J. Steroid Biochem. Molec. Biol. Vol. 43, No. 8, pp. 1003–1021, 1992
Printed in Great Britain.

0960-0760/92 $5.00 + 0.00

INHIBITORS OF *P*450-DEPENDENT STEROID BIOSYNTHESIS: FROM RESEARCH TO MEDICAL TREATMENT

H. VANDEN BOSSCHE
Department of Comparative Biochemistry—Janssen Research Foundation, 2340 Beerse, Belgium

Summary—A number of cytochrome *P*450-dependent enzymes are major targets for both steroidal and nonsteroidal compounds that may be of use in the treatment of a number of androgen-independent, androgen-, estrogen- and other steroid-dependent diseases. Compounds of interest are for example aminoglutethimide and derivatives; esters of 4-pyridineacetic acid; imidazole derivatives, such as ketoconazole, liarozole, fadrozole, CGS 18320 B; bis-chlorophenyl-pyrimidine analogues; triazole derivatives vorozole and CGS 20267, and steroidal aromatase inhibitors such as 4-hydroxyandrostenedione. Some of them (e.g. ketoconazole) triggered studies to find new possibilities in medical treatment. Others are of use clinically or under clinical evaluation to provide a palliative treatment and/or cure to patients with for example prostatic carcinoma, breast cancer, hypercortisolism and benign prostatic hyperplasia.

OUTLINE

INTRODUCTION

Steroid hormones not only play an important role in regulating growth, differentiation and function of organs such as the gonads, pituitary and secondary sex organs (for reviews see Refs [1, 2]) but, are also involved in the natural history of for example benign prostatic hyperplasia (BPH), many cancers, including those of the prostate, breast and endometrium, and Cushing's syndrome (for reviews see Refs [2–5]).

BPH is a disease in which the prostate resumes growth late in life. It is the most common nonmalignant proliferation abnormality found in any internal organ [6, and references therein]. The incidence of BPH rises from 23% in men at 40 years of age up to 88% by the ninth decade [6]. BPH is presently the second leading indication for surgery in men [7].

Hormonal imbalance plays an important role among the etiologic factors of BPH [8]. In both foetal and adult prostate, the cell proliferative steroid, 5α-dihydrotestosterone (DHT), is formed from testosterone by 5α-reductase [6]. Humans with 5α-reductase deficiency have prostates which are small or undetectable [6]. The proliferative response of the prostate is not only a function of androgens or androgen receptors [6]. For example, there is direct and indirect evidence that estrogens are involved in the pathobiology of BPH [6, 9, 10, and references therein]. Therefore, interference with estrogen synthesis and/or action together with inhibition of DHT synthesis may have beneficial effects in prostate pathology.

Prostatic carcinoma has surpassed lung cancer as the most commonly diagnosed cancer

Proceedings of the First International Symposium on A Molecular View of Steroid Biosynthesis and Metabolism, Jerusalem, Israel, 14–17 October 1991.

in U.S. males and is the second leading cause of cancer deaths in men [5]. At present, prostate cancer costs, in the U.S.A., more than 10^9 $ annually and results in more than 28,000 deaths [5, 11]. In western Europe prostatic carcinoma is the second most frequent cancer in men [12]. Adenocarcinomas of the prostate originates from the prostatic epithelium [12]. The prostate gland depends on androgens, specially DHT, both for development within the foetus and for maintenance in the adult [1, 5, 6, 13]. Although the mechanisms that regulate growth of normal and malignant prostate tissue are poorly understood, like the normal prostate, clinically manifested prostate cancer is, at least in part or for a certain period, under endocrine control [4, 14]. The studies of Huggins *et al.* [15] first revealed the impact of androgens on prostatic carcinoma and provided the rationale for the treatment of prostate cancer by androgen deprivation. At present a number of treatment regimens are available such as surgical and medical castration, androgen blockade and inhibition of androgen synthesis [11, 16].

Breast cancer is the most common malignancy affecting women in the Western world with 100,000 new cases diagnosed yearly in the U.S.A. [17]. It is the leading cause of death in women aged 35–54 years and is second only to cardiovascular disease as the cause of death in women over 55 years of age [18]. Approximately one third of the breast tumours are estrogen-dependent [17, 18]. Removal of the source of estrogens or their precursor molecules or interference with estrogen action at the tumour brings about tumour remissions, often lasting several years, in approx. 30% of women with advanced breast cancer [19]. When applied to early disease, such therapies can improve survival rates by about 20% [19]. Since estrogens have an important role in the growth of breast cancers [2, 17–23, and references therein] an important number of investigators focused their research on the development of compounds, steroidal as well as nonsteroidal, which reduce the production of estrogens by inhibiting the aromatase.

Clinical, epidemiological and experimental data suggest that endometrial adenocarcinoma is hormone-dependent [19, 24]. Endometrial carcinomas regressed in one quarter of patients upon "ablation" of estrogenic effects with either antiestrogens alone or in combination with aromatase inhibitors or progestins [19]. Aromatase inhibitors are also of potential use in benign disorders such as endometriosis, precocious puberty of primary ovarian and testicular origin and fibrocystic breast disease [19].

Cushing's syndrome is the result of the metabolic effects of persistent, inappropriate hypercortisolism [25]. The absolute etiology of adrenocortical adenomas from patients with Cushing's syndrome has not been elucidated. However, recent studies by Ogo *et al.* [25] have shown that the overproduction of cortisol in adrenocortical adenomas associated with Cushing's syndrome results from an increased expression of cytochrome *P*450 17α-hydroxylase (*P*450c17) mRNA.

It is the aim of this paper to describe nonsteroidal and steroidal inhibitors of *P*450-dependent steroidogenic enzymes and their current or potential therapeutic uses. For some of these inhibitors effect on other *P*450 systems, such as retinoic acid metabolism, will also be discussed. An overview of the steroid biosynthesis pathways is given in Fig. 1.

NONSTEROIDAL INHIBITORS

Aminoglutethimide

The antiepileptic agent, aminoglutethimide (AG) (Fig. 2) is the pioneer drug of the reversible competitive aromatase inhibitors [17, 21, 22, 26, 27]. It is an amino derivative of the hypnotic agent glutethimide. AG was initially recognized to be an inhibitor of the adrenal cholesterol side-chain cleavage enzyme (*P*450scc) [26, 28–30]. Since glutethimide has little or no effect on *P*450scc, it follows that the amine is necessary for this inhibitory activity [29].

AG and its two enantiomers were studied for binding to corpus luteum mitochondrial *P*450 and inhibition of *P*450scc [28, 29]. The half-maximal inhibition (IC_{50}-value) of cholesterol side-chain cleavage occurs at about 16 μM *d*-AG, but a 2.5 times higher concentration is needed to reach the IC_{50}-value with *l*-AG. With the racemic mixture an IC_{50}-value of 26 μM is found [28]. On interaction with *P*450, *d*-, *l*- and racemic AG elicited Type II low spin difference spectra with peaks at 426 and troughs at 392 nm. This indicates an interaction of the primary amine with the cytochrome near or with the heme prosthetic group. This correlates with electron paramagnetic resonance (EPR) changes of bovine adrenal mitochondrial *P*450s:

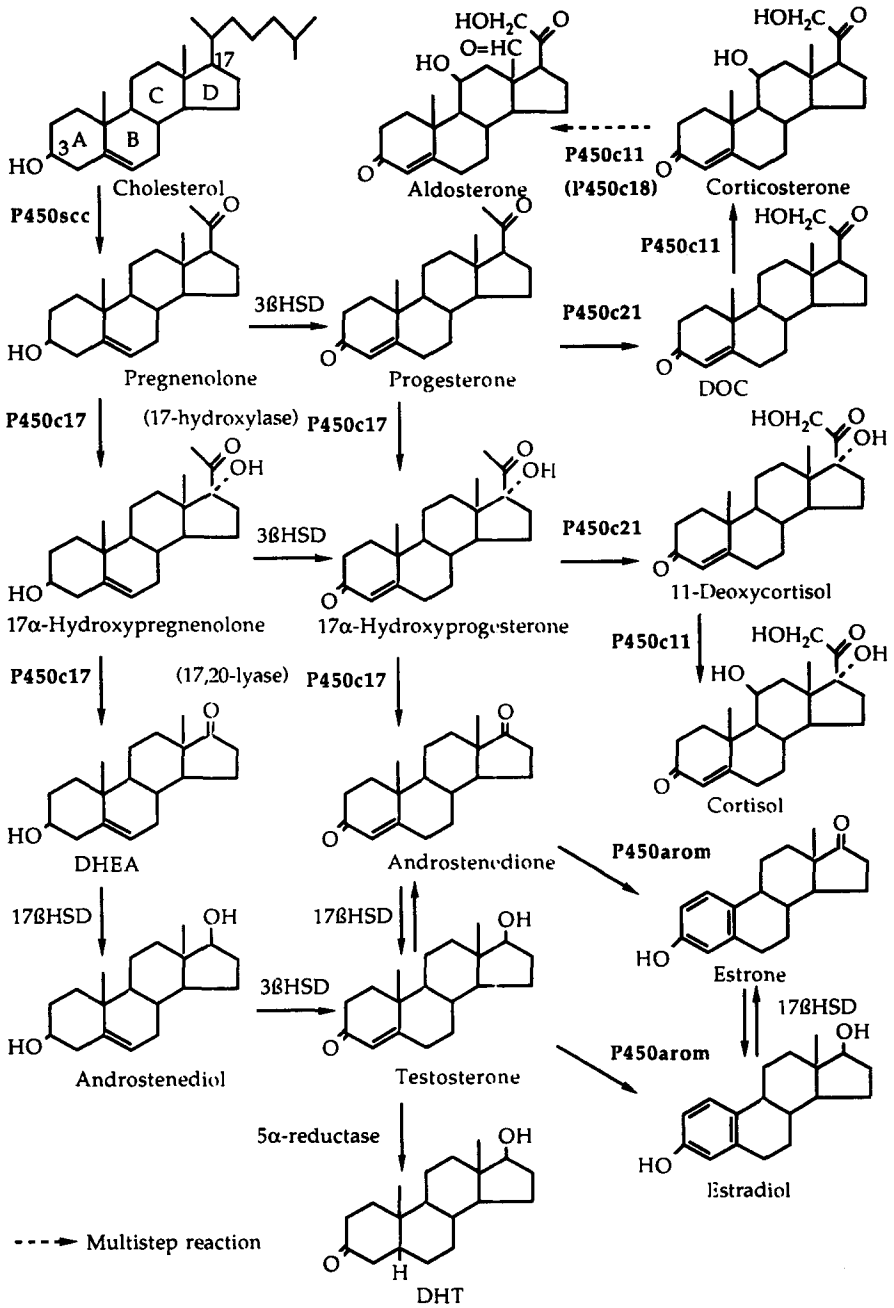

Fig. 1. Steroid biosynthesis pathway. SCC = side-chain cleavage; 17α = 17α-hydroxylase/17,20-lyase; Arom = aromatase; C21 = 21-hydroxylase; 11β = 11β-hydroxylase; C18 = 18-hydroxylase; 3βHSD = 3β-hydroxysteroid dehydrogenase + 4,5-isomerase; 17βHSD = 17β-hydroxysteroid dehydrogenase; DOC = 11-deoxycorticosterone; DHEA = dehydroepiandrosterone, DHT = dihydrotestosterone.

the native high spin Fe^{3+} state changing to an AG-bound low spin state [31]. Raman spectra of ferric *P*450scc complexed with AG are characteristic of low spin state [32]. Studies by Salhanick [29] suggest that AG acts by preventing reduction of *P*450, an obligate step preceding the oxidation of the substrate. AG not only binds to adrenal and corpus luteum mitochondrial *P*450, a Type II spectral change (minimum at 412 and maximum at 432 nm) was also induced by the addition of AG to rat brain mitochondria [33]. Pregnenolone synthesis from cholesterol by these brain mitochondria is inhibited by AG [33].

Testosterone synthesis from cholesterol in murine Leydig cells is also inhibited by AG [34]. Half-maximal inhibition of testosterone synthesis in unstimulated and hCG-stimulated cells is reached at 15.6 and 4.7 μM, respectively. This may originate from AG's effect on *P*450scc.

