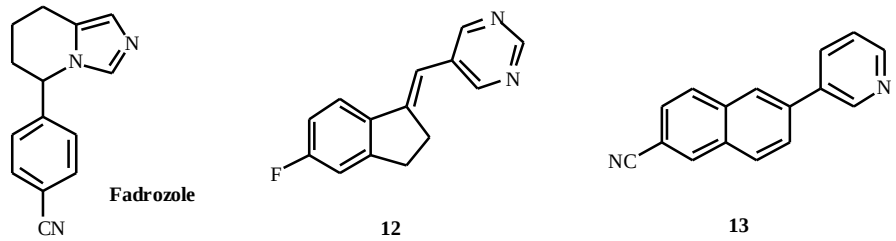
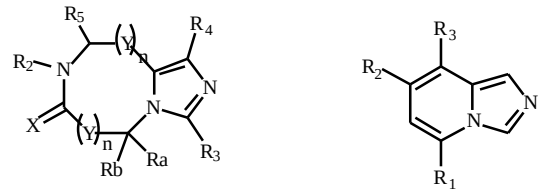


Fig. (11). Inhibition of aldosterone synthase (CYP11B2).

Early studies by Demers *et al.* [97] indicate that the aromatase inhibitor Fadrozole is an inhibitor of aldosterone formation. Further studies by Hartmann *et al.* [3] revealed that both CYP11B1 and CYP11B2 are inhibited by this compound. However, recent clincial studies with Fadrozole show (WO05099695) [98] the usefulness of this compound for the prevention or reduction of vascular access dysfunction in patients after catheter insertion (150 patients, 50 Fadrozole treatment and 100 placebo; effect shown after 3-month treatment).

The structural modifications (see Fig. (**12**)) of Fadrozole (WO04014914 [99] and WO04046145 [100]) mainly consisted in the introduction of a nitrogen in position 7 and an oxo-substitution in position 6.



X: O, H_2
Ra, Rb: H, alkyl, heteroalkyl, aryl, heteroaryl
R_2: $(CHR)_p$-R, R: alkyl, cycloalkyl, heterocyclyl

R_1: Cycloalkyl, heterocyclyl, phenyl
R_2,R_3: H, CF_3, O-alkyl or R_2, R_3 form ring

Fig. (12). Structural modifications of Fadrozole.

Various substituents were introduced in position 7, as depicted in Fig. (**13**), which resulted in very active compounds. The replacement of a 4-CN-phenyl ring (**14** / **15**) by a 3-bromo-phenyl group (**16**) led only to a small increase in potency, thus demonstrating that a nitrile group is not necessary for high activity. However, aromatization leads with **17** to a drop in activity. When looking at these data one has to bear in mind that differences between species (rat / human) and variations in substrate concentrations render a direct comparison rather difficult.

5. CURRENT & FUTUR DEVELOPMENTS

The use of aromatase inhibitors is currently restricted to postmenopausal patients and ovarian suppression is the most effective means of reducing estrogen production in premenopausal women. Tamoxifen is the established first

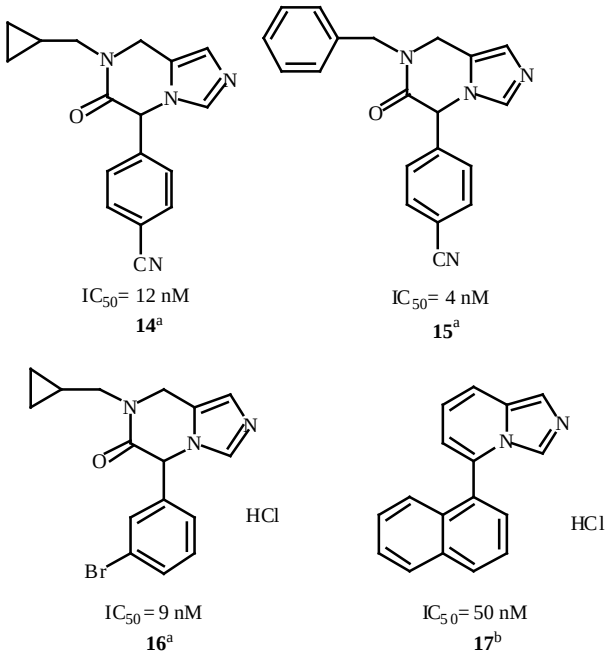


Fig. (13). Inhibition of aldosterone synthase by select imidazopyridines and imidazopyrazines. [a]From WO04014914 [99] [b]From WO04046145 [100] [c]Inhibition of cytosolic rat adrenal aldosterone synthase (4 10^{-6} M corticosterone).

line adjuvant therapy for hormone receptor positive postmenopausal patients with breast cancer. Despite this fact, recent results from randomized controlled studies have indicated that aromatase inhibitors like Anastrozole or Letrozole have superior antitumor efficacy and toxicity profiles compared with Tamoxifen in the treatment of postmenopausal women with node-negative hormone receptor positive breast cancer [101,102].

At present it seems that the most promising prospects for aromatase inhibitors exist in a combination of agents having a distinct mechanism of action, thus influencing various components of the malignant pathophysiological process. Such therapies could be represented by a combination of an aromatase inhibitor with an inhibitor of farnesyltransferase or COX2 [101-104]. Thus a main objective for future developments in the field of aromatase inhibitors is rather directed to the search for dual activity, as was already shown in this report with e.g. the simultaneous inhibition of aromatase and steroid sulfatase. Given the high activity and

selectivity that was already achieved with known inhibitors it is more desirable to investigate lead compounds with such an additional activity.

A combination therapy could also be beneficial for inhibitors of CYP17 which have shown activity *in vitro* for the potential treatment of prostate cancer. The negative psychological impact associated with orchiectomy highlights the need for medical alternatives to deprive circulating androgens. Amongst the different structures reported in this article the most promising ones seem to be represented by thiazole derivatives from Bayer Corporation [9]. Presently no data are available on preclinical or clinical results for such compounds. More advanced studies with the steroidal 17-hydroxylase-C17,20-lyase inhibitor Abiraterone have shown that the drug is safe, and serum levels of testosterone are reduced to 50 % at a dose of 500 mg [105]. Since the number of patients in these initial tests was rather low, further studies will be needed in order to evaluate the potential oncostatic effects of this compound. For the future it seems that the most important question to be answered will be, if inhibitors of CYP17 can compete with a combination treatment using androgen receptor antagonists and gonadotropin releasing hormone agonists.

Research in the field of aldosterone synthase inhibitors is still in an early stage. Thus at present it cannot be foreseen whether CYP11B2 will be replacing ACE inhibitors or aldosterone receptor antagonists in the treatment of certain cardiovascular diseases. The success in this area will largely depend on the degree of selectivity that can be achieved by active inhibitors of CYP11B2 with respect to other P450 enzymes.

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