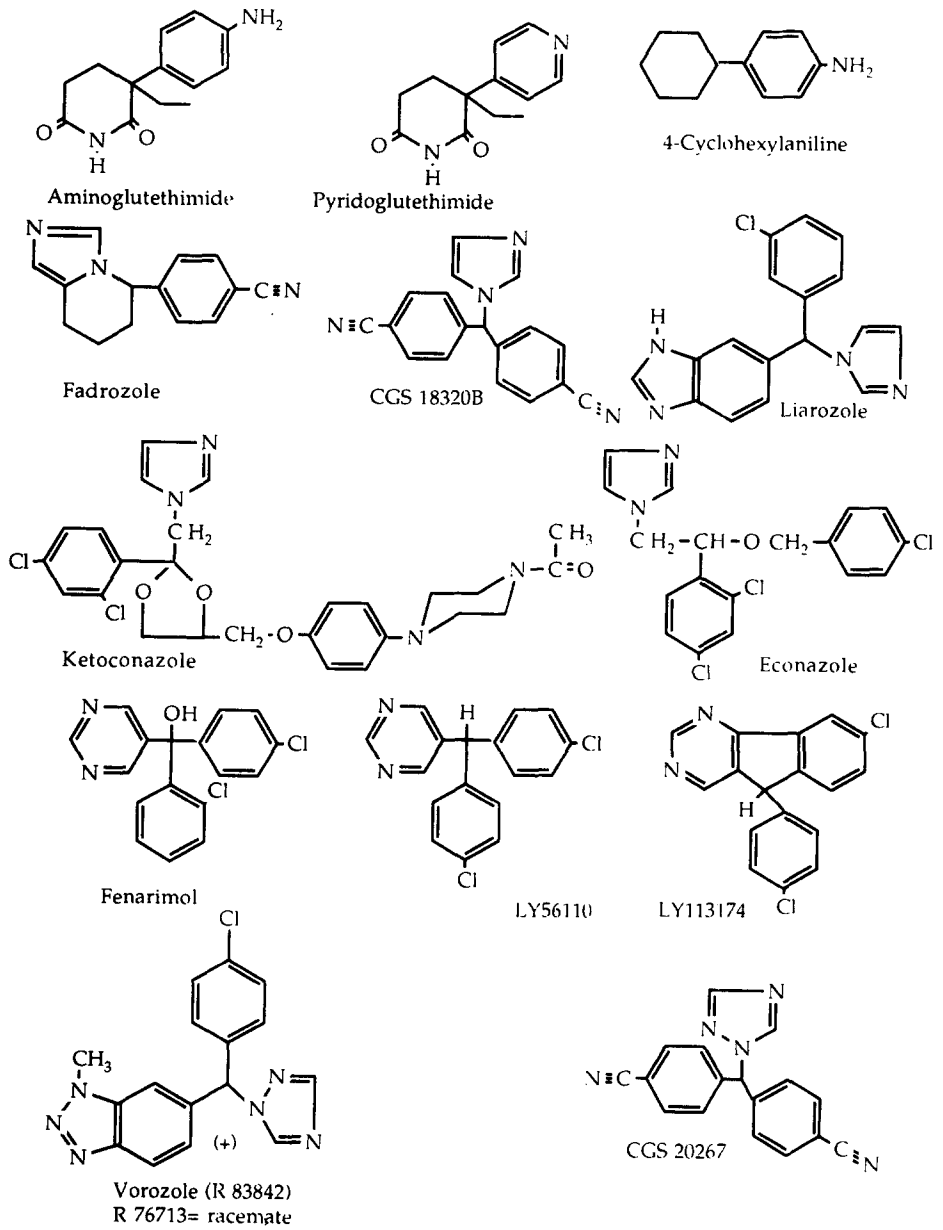

Fig. 2. Nonsteroidal inhibitors of *P*450-dependent reactions.

Indeed, Georgiou *et al.* [35] showed that AG inhibits *P*450scc activity in rat Leydig cells. Acute administration of AG to normal men lowers the plasma androgen levels through a direct testicular effect [36]. However, a reflex rise in luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels results [36]. Plasma LH increases to castrate levels in male dogs and testosterone secretion is thereby enhanced after an initial inhibitory action [26]. Thus, medical castration with AG as a means of blocking testicular function in men with prostatic carcinoma is not feasible [26]. However, based on its effects on adrenal *P*450scc activity, AG can be used as a medical adrenalectomy in men with prostatic carcinoma after orchiectomy [26]. Several clinical trials have indicated that in patients with prostate cancer, which has become refractory to orchiectomy, the combination of AG and glucocorticoid can induce palliation in approximately one-third of the patients [17].

AG-induced medical adrenalectomy is not only due to its effects on *P*450scc. The 11β-hydroxylase is also vulnerable to AG-inhibition [37]. Thus not only the synthesis of androstenedione but also that of cortisol is reduced. Consequently, supplementation of AG-treated patients with hydrocortisone is

required [17, 21, 22]. AG does not significantly influence the metabolism of hydrocortisone [26]. However, it accelerates the metabolism of dexamethasone and other synthetic glucocorticoids [26]. AG also inhibits *in vitro* the *P*450-dependent 18-hydroxylation of corticosterone by sheep and human adrenal homogenates [38]. Some observations pointed to an additional action at the 21-hydroxylase [26]. The inhibition of all these adrenal *P*450 enzymes results in a significant reduction in aldosterone production [17, 26]. However, as long as an adequate sodium intake is maintained in the diet mineralocorticoid treatment is usually not required [26].

As a consequence of its adrenal inhibitory actions AG is indicated for treatment of certain patients with Cushing's syndrome (for reviews see Refs [17, 26, 27]).

Since nearly all of the estrogens produced in postmenopausal women derive from peripheral aromatization of adrenal androgen precursors, AG was originally introduced for treating metastatic breast cancer as a form of medical adrenalectomy [17, 21, 22, 26, 27]. However, while these studies were ongoing, AG was found to be an effective inhibitor of the aromatase [39]. The concentration needed to block the aromatase activity was 0.3 μM being more than 10 times lower than that required to block cholesterol side-chain cleavage [26]. Subsequently it was found that AG-hydrocortisone (1000 mg AG + 40 mg hydrocortisone) also blocks peripheral conversion of androgens to estrogens *in vivo* [40]. Therefore, the primary cause of AG's antitumour effects seems to be its action on the peripheral conversion of androgens into estrogens. As for the inhibition of the cholesterol side-chain cleavage, the aromatase is also more sensitive to *d*-AG [29]. Fifty percent inhibition of estrogen synthesis was achieved with 8 μM *d*-AG whereas 0.31 mM was needed when *l*-AG was added to placental microsomes.

A dose-dependent inhibition of *in situ* aromatization in breast cancer tissue homogenates has been observed [17, and references therein]. Eighty percent inhibition was achieved at 30 μM AG, the mean plasma level reached after administration of 1000 mg AG.

In randomized clinical trials AG was found to be as effective as adrenalectomy and hypophysectomy. Furthermore, inhibition of estrogen synthesis with AG was found to have an almost identical antitimour effect to antagonism of estrogen action with tamoxifen [17]. However, the use of AG does not produce a consistent reduction of estrogen production in premenopausal women. Therefore its use is restricted to postmenopausal patients [17, 41].

Since AG is a nonspecific aromatase inhibitor and presents some problems with tolerability new and more selective aromatase inhibitors have been synthesized.

AG derivatives and esters of 4-pyridineacetic acid

A number of structural AG analogues have been synthesized. Examples are pyridoglutethimide [3-ethyl-3-(4-pyridyl)-piperidine-2,6-dione] (Fig. 2) [42] and the 3- and 4-alkylated derivatives such as 3-(4-aminophenyl)-3-isopentylpiperidine-2,6-dione [43]. Pyridoglutethimide is slightly less potent than AG, but it is a relatively selective inhibitor of the human aromatase without affecting *P*450scc [44]. This means that the drug can be used without the need for corticoid replacement [21, 44]. Tests of central nervous system (CNS) activity in animals indicate that in contrast with AG, pyridoglutethimide has no sedative properties [22].

4-Cyclohexylaniline (Fig. 2) is found to be an effective inhibitor of the aromatization of testosterone and androstenedione [45]. With human placental micrsomes, 4-cyclohexylaniline is an about 2 times more potent aromatase inhibitor than *d*-AG. *P*450scc from human placenta and bovine adrenals is about 24- and 16-fold less inhibited by 4-cyclohexylaniline than by *d*-AG [45]. The acetylation of both *d*-AG and 4-cyclohexylaniline resulted in the loss of inhibitory activity toward aromatase. This indicates that arylamine is essential for their inhibitory activity. Furthermore, the results of Kellis and Vickery [45] indicate that the 2,6-piperidinedione, nor the chiral 3-ethyl substituent of *d*-AG is required for the high affinity binding of *P*450arom. Since the glutarimide moiety may be responsible for AG's depressant action [29, 46] and skin rashes [29] the latter analogues represent a substantial improvement.

A variety of esters of 4-pyridineacetic acid derivatives have been prepared [47, 48]. Within this series of derivatives the most potent inhibitors of human placental aromatase are the (−)borneyl, (1*R*,2*R*,3*R*,5*S*)-(−)-isopinocampheyl and 1-adamantyl esters (IC_{50} = 0.089–0.096 μM). These esters also inhibit rat testicular 17α-hydroxylase (IC_{50} = 0.56–1.7 μM) and 17,20-lyase (IC_{50} = 0.61–2.2 μM). Within this series of derivatives the most potent inhibitor of rat testicular 17α-hydroxylase (IC_{50} = 0.28 μM)

Table 1. Effects of ketoconazole, liarzole and vorozole on *P*450-dependent reactions[a]

Species and tissues	Substrate	Product(s) formed	IC_{50} (μM) Ketoconazole	Liarozole	Vorozole
Testes					
S-10,000					
Rat	17OH,20	Androgens	0.26	0.22	1.8
	Pregnenolone	Androgens	6.00	3.20	>10 (23%)
Pig			3.00	4.15	>10 (0%)
Dog			5.41	0.26	
Bovine				0.27	
Human			0.34	0.26	>10 (29.3%)
Adrenals					
Bovine	Pregnenolone	11-Deoxy.	2.80	0.15	>10 (0%)
Microsomes		A.dione	0.40	12.70	>10 (19.6%)
Mitochondria	Cholesterol	Pregnenolone	1.7	>10 (0%)	>10 (0%)
	11-Deoxy.	Cortisol	3.8	1.4	64
Placenta					
Human	A.dione	Estrone	4.2	0.004	0.0014
Skin					
Rat	Retinoic acid	4-OH-RA	0.65	2.6	>10 (14%)
Liver					
Human					
Hep G2	Acetate	Cholesterol	0.52	5.0	>10
Chang			0.14	9.0	>10
Rat					
S-10,000	Mevalonate	Cholesterol	2.0	>10 (0%)	>10 (0%)
Microsomes	Cholesterol	7-OH-Chol.	0.2	>10 (7%)	>10 (7%)
C. albicans					
Cells	Acetate	Ergosterol	0.005		
S-10,000	Mevalonate	Ergosterol	0.07		

[a]Most of the results obtained with ketoconazole are taken from Vanden Bossche *et al.* [54, 70]. Those obtained with liarozole and vorozole are partly taken from Refs [71, 72] respectively. Effects on 17,20-lyase and 17-hydroxylase + 17,20-lyase in testes and adrenals [65, 72, 73], aromatase (substrate = [4-^{14}C]androstenedione) [72], 4-hydroxylation of retinoic acid [74], 7-hydroxylation of cholesterol [72] and ergosterol synthesis [73] were studied as described previously. Cholesterol synthesis was studied in human hepatoma cells (Hep G_2 cells ATCC HB 8065) [73], Chang liver cells (ATCC CCL 13) [75], and in a subcellular fraction (supernatant of a 10,000 *g* centrifugation, S-10,000) of male Wistar rat liver [76]. IC_{50}-value = concentration needed to reach 50% inhibition. 17OH,20 = 17-hydroxy,20-dihydroprogesterone; 11-Deoxy. = 11-deoxycortisol; A.dione = androstenedione; 4-OH-RA = 4-hydroxyretinoic acid; 7-OH-Chol. = 7-hydroxycholesterol. Figures in parentheses = % inhibition at 10 μM.

and 17,20-lyase ($IC_{50} = 0.26\,\mu M$) is (1*S*,2*S*, 3*S*,5*R*)-(+)-isopinocampheyl 4-pyridineacetic acid ester [47]. This ester is also a good inhibitor of the human placental aromastase ($IC_{50} = 0.12\,\mu M$). Although the 4-pyridineactic acid esters have good *in vitro* activity, their *in vivo* activity may be compromised by metabolic degradation by esterases [47]. Furthermore, since they are inhibitors of the 17α-hydroxylase, cortisol depletion can be expected.

Imidazole derivatives

Wilkinson *et al.* [49, 50] described a long list of imidazole derivatives as potent inhibitors of *P*450-dependent reactions in liver microsomes. The imidazoles investigated exhibited a Type II difference spectrum with a peak at 430–431 and a trough at 390–393 nm. This suggests that the unhindered nitrogen (N-3 in the imidazole ring) binds to the catalytic heme iron atom at the site occupied by the exchangeable sixth ligand. A great number of the present antifungal agents belong to the class of imidazole derivatives. Examples are miconazole, clotrimazole, econazole, imazalil, tioconazole, bifonazole, sulconazole and ketoconazole.

Ketoconazole. The introduction of the orally active imidazole derivative, ketoconazole (Fig. 2), represented a major advance in antifungal chemotherapy. Its antifungal activity originates from interaction with the *P*450-dependent demethylation of lanosterol or 24-methylenedihydrolanosterol, a key step in the synthesis of ergosterol (for reviews see Refs [51–55]). The effective daily dose of ketoconazole for most fungal infections is 200–400 mg [56]. The incidence of side effects is low. However, gynecomastia was reported in two patients during treatment with 200 mg ketoconazole daily [57] and in a few patients receiving high multiple doses (600–1200 mg/day) [58]. This rare side effect suggested that in such patients ketoconazole affected androgen synthesis. Measuring the testosterone serum levels, a dose related reversible decrease was observed [58–63].

Type II difference spectra obtained when increasing concentrations of ketoconazole were

added to piglet testes microsomes consisted of a maximum at about 433 nm, a minimum at 412 nm and an isobestic point at 423 nm [64]. Thus, ketoconazole might exert, at least partly, its inhibitory action on testosterone synthesis by interacting with the heme iron of *P*450s involved. It has been proven that ketoconazole inhibits the 17,20-lyase [64–78]. As shown in Table 1, ketoconazole is a more potent inhibitor of the 17,20-lyase than of the 17α-hydroxylase activity in subcellular fractions of rat testes. The 17α-hydroxylase/17,20-lyase catalysed androgen synthesis from pregnenolone in human testes is much more sensitive than that in rat, boar and dog testes (Table 1). Ketoconazole inhibits androgen synthesis when added to bovine adrenal microsomes ($IC_{50} = 0.4\ \mu M$) (Table 1). This inhibition coincided with an accumulation of pregnenolone and 11-deoxycorticosterone [64]. At higher concentrations, it also inhibited the synthesis of 11-deoxycortisol coinciding with a further increase in 11-deoxycorticosterone. This indicates again that the 17α-hydroxylase catalysed conversion of pregnenolone to 17α-hydroxyprogesterone is less sensitive than the 17,20-lyase. This difference in sensitivity was also found in human adrenal microsomes [77–80]. A marked inhibition of the 17,20-lyase and a moderate effect on the 17α-hydroxylase was also found in patients with metastatic prostate carcinoma on long term high dose ketoconazole therapy [81]. This difference in sensitivity is of interest, since in pig [82, 83], human [84], bovine [85] and rat [86] the microsomal 17-hydroxylase and 17,20-lyase activities result from the action of a single *P*450 (*P*450c17). This difference in sensitivity may originate from changes in the steroid–substrate–protein interaction during the two consecutive reactions. Indeed, site directed mutagenesis studies revealed that, at least in rat *P*450c17, the steroid–substrate–protein interaction changes during the two reactions [87].

The fact that ketoconazole interferes with testicular as well as adrenal androgen synthesis has made it a candidate for the treatment of androgen-dependent prostate carcinoma [63, 80, 88–93]. With the frequent administration of high doses of ketoconazole (400 mg every 8 h) symptomatic relief of bone pain and sustained clinical remissions occur in a significant number of patients with advanced prostate cancer [81]. However, at the high dose used, the principal side effect is gastric discomfort with nausea [90]. Thus, although striking clinical improvement is seen in many patients, ketoconazole's use is limited by the uncomfortable intake schedule and gastric discomfort. However, these investigations provided us with *P*450 systems to evaluate activity and predict possible toxicity and indicated the therapeutic potential of 17,20-lyase inhibitors in the treatment of prostatic cancer.

Ketoconazole is a poor inhibitor of the aromatase in both human placental microsomes ($IC_{50} = 4.2\ \mu M$; Table 1) and homogenates from human prostate ($IC_{50} = 4.7\ \mu M$) [94]. Thus, human prostatic aromatase is about 12 times less sensitive than androgen synthesis from pregnenolone in human testes. Although the demonstration of aromatase activity in human prostatic tissue raises the possibility of regulation of intraprostatic levels of estrogen independently of circulating plasma levels [94], it is doubtful that ketoconazole's effect on aromatase contributes to its beneficial effects in prostate pathology.

Since androgens are of causal significance in the etiology of hirsutism [95, 96], inhibitors of androgen synthesis, such as ketoconazole, may be of help in the treatment of this disease [92]. Successful therapy with ketoconazole has also been reported in a few children with precocious puberty and autonomous Leydig-cell hyperactivity with low basal and gonadotropin-releasing hormone-stimulated gonadotropin levels (for a review see Ref. [94]).

Since ketoconazole also inhibits, next to the 17-hydroxylase, the cholesterol side-chain cleavage and 11β-hydroxylase (Table 1) its use in Cushing's syndrome could be expected. Rapid clinical improvement has been observed after daily administration of 600–800 mg ketoconazole in pituitary-dependent Cushing's disease [97–99]. In a recent study ketoconazole's use as a palliative treatment was evaluated in 34 patients [100]. The data collected confirm that ketoconazole is valuable in the management of hypercortisolism, provided that patients are closely followed to exclude those who may develop liver toxicity and to prevent the occurrence of adrenal insufficiency [100].

Liarozole. The results obtained with ketoconazole in the treatment of prostatic cancer have triggered a multidisciplinary study to find new possibilities in medical treatment. A first product of these studies is liarozole (R 75251; Fig. 2; liarozole fumarate = R 85246). This imidazole derivative is devoid of antifungal activity, it is a poor inhibitor of cholesterol

synthesis, has no effect on cholesterol side-chain cleavage, but, shares with ketoconazole the effects on *P*450 isozymes involved in testicular androgen and adrenal cortisol synthesis (Table 1) [71, 101, 102]. As shown in Table 1 low concentrations of liarozole inhibit testicular androgen synthesis from 17-hydroxy,20-dihydroprogesterone by subcellular fractions of rat testes. Higher concentrations are needed to inhibit the synthesis of androgens from pregnenolone (17α-hydroxylase and 17,20-lyase activities). This indicates that androgen synthesis inhibition originates first from an effect on the 17,20-lyase. Liarozole and ketoconazole are almost equipotent inhibitors of androgen synthesis from pregnenolone by subcellular fractions from human testes (Table 1). However, as compared with ketoconazole, liarozole is a more potent inhibitor of the 17α-hydroxylase/17,20-lyase activity in dog testicular microsomes.

In dogs, liarozole at 2.5 mg/kg reduced plasma testosterone and androstenedione levels to castrate levels for at least 12 h (De Coster, personal communication). Values close to castrate levels are found in male volunteers 8 h after a single oral dose of 300 mg liarozole [102]. Mean plasma testosterone levels decreased from 23.6 to 3.3 nM. After 24 h testosterone levels were still decreased by 40% [102]. These results prove that liarozole is *in vitro* and *in vivo* an inhibitor of testicular androgen synthesis. Liarozole inhibits (Table 1) the synthesis of 11-deoxycortisol from pregnenolone in bovine adrenal microsomes proving that the adrenocortical 17α-hydroxylase activity of *P*450c17 is also sensitive. However, when compared with its effects on androgen synthesis by bovine testicular microsomes, almost 47 times more liarozole is needed to achieve 50% inhibition of androstenedione synthesis from pregnenolone by adrenocortical microsomes (Table 1). This indicates that in contrast with the 17,20-lyase activity of bovine testicular *P*450c17 the adrenal one is not a good target for liarozole. Nevertheless, in a pilot study, in orchiectomized stage D prostate cancer patients, liarozole (300 mg every 12 h) induced both subjective and objective improvement [12, 103]. In these patients adrenal androgen synthesis was not inhibited [104]. Furthermore, liarozole reduced growth of both androgen-dependent [105] and androgen-independent [106] prostatic adenocarcinoma in rats. These results suggest that liarozole's antitumoural effects may not be related to its inhibitory effects on androgen biosynthesis.

In contrast to ketoconazole, liarozole is a potent inhibitor of the aromatase in human placental microsomes (Table 1). Fifty percent inhibition of estrogen synthesis (estrone plus estradiol synthesis from [4-^{14}C]androstenedione) is already reached at 3.8 nM. In rat granulosa cells, a 50% inhibition of FSH-stimulated estradiol synthesis was achieved at 0.4 μM (measured by an estradiol radioimmunoassay) and estradiol plasma levels were significantly suppressed after a single oral intake of 300 mg liarozole by male volunteers [102].

Estrogens are described as prostatic growth stimulators and may play a role in prostatic pathology [10, 107, 108]. Futhermore, the aromatase inhibitors 4-hydroxy-androstene-3,17-dione (4-OHA) and 1-methyl-1,4-androstadiene-3,17-dione (atamestane, see later) are able to antagonize androstenedione-induced prostatic hypertrophy in dogs and cynomolgus monkeys, respectively [9, 109]. The benefit of estrogen depletion in prostate carcinoma remains to be determined, but it cannot be excluded that liarozole's effects on androgen-dependent and androgen-independent prostatic adenocarcinoma in rats and prostate cancer in men may partly originate from its effects on aromatase.

Effects of ketoconazole and liarozole on retinoic acid metabolism. As shown in Table 1, both liarozole [71] and ketoconazole [74] inhibit *in vitro* the *P*450-dependent 4-hydroxylation of retinoic acid in epidermal microsomes of neonatal rats. Ketoconazole and liarozole also inhibit this 4-hydroxylation step in liver microsomes from phenobarbital pretreated male Sprague–Dawley rats. Fifty percent inhibition is achieved at 0.7 and 3.1 μM, respectively (unpublished results). *In vivo*, ketoconazole and liarozole increased the biological half-life of exogenously administered retinoic acid and enhanced its endogenous plasma level [110, 111]. Increased plasma retinoic acid levels and cutaneous reactions similar to those observed after high doses of vitamin A were observed in orchiectomized stage D prostate cancer patients treated with liarozole [104]. Retinoids are shown to be of key importance in differentiation and morphogenesis. Upon exposure to retinoic acid, F9 mouse teratocarcinoma and HL60 human promyelocytic leukemia cells differentiate respectively to normal parietal endoderm and functional mature granulocytes (for a review see Ref. [112]). In F9 cells it has been proven that retinoic acid itself rather than its metabolites

mediates differentiation [113]. Since extensive metabolism occurs simultaneously with retinoid-induced differentiation, inhibition of the 4-hydroxylase will prolong retinoic acid's activities. Liarozole (10 μM) enhanced the effects of retinoic acid in F9 teratocarcinoma cells [106] an effect shared with ketoconazole [113, 114].

Based on these results it is tempting to speculate that ketoconazole's beneficial effects in prostate pathology may originate from its interaction with the 17,20-lyase activity and the *P*450 involved in the 4-hydroxylation of retinoic acid. The effects on the metabolism of endogenous retinoic acid and on aromatase may be involved in liarozole's antitumoural effects. Preclinical and clinical studies are needed to prove or reject this hypothesis.

Fadrozole and CGS 18320B. Two non-steroidal competitive aromatase inhibitors, fadrozole hydrochloride (CGS 16949A) and CGS 18320B (Fig. 2) have recently been introduced [22, 115, 116]. Fadrozole is a potent competitive inhibitor of human placental aromatase with a K_i of 1.6 ± 0.2 nM vs 700 ± 200 nM for *dl*-AG phosphate [115]. IC_{50}-values for inhibition of human placental and rat ovarian aromatase are 5 nM (fadrozole) and 3 nM (CGS 18320B) [117]. Using ovarian microsomes from immature rats, primed with pregnant mare's serum gonadotropin (PMSG), and measuring the conversion of testosterone into estradiol, IC_{50}-values of 1.7 and 200 nM were found for fadrozole and *dl*-AG phosphate, respectively [115]. The potency of the *l* isomer of fadrozole is equivalent to that of the racemate [118]. The IC_{50}-value of the *d* isomer is 39 nM [118]. The cholesterol side-chain cleavage in hamster ovarian tissue was inhibited by high concentrations of fadrozole and CGS 18320B with IC_{50}-values of 160 and >300 μM [117]. However, *in vitro* investigations with dispersed normal and hyperplastic human adrenocortical cells showed that fadrozole is a 11β-hydroxylase inhibitor (IC_{50}-values are between 0.1 and 1 μM), which inhibits ACTH-stimulated cortisol release [119]. Aldosterone release by ACTH-stimulated normal human adrenocortical cells was suppressed 50% by 1 nM fadrozole. Fadrozole also inhibited (91% at 0.1 μM) angiotensin II-stimulated aldosterone release by adrenal adenoma cells [119]. This suggests that fadrozole also inhibits the 18-hydroxylation of deoxycorticosterone (DOC) and/or the 18-oxidation of 18-hydroxy-DOC. Fifty percent inhibition of aldosterone production by rat adrenal tissue slices is achieved at 1 μM fadrozole and 6.1 μM CGS 18320B [117].

At a dose of 16 mg daily fadrozole hydrochloride significantly blunted cortisol responses to ACTH in postmenopausal patients with metastatic breast cancer [120]. At the same dose a significant suppression of both basal and ACTH-stimulated aldosterone production and a concomitant rise in the blood 18-hydroxycorticosterone/aldosterone ratio was observed [121]. This proves that fadrozole inhibits the terminal step in aldosterone biosynthesis. However, maximum inhibition of estrogen synthesis is already observed at a daily dose of 2 mg fadrozole [120, 122]. The degree of estrogen reduction was similar to that found in women treated with 1000 mg AG plus 40 mg hydrocortisone daily [120]. Postmenopausal women with breast cancer were treated for 4 weeks with 0.3, 1.0 and 2 mg fadrozole twice daily [123]. There was no significant difference between any of the doses in the suppression of estrone but highest suppression of estradiol levels were achieved at the 2 mg twice daily dose. No significant effects were noted on serum levels of testosterone, androstenedione, 17-hydroxyprogesterone or cortisol. However, serum aldosterone levels were significantly suppressed by 1 mg twice daily and further suppressed by 2 mg twice daily [123].

In a phase II study doses of 0.6 mg 3 times daily, 1 mg twice daily and 2 mg twice daily were compared [22]. Maximal suppression of plasma and urinary estrogens occurred at a dosage of 1 mg twice daily. Basal and ACTH-stimulated cortisol levels were unaffected. Basal aldosterone levels also remained stable upon administration of the 3 drug dosages. However, ACTH-stimulated aldosterone levels were significantly blunted [22].

Both fadrozole and CGS 18320B caused almost complete regression of 7,12-dimethyl benz(*a*) anthracene (DMBA)-induced mammary tumours in adult female Sprague–Dawley rats and also caused almost complete suppression of the appearance of new tumours. The ED_{50}-values of the antitumour effect of fadrozole and CGS 18320B were estimated to be 0.1–0.5 and ± 0.05 mg/kg/day, respectively [117]. The antitumour activity of fadrozole in patients is not yet well defined. In a phase I study [122] 2 of the 16 patients, who were evaluable for antitumour response, had a partial response and 7 patients had stable disease for 5,

6, 7, 8, 8, 11 and 24+ months, respectively. In the phase II trial [22], 1 complete regression and 3 partial regressions were observed in the 18 patients treated. In a recent study 80 postmenopausal women with metastatic breast cancer were treated daily with 1 or 4 mg [124]. Seventy-eight patients were assessable for side effects and response. Side effects were limited to hot flashes in 28%, nausea in 13%, fatigue in 8% and mild loss of appetite in 5% of patients. There was no change in serum electrolytes and calcium during the course of the study. Eight patients had a complete response, and 10 patients had a partial response. In addition 45% of the patients had a no change status.

Imidazole antifungals inhibiting aromatase. Other potent aromatase inhibitors are found in the class of imidazole antifungals. In contrast with ketoconazole, the topically used imidazole antifungal, econazole (Fig. 2) is a potent inhibitor of the aromatase in human placental microsomes [125] and of the human placental *P*450arom expressed in transfected COS-1 cells [126]. In the latter cells an IC_{50}-value of 40 nM was found ([^{3}H]androstenedione was used as substrate) [126]. Using human placenta microsomes and [4-^{14}C]androstenedione (40 nM) as substrate we reached 50% inhibition with 1.8 nM econazole and 6.8 nM miconazole, whereas 4.2 μM of ketoconazole was needed. Mason *et al.* [125] using [^{3}H]androstenedione as substrate compared the inhibitory effects of econazole with those of imazalil, miconazole, prochloraz, clotrimazole, ketoconazole, and aminoglutethimide on the human placenta microsomal aromatase. Fifty percent inhibition was achieved at 0.03, 0.15, 0.6, 0.7, 1.8, 60 and 45 μM, respectively.

Bis-chlorophenyl-pyrimidine analogues

The interest in the development of bis-chlorophenyl-pyrimidine analogues as aromatase inhibitors started with the agricultural fungicide fenarimol (Fig. 2) [127]. This 14α-demethylase inhibitor [52] was found to cause a decrease in fertility in rats which was associated with an impairment of male sexual behaviour. Subsequently, it was found that fenarimol inhibited the aromatase activity within the hypothalamic–preoptic area–amygdala region of the brain [127]. Fenarimol also inhibits *in vitro* estrogen synthesis by ovarian microsomes from rats treated with PMSG ($IC_{50} = 4.1$ μM) [127].

The inhibitory activity of the ovarian aromatase increased more than 70 times when the hydroxyl group of fenarimol was replaced by a proton and both chlorines are placed in the 4-position (LY56110, Fig. 2) [127–129]. LY56110 induced a reversed Type I binding spectrum, which is indicative of interaction with the Fe of *P*450 and the nitrogen of the pyrimidine moiety [129]. However, despite its good activity, both *in vitro* and *in vivo*, it had to be abandoned due to its ability to induce hepatic microsomal enzymes and its unusually long biological half-life [127]. Structural modifications resulted in the development of the indenopyrimidine LY113174 (Fig. 2). The latter compound inhibited rat ovarian aromatase *in vitro* with an IC_{50} of 24 nM [127]. The plasma radioactivity half-life for LY113174 was estimated to be $13\frac{1}{2}$ h which is considerably less than the 49 h found with LY56110. Although LY113174 increased relative liver weight and the microsomal protein content in female rats, this was not associated with an increase in *P*450 [127].

Triazole derivatives

The knowledge gathered with ketoconazole and liarozole contributed to the finding of new *P*450 inhibitors that interfere with estrogen biosynthesis. The search for a potent and selective aromatase inhibitor led to the synthesis of the triazole derivative, R76713 (Fig. 2) [72, 130, 131]. R 76713 is a potent inhibitor of the aromatase in human placental microsomes. Fifty percent inhibition of the conversion of [4-^{14}C]androst-4-ene-3,17-dione into estrogens by human placental microsomes is reached at 2.59 ± 1.37 nM, i.e. at concentrations 549 times lower than that needed for AG [72]. This IC_{50}-value corresponds very well with the 5.1, 3, 3.7, 7.6 and 10 nM found for rat ovarian homogenates [132], cultured rat granulosa cells [130], cultured human adipose stromal cells [131], cultured human choriocarcinoma JEG-3 cells [134] and cultured human ovarian granulosa cells [133], respectively. Kinetic analysis implies that there is a competitive part in the inhibition of human placental ($K_i = 1.3$ nM) [73] and JEG-3 cell ($K_i =$ 0.43 nM) [134] aromatase by R 76713. JEG-3 tumour aromatase measured *ex vivo*, 2 h after a single administration of R 76713, to nude mice was dose-dependently inhibited. An ED_{50}-value of 0.05 mg/kg was calculated [134]. Spectrophotometric analysis indicates that R76713 competes at low concentrations with carbon monoxide for binding to the heme iron of *P*450

present in human placenta microsomes [72]. Binding to *P*450 was further proven by adding increasing concentrations of R 76713 to microsomal suspensions previously equilibrated with androstenedione. The reversed Type 1 spectral changes observed, suggest that this triazole derivative is able to replace the substrate from its binding place [72].

In vivo, a single oral dose of 1 mg/kg lowered plasma estradiol levels of rats primed with PMSG by more than 90% for at least 16 h [130]. Oral administration of 1 mg/kg of R 76713 twice daily for 12 days reduced plasma estradiol to levels comparable to those obtained with ovariectomy [131]. Plasma progesterone levels decreased and LH levels slightly increased by both less than after ovariectomy [131]. Fifty percent inhibition of peripheral aromatization in male cynomolgus monkeys was obtained at an intravenous dose of 0.13 μg/kg [131].

R 76713 is in contrast to ketoconazole and some other azole antifungals, devoid of any effect on the *P*45014DM-dependent cholesterol synthesis [72]. It has at 10^{-5}M almost no effect on microsomal *P*450(s) of *C. albicans* [101]. In contrast with ketoconazole but similar to liarozole, R 76713 is devoid of effects on the cholesterol side chain cleavage (*P*450scc) [72, 135, 136]. At 10 μM it has a slight effect on bovine adrenal androgen and 11-deoxycortisol synthesis, suggesting that R 76713 is a poor inhibitor of both the adrenal 17-hydroxylase and 17,20-lyase [72]. R 76713 is also a poor inhibitor of androgen synthesis by subcellular fractions of Wistar rat testis; 50% inhibition is reached at $\pm 2\,\mu$M i.e. about 770 times higher than the concentration needed to achieve 50% inhibition of the aromatase [72]. A $\pm 17\%$ decrease in cortisol synthesis is observed at 10 μM only [72]. Fifty percent inhibition of cortisol, corticosterone and aldosterone production by cultured human adrenal cells was only reached at concentrations $> 10\,\mu$M [131, 133]. R 76713 is a poor inhibitor of the regio- and stereoselective *P*450-dependent hydroxylations of steroids by rat liver microsomes [72]. No changes in plasma progesterone, testosterone, 11-deoxycorticosterone, corticosterone or aldosterone levels were detected, 2 h after a single oral administration of 20 mg/kg of R 76713 to rats injected with PMSG, LHRH or ACTH or fed a sodium-deprived diet [131]. The plasma renin activity levels were also not altered [131]. R 76713 did not show any *in vitro* or *in vivo* estrogenic or antiestrogenic property [130, 131].

R 76713 treatment is comparable to ovariectomy in causing almost complete regression of DMBA- or NMU (dimethylnitrosourea)-induced mammary tumours in Sprague–Dawley and Wistar rats, respectively [131, 137, 138]. Three weeks oral treatment of rats with R 76713 (0.3–5 mg/kg) caused regression of experimental endometrial autotransplants [131].

Results of a preclinical study in male and female (premenopausal) volunteers prove that R 76713 is a potent inhibitor of estrogen synthesis [131]. Twenty-six patients with advanced postmenopausal breast cancer, all heavily pretreated for their disease, received either 2.5 or 5 mg R 76713 as a single oral dose once daily [139]. An ACTH test performed before and after 4 weeks of treatment proved that R 76713 does not affect cortisol or aldosterone plasma levels.

The aromatase inhibitory effect of R 76713 is largely due to its (+)-S-enantioner, R 83842 (Vorozole). For human placental microsomal aromatase vorozole ($IC_{50} = 1.4$ nM, Table 1) is about 1.9- and 32-times more active than the racemate and the (−)-R-enantiomer (R 83839) [72]. Vorozole is about 30- and 1029-times more active than 4-hydroxyandrostenedione (see later) and AG [72]. The high potency of vorozole might originate from its high affinity for the microsomal *P*450. Indeed, as compared to R 76713 and R 83839, the (+) enantiomer forms a more stable *P*450–drug complex. As shown in Table 1, vorozole is not only a potent aromatase inhibitor, it also is a selective one. Fifty percent inhibition of the conversion of 17-hydroxy,20-dihydroprogesterone to androgens (17,20-lyase) by a subcellular fraction of Wistar rat testes was achieved at 1.8 μM, i.e. at a concentration 1300-times higher than that needed to reach 50% inhibition of the human placental aromatase. With pregnenolone as substrate (a measure for both the 17-hydroxylase and 17,20-lyase activities of *P*450c17) only minor effects on androgen synthesis by rat, pig and human testes, are observed (Table 1). Sixty-four micromolar is needed to get 50% inhibition of the 11β-hydroxylase. Thus, vorozole differs from fadrozole, which is a relatively potent inhibitor of this mitochondrial *P*450-dependent enzyme (see above). Up to 10 μM, vorozole, R 76713 and R 83839 have no statistically significant effect on the regio- and stereoselective

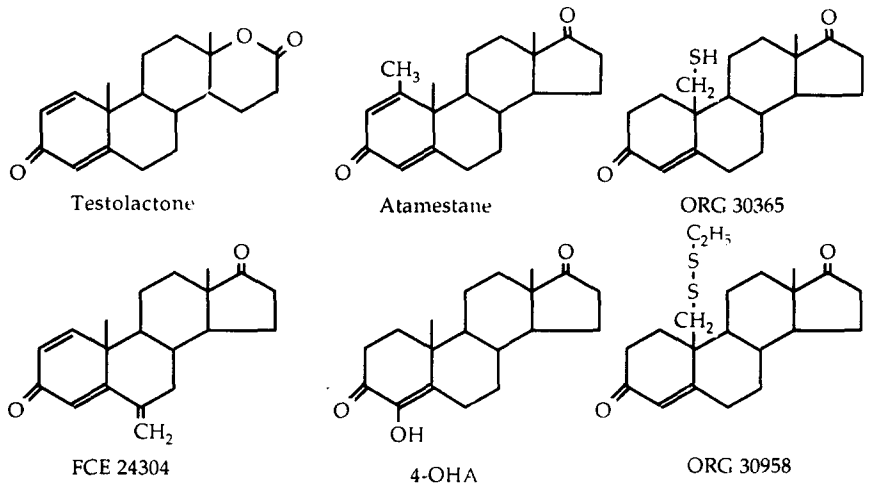

Fig. 3. Steroidal inhibitors of *P*450-dependent reactions. 4-OHA = 4-hydroxyandrostenedione.

oxidations of testosterone in male rat liver microsomes [72].

The potency and selectivity of vorozole suggest that it may represent a major improvement over AG for treatment of patients with estrogen-dependent diseases. Clinical studies in advanced postmenopausal breast cancer have been initiated.

A triazole derivative of CGS 18320, CGS 20267 (Fig. 2), is also under clinical study. CGS 20267 appears to be active at a dosage as low as 0.2 mg given daily to men [140]. In animal studies, this triazole does not interact with the cholesterol side-chain cleavage, 11β-hydroxylase or 17,20-lyase [140].

STEROIDAL INHIBITORS

The aromatase inhibitors, discussed so far, are nonsteroidal competitive aromatase inhibitors. They bind reversibly to the active site of the enzyme and prevent estrogen synthesis only as long as they occupy the catalytic site. A great number of steroidal compounds structurally related to the natural substrate, have been developed (for reviews see Refs [22, 141, 142]). These compounds can be classified as irreversible mechanism based inhibitors. They initially compete with androstenedione or testosterone for binding to the active site of *P*450arom but are then converted by the enzyme into alkylating agents which form covalent bonds at or near the active site. Thus, the enzyme becomes inactivated as a consequence of its own mechanism of action. Such inhibitors have been called "suicide" inhibitors [21, 22, 141–143]. Covey [141] evaluated the activity of 87 steroidal inhibitors (a few of them will be described here) and discussed the possible aromatase inactivation mechanisms.

Testolactone

A steroidal aromatase inhibitor, clinically used, since the beginning of the sixties, in the palliative treatment of postmenopausal breast cancer, is Δ^1-testolactone (*D*-homo-17α-oxa-androsta-1,4-diene-3,17-dione, Fig. 3) [144]. Knowledge of its efficacy for the treatment of breast cancer preceded the discovery that testolactone lowers circulating estrogen levels [145]. It was found to be a weak-binding, slow inactivator of the aromatase in placental microsomes [141]. Apparently the modified D-ring of this $\Delta^{1,4}$-3-ketosteroid prevents its tight binding to *P*450arom [141]. Encouraging results with Δ^1-testolactone (100 mg b.i.d.) were obtained in a relatively small group of patients with BPH [146]. These results suggest that aromatase inhibitors may be of use in the treatment of BPH.

Atamestane

Another $\Delta^{1,4}$-3-ketosteroid with a methyl group at C-1, atamestane (SH 489, ZK 95639, Fig. 3) shows a much higher affinity ($K_i = 0.082\ \mu$M) for human placental aromatase than testolactone ($K_i = 20\ \mu$M) [9, 147–149]. The presence of the 1-methyl group prevents ready aromatization of the A-ring while improving the noncovalent binding to the enzyme [147].

Other *P*450-dependent enzymes of steroid synthesis (adrenals) or metabolism (5α-reductase) are not inhibited [9]. Atamestane is a

substrate for liver 5β-reductase and for 17β-hydroxysteroid dehydrogenases of liver and testis in rat and guinea pig [150]. Given subcutaneously to PMSG-stimulated rats, atamestane was found to give a dose-dependent reduction in serum estradiol [9]. However, its effect was negligible when given orally at a dose of 100 mg/kg [143].

Atamestane inhibits estrogen-induced hyperplastic changes in the fibromuscular stroma of the prostate in androstenedione-treated dogs and monkeys [9, 109, 151]. In male volunteers and patients with BPH, atamestane at an oral dose of 10 mg/kg/day (7 days) induced a maximum mean reduction of serum estrogen levels of about 65% [150]. Twenty-four hours after treatment the BPH patients showed 71–78% reduction of serum estrone levels. Studies are planned to establish the long-term safety and to evaluate the potential of atamestane to alter the natural history of BPH [150].

ORG 30958, ORG 30365 and FCE 24304

More recently 19-(ethyldithio)-androst-4-ene-3,17-dione (ORG 30958, Fig. 3) has been reported to be both a potent competitive and irreversible inhibitor of the aromatase [152]. ORG 30958 itself is *in vitro* a very weak aromatase inhibitor. This compound is assumed to be converted into 19-mercapto-androst-4-ene-3,17-dione (ORG 30365, Fig. 3), a potent irreversible aromatase inhibitor [152]. The latter compound is as compared with atamestane, an 8-times more active inhibitor of the human placental microsomal aromatase. The prodrug ORG 30958, is also more active than atamestane in rat and dog models. It does not display other hormonal effects [152].

Another irreversible aromatase inhibitor is 6-methyleneandrosta-1,4-diene-3,17-dione (FCE 24304, Fig. 3) [143, 153]. This compound is an effective inhibitor when administered both orally ($ED_{50} = 3.7$ mg/kg) and subcutaneously ($ED_{50} = 1.8$ mg/kg) to rats [143].

4-OHA

The so far best studied example of this class of inhibitors is 4-OHA (CGP 32349, lentaron, Fig. 3) [22, 46, 94, 109, 141–143, 154–158, and references therein]. 4-OHA is a relatively potent competitive aromatase inhibitor which also causes inactivation of the enzyme *in vitro* [159]. In laboratory experiments, 4-OHA was found to inhibit human placental [46, 72, 154, 155, 159, 160] and rat ovarian aromatase [46, 154, 155, 160] and to reduce estradiol synthesis in granulosa cells from preovulatory follicles of rats and humans [161]. Studies of Ayub and Levell [94] indicate that 4-OHA also inhibits human prostatic aromatase activity. 4-OHA administered (subcutaneously) to normal cycling rats reduced estrogen levels in plasma from ovarian vein blood and aromatase activity of ovarian homogenates [46, 156, 160] and was found to inhibit peripheral aromatization in male rhesus monkeys [47, 156, 157, 163]. Inhibition of peripheral aromatization was confirmed in postmenopausal women with breast cancer [163]. Aromatase activity in the breast tumour of these patients was also found to be inhibited in 3 out of 7 patients [163]. 4-OHA also produced tumour regression in DMBA- [46, 154, 164] and NMU-treated rats [46].

In cycling animals, 4-OHA also directly inhibits gonadotropin production and reduces estrogen and progesterone receptors [157]. These effects appear to be mediated by interaction with androgen but not with estrogen receptors [157] and appears to be associated with the weak androgenic activity of 4-OHA [158]. The latter activity may contribute to reducing the growth stimulating effects of estrogen.

In the initial endocrine study, postmenopausal breast cancer patients received 500–1000 mg 4-OHA by weekly intramuscular (i.m.) injection [165]. During therapy estradiol levels fell from 7.2 to 2.6–2.8 pg/ml. A single i.m. injection of 500 mg 4-OHA suppressed estradiol levels to a mean of 36.3% of baseline 4–7 days later in 14 patients [166]. No effect of 4-OHA treatment was observed on serum gonadotropin, sex steroid binding protein or dehydroepiandrosterone sulfate levels [157]. In a recent overview, Brodie [158] summarizes the results of three clinical trials on 465 breast cancer patients. The patients received either 500 i.m. weekly or biweekly, or 250 mg biweekly. The response rates were not significantly different between the different doses and frequency of administration. Overall 26% of patients experienced complete or partial regression of their tumours, in a further 24.6% of patients the disease was stabilized and the disease progressed in the remaining women [158].

In contrast with Brodie *et al.* [167], Ayub and Levell [94] were able to measure the conversion of androstenedione to estrone and estradiol in human prostatic tissue. As already mentioned

above, 4-OHA inhibits human prostatic aromatase activity [94] and 4-OHA is able to antagonize androstenedione induced prostatic hypertrophy in dogs [10, 109]. Furthermore, Shearer *et al.* [168] reported that 12 of 19 patients with advanced, hormone resistant, prostatic cancer and who had relapsed following castration and other therapies, gained significant pain reflief following weekly i.m. injections of 500 mg 4-OHA. These preliminary results suggest that 4-OHA can be of use in the palliation of patients with advanced prostatic cancer.

CONCLUSION

The examples, taken out of the long list of present available nonsteroidal and steroidal inhibitors, demonstrate that *P*450 systems can be exploited in the construction of compounds that may become important tools in chemotherapeutic applications. The study of interactions of compounds, such as ketoconazole and liarozole, with *P*450s resulted in the finding of new targets (i.e. the *P*450-dependent 17,20-lyase and catabolism of retinoic acid) which can be exploited in the search for new possibilities in medical treatment.

Acknowledgements—The author wishes to thank I. Beyens, J. Gorrens, P. Marichal, I. Roels and G. Willemsens for the unpublished results used. He also thanks Drs R. De Coster and M. Janssen for their critical review of the manuscript.

REFERENCES

1. Moradian A. D., Morely J. E. and Korenman S. G.: Biological actions of androgens. *Endocrine Rev.* **8** (1987) 1–28.
2. Miller W. R. and O'Neill J. S.: The significance of steroid metabolism in human cancer. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 317–325.
3. Freeman D. A.: Steroid hormone-producing tumors in man. *Endocrine Rev.* **7** (1986) 204–220.
4. Bartsch W., Klein H., Schiemann U., Bauer H. W. and Voigt K. D.: Enzymes of androgen formation and degradation in the human prostate. *Ann. N. Y. Acad. Sci.* **595** (1990) 53–66.
5. Chiarodo A.: National cancer institute roundtable on prostate cancer: future research directions. *Cancer Res.* **51** (1991) 2498–2505.
6. Cunha G. R., Donjacour A. A., Cooke P. A., Mee S., Bigsby R. M., Higgins S. J. and Sugimura Y.: The endocrinology and developmental biology of the prostate. *Endocrine Rev.* **8** (1987) 338–362.
7. Stoner E.: The clinical development of a 5α-reductase inhibitor, finasteride. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 375–378.
8. Sciarra F., Toscano V., Concolino G. and Di Silverio F.: Antiandrogens: clinical applications. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 349–362.
9. Henderson D., Habenicht U. F., Nishino Y., Kerb U. and El Etreby M. F.: Aromatase inhibitors and benign prostatic hyperplasia. *J. Steroid Biochem.* **25** (1986) 867–876.
10. Karr J. P., Kaburagi Y., Mann C. F. and Sandberg A. A.: The potential significance of aromatase in the ethiology and treatment of prostatic disease. *Steroids* **50** (1987) 451–457.
11. Gittes R. F.: Carcinoma of the prostate. *New Engl. J. Med.* **324** (1991) 236–245.
12. Denis L. J.: Controversies in the management of localised and metastatic prostatic cancer. *Eur. J. Cancer* **27** (1991) 333–341
13. Martini L., Celotti F., Lechuga M. J., Melcangi R. C., Motta M., Negri-Cesi P., Poletti A. and Zoppi S.: Androgen metabolism in different target tissues. *Ann N. Y. Acad. Sci.* **595** (1990) 184–198.
14. Pierrepoint C. G., Turkes A. O., Walker K. J., Harper M. E., Wilson D. W., Peeling W. B. and Griffiths K.: Endocrine factors in the treatment of prostatic cancer. In *EORTC Genitourinary Group Monograph 2, Part A: Therapeutic Principles in Metastatic Prostatic Cancer* (Edited by F. H. Schroeder and B. Richards). Alan R. Liss, New York (1985) pp. 51–72.
15. Huggins C., Stevens R. E. and Hodges C. V.: Studies on prostatic cancer. II. Effects of castration on advanced carcinoma of the prostate gland. *Archs Surg.* **43** (1941) 209–223.
16. Grayhack J. T., Keeler T. C. and Kozlowski J. M.: Carcinoma of the prostate: Hormonal therapy. *Cancer* **60** (1987) 589–601.
17. Manni A. and Santen R. J.: Clinical uses of aromatase inhibitors. In *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action* (Edited by B. J. A. Furr and A. E. Wakeling). Baillière Tindall, London (1987) pp. 271–287.
18. Jackson I. M. and Lowery C.: Clinical uses of antiestrogens. In *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action* (Edited by B. J. A. Furr and A. E. Wakeling). Baillière Tindall, London (1987) pp. 87–105.
19. Santen R. J.: Potential clinical use of new aromatase inhibitors. *Steroids* **50** (1987) 575–593.
20. Nicholson R. I., Walker K. J., Bouzubar N., Wills R. J., Gee J. M. W., Rushmere N. K. and Davies P.: Estrogen deprivation in breast cancer. Clinical, experimental, and biological aspects. *Ann. N. Y. Acad Sci.* **595** (1990) 316–327.
21. Miller W. R.: Endocrine treatment for breast cancers: biological rationale and current progress. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 467–480.
22. Santen R. J., Manni A., Harvey H. and Redmond C.: Endocrine treatment of breast cancer in women. *Endocrine Rev.* **11** (1990) 221–265.
23. James V. H. T., Reed M. J., Lai L. C, Ghilchik M. W., Tait G. H., Newton C. J. and Coldham N. G.: Regulation of estrogen concentrations in human breast tissues. *Ann. N. Y. Acad. Sci.* **595** (1990) 227–235.
24. Dell' Acqua S., De Cicco Nardone F., Benedetto M. T., Iacobelli S., Rossiello F., Bongiorno M. and Margariti P. A.: Hormone receptors and enzymatic activities in human endometrial adenocarcinoma. *Ann. N. Y. Acad. Sci.* **595** (1990) 334–347.
25. Ogo, A., Haji M., Ohashi M. and Nawata H.: Markedly increased expression of cytochrome *P*-450 17α-hydroxylase (*P*450c17) mRNA in adrenocortical adenomas from patients with Cushing's syndrome. *Molec. Cell Endocr.* **80** (1991) 83–89.
26. Santen R. J. and Misbin R. I.: Aminoglutethimide: review of pharmacology and clinical uses. *Pharmacotherapy* **1** (1981) 95–120.

27. Shaw M. A., Nicholls P. J. and Smith H. J.: Aminoglutethimide and ketoconazole: historical perspectives and future prospects. *J. Steroid Biochem.* **31** (1988) 137–146.
28. Uzgiris V. I., Whipple C. A. and Salhanick H. A.: Stereoselective inhibition of cholesterol side chain cleavage by enantiomers of aminoglutethimide. *Endocrinology* **101** (1977) 89–92.
29. Salhanick H. A.: Basic studies on aminoglutethimide. *Cancer Res.* **42** (Suppl. 2) (1982) 3315S–3321S.
30. Nadjafi C.: Aminoglutethimide chemistry, pharmacokinetics, pharmacology and mechanism of action. *Acta Clin. Belgica* **41** (Suppl. II) (1986) 3–11.
31. Jefcoate C. R., Orme-Johnson W. H. and Beinert H.: Cytochrome *P*-450 of bovine adrenal mitochondria. *J. Biol. Chem.* **251** (1976) 3706–3715.
32. Tsubaki M., Hiwatashi A. and Ichikawa Y.: Effects of cholesterol analogues and inhibitors on the heme moiety of cytochrome $P\text{-}450_{SCC}$: a resonance Raman study. *Biochemistry* **26** (1987) 4535–4540.
33. Walther B., Ghersi-Egea J-F., Minn A. and Siest G.: Brain mitochondrial cytochrome $P\text{-}450_{SCC}$: spectral and catalytic properties. *Archs Biochem. Biophys.* **254** (1987) 592–596.
34. Hou J. W., Delwood C., Collins C. and Schleicher R. L.: Sources of cholesterol for testosterone biosynthesis in murine Leydig cells. *Endocrinology* **127** (1990) 2047–2055.
35. Georgiou M., Perkins L. M. and Payne A. H.: Steroid synthesis-dependent, oxygen-mediated damage of mitochondrial and microsomal cytochrome *P*-450 enzymes in rat Leydig cell cultures. *Endocrinology* **121** (1987) 1390–1399.
36. Santen R. J., Cohn N., Misbin R., Samojlik E. and Foltz E.: Acute effects of aminoglutethimide on testicular steroidogenesis in normal men. *J. Clin. Endocr. Metab.* **49** (1979) 631–634.
37. Touitou Y., Bogdan A., Legrand J. C. and Desgrez P.: Aminoglutethimide and glutethimide: effects on 18-hydroxycorticosterone biosynthesis by human and sheep adrenals *in vitro*. *Acta Endocr.* **80** (1975) 517–526.
38. Dexter R. N., Fishman L. M., Ney R. L. and Liddle G. W.: Inhibition of adrenal corticosteroid synthesis by aminoglutethimide: studies of the mechanism of action. *J. Clin. Endocr. Metab.* **27** (1967) 473–480.
39. Thompson E. A. and Siiteri P. K.: The involvement of human placental microsomal cytochrome *P*-450 in aromatization. *J. Biol. Chem.* **249** (1974) 5373–5378.
40. Santen R. J., Santner S., Davis B., Veldhuis J., Samojlik E. and Ruby E.: Aminoglutethemide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. *J. Clin. Endocr. Metab.* **47** (1978) 1257–1265.
41. Harris A. L., Dowsett M., Jefcoate S. L., McKinna J. A., Morgan M. and Smith J. E.: Endocrine and therapeutic effects of aminoglutethimide on premenopausal patients with breast cancer. *J. Clin. Endocr. Metab.* **55** (1982) 718–722.
42. Foster A. B., Jarman M., Leung C.-S., Rowlands M. G., Taylor G. N., Plevey R. G. and Sampson P.: Analogues of aminoglutethimide: selective inhibition of aromatase. *J. Med. Chem.* **28** (1985) 200–204.
43. Hartman R. W. and Batzl C.: Synthesis and evaluation of mammary tumor inhibiting activity of 3-alkylated 3-(4-aminophenyl) piperidine-2,6-diones. *J. Med. Chem.* **29** (1986) 1362–1369.
44. Leung C. S., Rowlands M. G., Jorman M., Foster A. B., Griggs L. S. and Wilman D. E. V.: Analogues of 3-ethyl-3-(4-pyridyl)piperidine-2,6-diones as selective inhibitors of aromatase: derivatives with variable 1-alkyl and 3-alkyl substituents. *J. Med. Chem.* **30** (1987) 1550–1554.
45. Kellis J. T. and Vickery L. E.: Inhibition of estrogen synthase (aromatase) by 4-cyclohexylaniline. *Endocrinology* **114** (1984) 2128–2137.
46. Brodie A. H. M.: Pharmacology of aromatase inhibitors. In *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action* (Edited by B. J. A. Furr and A. E. Wakeling). Baillière Tindall, London (1987) pp. 255–270.
47. McCague R., Rowlands M. G., Barrie S. E. and Houghton J.: Inhibition of enzymes of estrogen and androgen biosynthesis by esters of 4-pyridylacetic acid. *J. Med. Chem.* **33** (1990) 3050–3055.
48. Laughton C. A. and Neidle S.: Inhibitors of the *P*450 enzymes aromatase and lyase. Crystallographic and molecular modeling studies suggest structural features of pyridylacetic acid derivatives responsible for differences in enzyme inhibitory activity. *J. Med. Chem.* **33** (1990) 3055–3060.
49. Wilkinson C. F., Hetnarski K. and Yellin T. O.: Imidazole derivatives—A new class of microsomal enzyme inhibitors. *Biochem. Pharmac.* **21** (1972) 3187–3192.
50. Wilkinson C. F., Hetnarski K. and Hicks L. J.: Substituted imidazoles as inhibitors of microsomal oxidation and insecticide synergists. *Pest. Biochem. Physiol.* **4** (1974) 299–312.
51. Vanden Bossche H.: Biochemical targets for antifungal azole derivatives: hypothesis on the mode of action. In *Current Topics in Medical Mycology* (Edited by M. R. McGinnis). Springer Verlag, New York, Vol. 1 (1985) pp. 313–351.
52. Vanden Bossche H.: Mode of action of pyridine, pyrimidine and azole antifungals. In *Sterol Biosynthesis Inhibitors. Pharmaceutical and Agrochemical Aspects* (Edited by D. Berg and M. Plempel). Ellis Horwood, Chichester, England (1988) pp. 79–119.
53. Yoshida Y.: Cytochrome *P*450 of fungi: primary target for azole antifungal agents. In *Current Topics in Medical Mycology* (Edited by M. R. McGinnis). Springer Verlag, New York, Vol. 2 (1988) pp. 388–418.
54. Vanden Bossche H., Marichal P., Gorrens J., Coene M.-C., Willemsens G., Bellens D., Roels I., Moereels H. and Janssen P. A. J.: Biochemical approaches to selective antifungal activity. Focus on azole antifungals. *Mycoses* (Suppl. 1) **32** (1989) 35–52.
55. Köller W.: Antifungal agents with target sites in sterol functions and biosynthesis. In *Target Sites of Fungicide Action* (Edited by W. Köller). CRC Press, Boca Raton, FL (1992) pp. 119–206.
56. Graybill J. R.: The modern revolution in antifungal drug therapy. In *Mycoses in AIDS Patients* (Edited by H. Vanden Bossche, D. W. R. Mackenzie, G. Cauwenbergh, J. Van Cutsem, E. Drouhet and B. Dupont). Plenum Press, New York (1990) pp. 265–277.
57. De Felice R., Johnson D. G. and Galgiani J. N.: Gynecomastia with ketoconazole. *Antimicrob. Ag. Chemother.* **19** (1981) 1073–1074.
58. Pont A., Williams P. L., Azhar S. and Reirz R. E.: Ketoconazole blocks testosterone synthesis. *Archs Int. Med.* **142** (1982) 2137–2140.
59. Schürmeyer Th. and Nieschlag E.: Ketoconazole-induced drop in serum and saliva testosterone. *Lancet* **13** (1982) 1098–1099.
60. Santen R. J., Vanden Bossche H., Symoens J., Brugmans J. and De Coster R.: Site of action of low dose ketoconazole on androgen biosynthesis in men. *J. Clin. Endocr. Metab.* **57** (1983) 732–736.
61. De Coster R., Caers I., Coene M.-C., Amery W., Beerens D. and Haelterman C.: Effects of high dose ketoconazole therapy on the main plasma testicular

and adrenal steroids in previously untreated prostatic cancer patients. *Clin. Endocr.* **24** (1986) 657–664.
62. Feldman D.: Ketoconazole and other imidazole derivatives as inhibitors of steroidogenesis. *Endocrine Rev.* 7 (1986) 409–420.
63. Vanden Bossche H., De Coster R. and Amery W.: Pharmacological and clinical uses of ketoconazole. In *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action* (Edited by B. J. A. Furr and A. E. Wakeling). Baillière Tindall, London (1987) pp. 288–307
64. Vanden Bossche H., Lauwers W., Willemsens G. and Cools W.: Ketoconazole an inhibitor of the cytochrome *P*-450 dependent testosterone biosynthesis. In *EORTC Genitourinary Group Monograph 2, Part A: Therapeutic Principles in Metastatic Prostatic Cancer* (Edited by F. H. Schroeder and B. Richards). Alan R. Liss, New. York (1985) pp. 187–196.
65. Vanden Bossche H., Lauwers W., Willemsens G. and Cools W.: The cytochrome *P*-450 dependent C17,20-lyase in subcellular fractions of the rat testis: differences in sensitivity to ketoconazole and itraconazole. In *Microsomes and Drug Oxidations* (Edited by A. R. Boobis, J. Caldwell, F. de Matteis and C. R. Elcombe). Taylor and Francis, London (1985) pp. 63–73.
66. Lauwers W. F. J., Le Jeune L., Vanden Bossche H. and Willemsens G.: Identification of 17α,20-dihydroxyprogesterone in testicular extracts after incubation with ketoconazole. *Biomet. Mass Spectrosc.* **12** (1985) 296–301
67. Rajfer J., Sikka S., Xie H. W. and Swerdloff R. S.: Effect of ketoconazole on steroid production in rat testis. *Steroids* **46** (1985) 867–881.
68. Kan P., Hirst M. A. and Feldman D.: Inhibition of steroidogenic cytochrome *P*-450 enzymes in rat testis by ketoconazole and related imidazole anti-fungal drugs. *J. Steroid Biochem.* **23** (1985) 1023–1029.
69. Higashi Y., Omura M., Suzuki K., Inano H. and Oshima H.: Ketoconazole as a possible universal inhibitor of cytochrome *P*-450 dependent enzymes: its mode of inhibition. *Endocrinology (Jap.)* **34** (1987) 105–115.
70. Vanden Bossche H., Marichal P., Gorrens J., Bellens D., Coene M.-C., Lauwers W., Le Jeune L., Moereels H. and Janssen P. A. J.: Mode of action of antifungals of use in immunocompromised patients. Focus on *Candida glabrata* and *Histoplasma capsulatum*. In *Mycoses in AIDS Patients* (Edited by H. Vanden Bossche, D. W. R. Mackenzie, G. Cauwenbergh, J. Van Cutsem, E. Drouhet and B. Dupont). Plenum Press, New York (1990) pp. 223–243.
71. Vanden Bossche H. and Moereels H.: 17α-Hydroxylase/17,20-lyase. In *Design of Enzyme Inhibitors as Drugs* (Edited by M. Sandler and J. Smith). Oxford University Press, Oxford, Vol. 2. In press.
72. Vanden Bossche H., Willemsens G., Roels I., Bellens D., Moereels H., Le Jeune L., Lauwers W. and Janssen P. A. J.: R 76713 and its enantiomers: selective, nosteroidal inhibitors of the cytochrome *P*450-dependent oestrogen synthesis. *Biochem. Pharmac.* **40** (1990) 1707–1718.
73. Vanden Bossche H., Marichal P., Willemsens G., Bellens D., Gorrens J., Roels I., Coene M.-C., Le Jeune L. and Janssen P. A. J.: Saperconazole: a selective inhibitor of the cytochrome *P*-450-dependent ergosterol synthesis in *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton mentagrophytes*. *Mycoses* **33** (1990) 35–352.
74. Vanden Bossche H., Willemsens G. and Janssen P. A. J.: Cytochrome *P*-450-dependent metabolism of retinoic acid in rat skin microsomes: inhibition by ketoconazole. *Skin Pharmac.* **1** (1988) 176–185.
75. Vanden Bossche H., Willemsens G., Bellens D. and Janssen P. A. J.: Ridogrel: a selective inhibitor of the cytochrome *P*450-dependent thromboxane synthesis. *Biochem. Pharmac.* **43** (1992) 739–744.
76. Vanden Bossche H., Willemsens G., Marichal P., Cools W. and Lauwers W.: The molecular basis for the antifungal activities of N-substituted azole derivatives, focus on R 51211. In *Mode of Action of Antifungal Agents* (Edited by A. P. J. Trinci and J. F. Ryley). Cambridge University Press, Cambridge (1984) pp. 321–341.
77. Couch R. M., Muller J., Perry Y. S. and Winter J. S. D.: Kinetic analysis of inhibition of human adrenal steroidogenesis by ketoconazole. *J. Clin. Endocr. Metab.* **65** (1987) 551–554.
78. Ayub M. and Levell M. J.: Inhibition of human adrenal steroidogenic enzymes *in vitro* by imidazole drugs including ketoconazole. *J. Steroid Biochem.* **32** (1989) 515–524.
79. Trachtenberg J. and Zadra J.: Steroid synthesis inhibition by ketoconazole: sites of action. *Clin. Invest. Med.* **11** (1988) 1–5.
80. Engelhardt D., Weber M. M., Miksch T., Abedinpour F. and Jespers C.: The influence of ketoconazole on human adrenal steroidogenesis: incubation studies with tissue slices. *Clin. Endocr.* **35** (1991) 163–168.
81. Trachtenberg J., Halpern N. and Pont A.: Ketoconazole a novel and rapid treatment for advanced prostatic cancer. *J. Urol.* **130** (1983) 152–153.
82. Nakajin S., Shively J. E., Yuan P.-M. and Hall P. F.: Microsomal cytochrome *P*-450 from neonatal pig testis: two enzymatic activities (17α-hydroxylase/17,20-lyase) associated with one protein. *Biochemistry* **20** (1981) 4037–4042.
83. Nakajin S., Shinoda M., Haniu M., Shively J. E. and Hall P. F.: C_{21} steroid side-chain cleavage enzyme from porcine adrenal microsomes. Purification and characterization of the 17α-hydroxylase/C17,20-lyase cytochrome *P*-450. *J. Biol. Chem.* **259** (1984) 3971–3976.
84. Bradshaw K. D., Waterman M. R., Couch R. T., Simpson E. R. and Zuber M. X.: Characterization of complementary deoxyribonucleic acid for human adrenocortical 17α-hydroxylase: probe for analysis of 17α-hydroxylase deficiency. *Molec. Endocr.* **1** (1987) 348–354.
85. Zuber M. X., Simpson E. R. and Waterman M. R.: Expression of bovine 17α-hydroxylase cytochrome *P*-450 cDNA in nonsteroidogenic (COS 1) cells. *Science* **234** (1986) 1258–1261.
86. Fevold H. R., Lorence M. C., McCarthy J. L., Trant J. M., Kagimoto M., Waterman M. R. and Mason J. I.: Rat $P450_{17\alpha}$ from testis: characterization of a full-length cDNA encoding a unique steroid hydroxylase capable of catalyzing both Δ^4- and Δ^5-steroid-17,20-lyase reactions. *Molec. Endocr.* **3** (1989) 968–975.
87. Kitamura M., Buczko E. and Dufau M. L.: Dissociation of hydroxylase and lyase activities by site-directed mutagenesis of the rat $P450_{17\alpha}$. *Molec. Endocr.* **5** (1991) 1373–1380.
88. Heyns W., Drochmans A., vander Schueren E. and Verhoeven G.: Endocrine effects of high dose ketoconazole therapy in advanced prostatic cancer. *Acta Endocr.* **110** (1985) 276–283.
89. Denis L. J., Chaban M. and Mahler Ch.: Clinical application of ketoconazole in prostatic cancer. In *EORTC Genitourinary Group Monograph 2. Part A: Therapeutic Principles in Metastatic Prostatic Cancer* (Edited by F. H. Schroeder and B. Richards). Alan R. Liss, New York (1985) pp. 319–326.
90. Amery W. K., De Coster R. and Caers I.: Ketoconazole: from an antimycotic to a drug for prostate cancer. *Drug Dev. Res.* **8** (1986) 299–307.

91. Tapazoglou E., Subramanian M. G., Al-Sarraf M., Kresge C. and Decker D. A.: High-dose ketoconazole therapy in patients with metastatic prostate cancer. *Am. J. Clin. Oncol.* **9** (1986) 369–375.
92. Sonino N.: The use of ketoconazole as an inhibitor of steroid production. *New Engl. J. Med.* **317** (1987) 812–818.
93. Lowe F. C. and Bamberger M. H.: Indications for use of ketoconazole in management of metastatic prostate cancer. *Urology* **36** (1990) 541–545.
94. Ayub M. and Levell M. J.: The inhibition of human prostatic aromatase activity by imidazole drugs including ketoconazole and 4-hydroxyandrostenedione. *Biochem. Pharmac.* **40** (1990) 1569–1575.
95. Neumann F.: Pharmacology and clinical uses of cyproterone acetate. In *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action* (Edited by B. J. A. Furr and A. E. Wakeling). Baillière Tindall, London (1987) pp. 132–159.
96. Parker L. N.: Control of adrenal secretion. *Endocr. Met. Clin. N. Am.* **20** (1991) 401–421.
97. Angeli A. and Frairia R.: Ketoconazole therapy in Cushing's disease. *Lancet* **i** (1985) 821.
98. Sonino N., Boscaro M., Merola G. and Mantero F.: Prolonged treatment of Cushing's disease by ketoconazole. *J. Clin. Endocr. Metab.* **61** (1985) 718–722.
99. Loli P., Berselli M. E. and Tagliaferry M.: Use of ketoconazole in the treatment of Cushing's syndrome. *J. Clin. Endocr. Metab.* **63** (1986) 1365–1371.
100. Sonino N., Boscaro M., Paoletta A., Mantero F. and Zilioto D.: Ketoconazole treatment in Cushing's syndrome: Experience in 34 patients. *Clin. Endocr.* **35** (1991) 347–352.
101. Vanden Bossche H., Willemsens G., Bellens D., Roels I. and Janssen P. A. J.: From 14α-demethylase inhibitors in fungal cells to androgen and oestrogen biosynthesis inhibitors in mammalian cells. *Biochem. Soc. Trans.* **18** (1990) 10–13.
102. Bruynseels J., De Coster R., Van Rooy P., Wouters W., Coene M.-C., Snoeck E., Raeymaekers A., Freyne E., Sanz G., Vanden Bussche G., Vanden Bossche H., Willemsens G. and Janssen P.A. J.: R 75251, a new inhibitor of steroid synthesis. *The Prostate* **16** (1990) 345–357.
103. Mahler C., Denis L. and De Coster R.: The effects of a new imidazole derivative in advanced prostatic cancer. A preliminary report. In *Therapeutic Progress in Urological Cancers* (Edited by G. P. Murphy and S. Khoury). Alan R. Liss, New York (1989) pp. 205–209.
104. De Coster R., Van Ginckel R, van Moorselaar R. J. A., Schalken J. A., De Bruyne F. M. J., Bruynseels J. and Denis L.: Antitumoral effects of R 75251 in prostatic carcinoma experimental and clinical studies. *Proc. 82nd Meet. Am. Assoc. Cancer Res.* (1991) 213.
105. Van Ginckel R., De Coster R., Wouters W., Vanherck W., van der Veer R., Goeminne N., Jagers E., Van Cauteren H., Wouters L., Distelmans W. and Janssen P. A. J.: Antitumoral effect of R 75251 on the growth of transplantable R 3327 prostatic adenocarcinoma in rats. *The Prostate* **16** (1990) 313–323.
106. De Coster R., Wouters W., Van Ginckel R., End D., Krekels M., Coene M.-C. and Bowden C.: Experimental studies with liarozole (R 75251): an antitumoral agent which inhibits retinoic acid breakdown. *J. Steroid Biochem. Molec. Biol.* **43** (1992) 197–201.
107. Kaburagi Y., Marino M. B., Kirdani R. Y., Greco J. P., Karr J. P. and Sandberg A. A.: The possibility of aromatization of androgen in human prostate. *J. Steroid Biochem.* **26** (1987) 739–742.
108. Bélanger C., Veilleux R. and Labrie F.: Stimulatory effects of androgens, estrogens, progestins, and dexamethasone on the growth of the LNCaP human prostate cancer cells. *Ann. N. Y. Acad. Sci.* **595** (1990) 399–402.
109. Habenicht U. F., Schwartz K., Schweikert H. U., Neumann F. and El Etreby M. F.: Development of a model for the induction of estrogen-related prostatic hyperplasia in the dog and its response to the aromatase inhibitor 4-hydroxy-androstene-3,17-dione. *The Prostate* **8** (1986) 181–194.
110. Van Wauwe J. P., Coene M.-C., Goossens J., Van Nijen G., Cools W. and Janssen P. A. J.: Ketoconazole inhibits the *in vitro* and *in vivo* metabolism of all-*trans*-retinoic acid. *J. Pharmac. Exp. Ther.* **245** (1988) 718–722.
111. Van Wauwe J. P., Coene M.-C., Goossens J., Cools W. and Monbaliu J.: Effects of cytochrome *P*-450 inhibitors on the *in vivo* metabolism of all-*trans*-retinoic acid in rats. *J. Pharmac. Exp. Ther.* **252** (1990) 365–369.
112. de Thé H. and Dejean A.: The retinoic acid receptors. In *Retinoids: 10 Years On* (Edited by J. H. Saurat). S. Karger, Basel (1991) pp. 2–9.
113. Williams J. B. and Napoli J. L.: Metabolism of retinoic acid and retinol during differentiation of F9 embryonal carcinoma cells. *Proc. Natn. Acad. Sci. U.S.A.* **82** (1985) 4658–4662.
114. Williams J. B. and Napoli J. L.: Inhibition of retinoic acid metabolism by imidazole antimycotics in F9 embryonnal carcinoma cells. *Biochem. Pharmac.* **36** (1987) 1386–1388.
115. Steele R. E., Mellor L. B., Sawyer W. K., Wasvary J. M. and Browne R. E.: *In vitro* and *in vivo* studies demonstrating potent and selective estrogen inhibition with the nonsteroidal aromatase inhibitor CGS 16949A. *Steroids* **50** (1987) 147–161.
116. Walters J. R., Juniewicz P. E., Oesterling J. E., Mendis-Handagama S. M. L. C., Zirkin B. R. and Ewing L. L.: The effect of inhibition of aromatase enzyme activity on Leydig cell number and ultrastructure in Beagles. *Endocrinology* **123** (1988) 2223–2229.
117. Bhatnagar A. S., Häusler A., Schieweck K., Browne L. J., Bowman R. and Steele R. E.: Novel aromatase inhibitors. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 363–367.
118. Browne L. J., Gude C., Rodriguez H., Steele R. E. and Bhatnager A.: Fadrozole hydrochloride: a potent, selective, nonsteroidal inhibitor of aromatase for the treatment of estrogen-dependent disease. *J. Med. Chem.* **34** (1991) 725–736.
119. Lamberts S. W. J., Bruining H. A., Marzouk H., Zuiderwijk J., Uiterlinden P., Blijd J. J., Hackeng W. H. L. and De Jong F. H.: The new aromatase inhibitor CGS 165949A suppresses aldosterone and cortisol production by human adrenal cells *in vitro*. *J. Clin. Endocr. Metab.* **69** (1989) 896–901.
120. Santen R. J., Demers L. M., Adlercreutz H., Harvey H., Santner S., Sanders S. and Lipton A.: Inhibition of aromatase with CGS 16949A in post menopausal women. *J. Clin. Endocr. Metab.* **68** (1989) 99–106.
121. Demers L. M., Melby J. C., Wilson T. E, Lipton A., Harvey H. A. and Santen R. J.: The effects of CGS 16949A, an aromatase inhibitor on adrenal mineralocorticoid biosynthesis. *J. Clin. Endocr. Metab.* **70** (1990) 1162–1166.
122. Lipton A., Harvey H. A., Demers L. M., Hanagan J. R., Mulagha M. T., Kochak G. M., Fitzsimmons S., Sanders S. I. and Santen R. J.: A phase I trial of CGS 16949A a new aromatase inhibitor. *Cancer* **65** (1990) 1279–1285.
123. Dowsett M., Stein R. C., Mehta A. and Coombes R. C.: Potency and selectivity of the non-steroidal aromatase inhibitor CGS 16949A in postmenopausal breast cancer patients. *Clin. Endocr.* **32** (1990) 623–634.
124. Raats J. I., Falkson G. and Falkson H. C.: A study of fadrozole, a new aromatase inhibitor, in post-

menopausal women with advanced metastatic breast cancer. *J. Clin. Oncol.* **10** (1992) 111–116.
125. Mason J. I., Carr B. R. and Murray B. A.: Imidazole antimycotics: selective inhibitors of steroid aromatization and progesterone hydroxylation. *Steroids* **50** (1987) 179–189.
126. Corbin C. J., Graham-Lorence S., McPhaul M. J., Mason J. I., Mendelson C. R. and Simpson E. R.: Isolation of a full-length cDNA insert encoding human aromatase system cytochrome *P*450 and its expression in nonsteroidogenic cells. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1988) 8948–8952.
127. Hirsch K. S., Jones C. D., Lindstrom T. D., Stamm N. B., Sutton G. P., Taylor H. M. and Weaver D. E.: Discovery and development of a novel class of nonsteroidal aromatase inhibitors. *Steroids* **50** (1987) 201–217.
128. Taylor H. M., Jones C. D., Davenport J. D., Hirsch K. S. and Weaver D. E.: Aromatase inhibition by 5-substituted pyrimidines and dihydropyrimidines. *J. Med. Chem* **30** (1987) 1359–1365.
129. Jones C. D., Winter M. A., Hirsch K. S., Stamm N., Taylor H. M., Holden H. E., Davenport J. D., Krumkalns E. V. and Suhr R. G.: Estrogen synthetase inhibitors: 2. Comparison of the *in vitro* aromatase inhibitory activity for a variety of nitrogen heterocycles substituted with diarylmethane or diarylmethanol groups. *J. Med. Chem* **33** (1990) 416–429.
130. Wouters W., De Coster R., Krekels M., van Dun J., Beerens D., Haelterman C., Raeymaekers A., Freyne E., Van Gelder J., Venet M. and Janssen P. A. J.: R 76713, a new specific non-steroidal aromatase inhibitor. *J. Steroid Biochem.* **32** (1989) 781–788.
131. De Coster R., Wouters W., Bowden C. R., Vanden Bossche H., Bruynseels J., Tuman R. W., Van Ginckel R., Snoeck E., Van Peer A. and Janssen P. A. J.: New nonsteroidal aromatase inhibitors: Focus on R 76713. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 335–341.
132. Krekels M. D. W. G., Wouters W. and De Coster R.: Aromatase inhibitor by R 76713: a kinetic analysis in rat ovarian homogenates. *Steroids* **55** (1990) 69–73.
133. Wouters W., De Coster R., Beerens D., Doolaege R., Guiwez J. A., Van Camp K., Vanden Pas H. and Van Herendael B.: Potency and selectivity of the aromatase inhibitor R 76713. A study of human ovarian, adipose stromal, testicular and adrenal cells. *J. Steroid Biochem.* **36** (1990) 57–65.
134. Krekels M. D. W. G., Wouters W., De Coster R., Van Ginckel R., Leonaers A. and Janssen P. A. J.: Aromatase in the human choriocarcinoma JEG-3: inhibition by R 76713 in cultured cells and in tumors grown in nude mice. *J. Steroid Biochem. Molec. Biol.* **38** (1991) 415–422.
135. Vanden Bossche H., Marichal P., Willemsens G. and Janssen P. A. J.: Effects of inhibitors on the *P*450-dependent metabolism of endogenous compounds in fungi, protozoa, plants and vertebrates. In *Molecular Aspects of Monooxygenases and Bioactivation of Toxic Compounds* (Edited by E. Arinç, J. B. Schenkman and E. Hodgson). Plenum Press, New York (1991) pp. 345–363.
136. Vanden Bossche H. and Janssen P. A. J.: Target sites of sterol biosynthesis inhibitors: secondary activities on cytochrome *P*-450-dependent reactions. In *Target Sites of Fungicide Action* (Edited by W. Köller). CRC Press, Boca Raton, FL (1992) pp. 227–254.
137. De Coster R., Van Ginckel R., Wouters W., Goeminne N., Vanherck W. and Byloos M.: Endocrine and anti-tumoral effects of R 76713 in rats. *J. Enzym. Inhib.* **4** (1990) 159–167.
138. De Coster R., Van Ginckel R. F., Callens M. J. L., Goeminne N. K. G. and Janssens B. L. E.: Anti-tumoral and endocrine effects of (+)-vorozole in rats bearing dimethylbenzanthracene-induced mammary tumors. *Cancer Res.* **52** (1992) 1240–1244.
139. Bruynseels J., De Coster R., Ceuppens M. and Borms M.: R 76713: nonsteroidal aromatase inhibitor. Phase I open pilot trial in the endocrine treatment of advanced postmenopausal breast cancer. Part I: Efficacy and pharmacodynamics—First report. Janssen Research Foundation. Clinical Research Report R 76713 (N 79273) (1991).
140. Santen R. J.: Clinical use of aromatase inhibitors in human breast carcinoma. *J. Steroid Biochem. Molec. Biol.* **40** (1991) 247–253.
141. Covey D. F.: Aromatase inhibitors: specific inhibitors of oestrogen biosynthesis. In *Sterol Biosynthesis Inhibitors. Pharmaceutical and Agrochemical Aspects* (Edited by D. Berg and M. Plempel). Ellis Horwood, Chichester, England (1988) pp. 534–571.
142. Banting L., Nicholls P. J., Shaw M. A. and Smith H. J.: Recent developments in aromatase inhibition as potential treatment for oestrogen-dependent breast cancer. In *Progress in Medicinal Chemistry* (Edited by G. P. Ellis and G. B. West). Elsevier, Amsterdam, Vol. 26 (1989) pp. 253–298.
143. Di Salle E., Giudici D., Briatico G. and Ornati G.: Novel irreversible aromatase inhibitors. *Ann. N. Y. Acad. Sci.* **595** (1990) 357–367.
144. Segaloff A., Weeth J. B., Meyer K. K., Rongone E. L. and Cunningham M. E. G.: Hormonal therapy in cancer of the breast. XIX. Effect of oral administration of Δ^1-testolactone on clinical course an hormonal excretion. *Cancer* **15** (1962) 633–635.
145. Barone R. M., Shamonki I. M., Siiteri P. K. and Judd H. L.: Inhibition of the peripheral aromatization of androstenedione to estrone in postmenopausal women with breast cancer using Δ^1-testolactone. *J. Clin. Endocr. Metab.* **49** (1979) 672–676.
146. Schweikert H.-U. and Tunn U. W.: Effects of the aromatase inhibitor testolactone on human benign prostate hyperplasia. *Steroids* **50** (1987) 191–200.
147. Henderson D., Norbisrath G. and Kerb U.: 1-methyl-1,4-androstadiene-3,17-dione (SH 489): characterization of an irreversible inhibitor of estrogen biosynthesis. *J. Steroid Biochem.* **24** (1986) 303–306.
148. Henderson D.: Aromatase inhibitors: their biochemistry and clinical potential. *J. Steroid Biochem.* **27** (1987) 905–914.
149. Henderson D., Habenicht U. F., Nishino Y. and Etreby M. F.: Estrogens and benign prostatic hyperplasia: the basis for aromatase inhibitor therapy. *Steroids* **50** (1987) 219–233.
150. El Etreby M. F., Nishino Y., Habenicht U.-F. and Henderson D.: Atamestane, a new aromatase inhibitor for the management of benign prostatic hyperplasia. *J. Androl.* **12** (1991) 403–414.
151. Habenicht U.-F. and El Etreby M. F.: Rationale for using aromatase inhibitors to manage benign prostatic hyperplasia. Experimental studies. *J. Androl.* **12** (1991) 395–402.
152. Geelen J. A. A., Deckers G. H., Van Der Wardt J. T. H., Loozen H. J. J., Tax L. J. W. and Kloosterboer H. J.: 19-(Ethyldithio)-androst-4-ene-3,17-dione (ORG 30958): a potent aromatase inhibitor *in vivo*. *J. Steroid Biochem. Molec. Biol.* **38** (1991) 181–188.
153. Di Salle E., Giudici D., Briatico G., D'Alessio, Villa V. and Lombardi P.: 4-aminoandrostenedione derivatives: a novel class of irreversible aromatase inhibitors. Comparison with FCE 24304 and 4-hydroxyandrostenedione. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 369–374.
154. Brodie A. H. M.: Aromatase inhibition and its pharmacologic implications. *Biochem. Pharmac.* **34** (1985) 3213–3219.

155. Brodie A. H. M., Wing L. Y., Goss P., Dowsett M. and Coombes R. C.: Aromatase inhibitors and their potential clinical significance. *J. Steroid Biochem.* **25** (1986) 859–865.
156. Brodie A. H. M., Coombes R. C. and Dowsett M.: Aromatase inhibitors: basic and clinical studies. *J. Steroid Biochem.* **27** (1986) 899–903.
157. Brodie A. H. M., Banks P. K., Inkster S. E., Dowsett M. and Coombes R. C.: Aromatase inhibitors and hormone-dependent cancers. *J. Steroid Biochem. Molec. Biol.* **37** (1990) 327–333.
158. Brodie A.: Aromatase and its inhibitors—an overview. *J. Steroid Biochem. Molec. Biol.* **40** (1991) 255–261.
159. Brodie A. H. M., Garrett W. M., Hendrickson J. R, Tsai-Morris C.-H., Marcotte P. A. and Robinson C. H.: Inactivation of aromatase *in vitro* by 4-hydroxyandrostenedione and 4-acetoxyandrostenedione and their sustained effects *in vivo*. *Steroids* **38** (1991) 693–701.
160. Brodie A. H. M. and Wing L. Y.: *In vitro* and *in vivo* studies with aromatase inhibitor 4-hydroxyandrostenedione. *Steroids* **50** (1987) 89–103.
161. Koos R. D., Le Maire W. J., Hung T. T. and Brodie A. H. M.: Comparison of the effect of 4-hydroxy-4-androstene-3,17-dione on aromatase activity in granulosa cells from preovulatory follicles of rats, rabbits and humans. *Steroids* **45** (1985) 143–150.
162. Brodie A. H. M. and Longcope C.: Inhibition of peripheral aromatization by aromatase inhibitors, 4-hydroxy- and 4-acetoxy-androstenedione. *Endocrinology* **106** (1980) 19–21.
163. Reed M., Lai L. C., Owen A. M., Singh A., Coldham N. G., Purihit A., Ghilchik M. W, Shaikh N. A. and James V. H. T.: The effect of treatment with 4-hydroxyandrostenedione on the peripheral conversion of androstenedione to oestrone and in vitro tumor aromatase activity in postmenopausal women with breast cancer. *Cancer Res.* **50** (1990) 193–196.
164. Brodie A. H. M., Garrett W. M., Hendrickson J. R. and Tsai-Morris C.-H.: Effects of aromatase inhibitor 4-hydroxyandrostenedione and other compounds in the 7,12-dimethylbenz(*a*)anthracene-induced breast carcinoma model. *Cancer Res.* **42** (1982) 3360s–3364s.
165. Coombes R. C., Goss P., Dowsett M., Gazet J. C. and Brodie A. M. H.: 4-Hydroxyandrostenedione treatment of postmenopausal patients with advanced breast cancer. *Lancet* **ii** (1984) 1237–1239.
166. Dowsett M., Goss P., Powles T. J., Hutchunson G., Cunningham D., Jarman M. I., Brodie A. M. H., Jefcoate S. L. and Coombes R. C.: Use of the aromatase inhibitor 4-hydroxyandrostenedione in postmenopausal breast cancer; optimization of therapeutic dose and route. *Cancer Res.* **47** (1987) 1957–1961.
167. Brodie A. M. H., Son C., King D. A., Meyer K. M. and Inkster S. F.: Lack of evidence for aromatase in human prostatic tissues: effects of 4-hydroxyandrostenedione and other inhibitors on androgen metabolism. *Cancer Res.* **49** (1989) 6551–6555.
168. Shearer R. J., Davies J. H., Dowsett M., Malone P. R., Hedley A., Cunningham D. and Coombes R. C.: Aromatase inhibition in advanced prostatic cancer: preliminary communication. *Br. J. Cancer* **62** (1990) 275–276.