Pharmacological Options for Treatment of Hyperandrogenic Disorders

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Abstract: Hyperandrogenic disorders are frequent in women. The most common cause is polycystic ovary syndrome, a condition found up to 7% in women of reproductive age. The effects of testosterone and dihydrotestosterone are elicited *via* androgen receptors. Androgen receptor acts as a ligand-dependent transcription factor that regulates the expression of several target genes. There are several pharmacological possibilities for the treatment of androgen excess, as inhibition of the biologic activity of androgens can be carried out at different levels. The androgen receptor, the 5α-reductase enzyme, and the hypothalamic-pituitary-gonad axis are the most frequent targets of antiandrogenic therapies. This review summarizes the structural and chemical features of currently available antiandrogenic drugs, including cyproterone acetate, spironolactone, flutamide and finasteride. Also, it presents some recent advances in the chemistry and pharmacology of novel steroidal and non-steroidal antiandrogens, and 5α–reductase inhibitors. Finally, recent knowledge on non-classical antiandrogenic drugs, such as insulin-sensitizers, ketoconazole, and GnRH-agonists are briefly discussed.

Key Words: Antiandrogen therapy, 5α–reductase inhibitors, androgen receptor antagonists, androgen excess.

INTRODUCTION

Higher androgen levels in women can produce hirsutism or, in cases of severe androgen excess, frank virilisation characterized by male pattern alopecia, clitoromegaly, deepening in the voice and increased muscle mass. Elevated androgens can be associated with cardiovascular risk factors, as high free testosterone fraction in women may play a role in the development of metabolic syndrome. Ovulatory deficiencies which are commonly associated with hyperandrogenism can lead to infertility and oligo- or amenorrhea with consequent endometrial hyperplasia. Hyperandrogenic state can arise from polycystic ovary syndrome (PCOS), Cushing-syndrome, hyperprolactinemia, acromegaly, thyroid dysfunction, classical and non-classical forms of congenital adrenal hyperplasia, hyperthecosis, hyperandrogenic-insulin resistant-acanthosis nigricans (HAIRAN) syndrome, and androgen producing adrenal or ovarian tumors (Table **1**). The majority of women with androgen excess are diagnosed as having PCOS. In PCOS gonadotropin-dependent functional ovarian hyperandrogenism is the major source of the androgen overproduction [1].

Table 1. Endocrine Diseases Causing Hyperandrogenism in Women

Polycystic Ovary Syndrome (PCOS)	Acromegaly
Hyperprolactinemia	Hyperthecosis
Ovarian tumors	Adrenal tumors
Cortisone reductase deficiency	Thyroid dysfunction
Glucocorticoid resistance	Cushing`s syndrome
Classical and non-classical forms of congenital adrenal hyperplasia 21-hydroxylase deficiency 11β-hydroxylase deficiency 3β hydroxyl dehydrogenase deficiency	Hyperandrogenic-insulin resistant – acanthosis nigricans (HAIRAN) syndrome

I. OVERVIEW OF THE BIOSYNTHESIS, SECRETION, REGULATION AND ACTION OF ANDROGENS

The major circulating androgen is testosterone (**1**). It is produced by ovarian theca cells and adrenal zona fasciculata cells, and by peripheral conversion of androgen precursors androstendione (**2**) and dehydroepiandrosterone(-sulphate) (**3**) in fat and skin [2] (Fig. (**1**)). In plasma, testosterone is largely bound to proteins, mainly albumin and sex hormone-binding globulin (SHBG). The term of bioavailable testosterone in plasma refers to free testosterone and a portion of albumin-bound testosterone [3].

Ovarian androgen secretion is regulated mainly by luteinizing hormone (LH). Insulin and insulin-like growth factor-1 can stimulate androgen production and secretion of the ovarian theca layer [4]. In androgen-sensitive target tissues testosterone is converted to dihydrotestosterone (DHT) (**4**). DHT is produced by the reduction of testosterone (T) under catalysis of the membrane-bound, NADPH-dependent 5α-reductase enzyme (5α-R). There are two 5-α-reductase isoenzymes (5α-reductase-1 and 5α-reductase-2) which cata-

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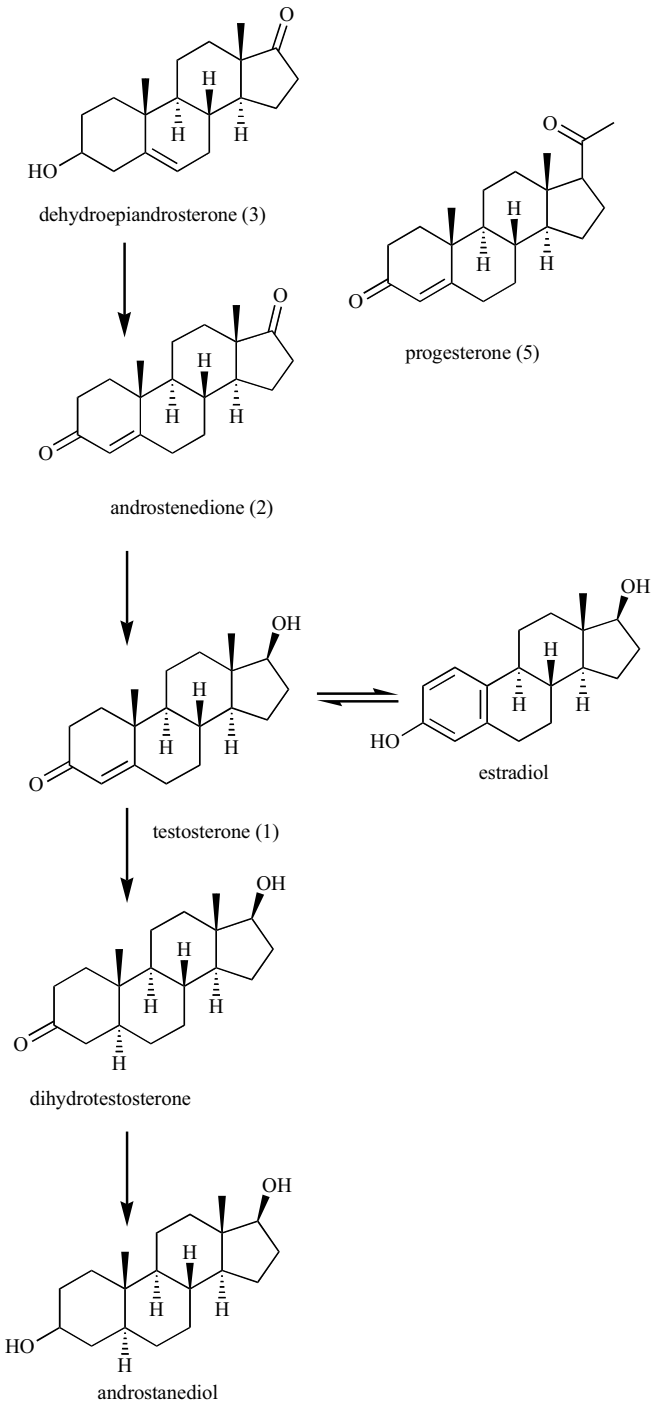

Fig. (1). Biosynthesis of testosterone from androgen precursors.

lyze a selective and irreversible reduction of 4-ene-3-oxosteroids to the corresponding 5α-3-oxosteroids (Fig. (**2**)) [5]. The 5α-reductase enzyme 3-oxo-steroid-4-enedehydrogenase is a hydrophobic protein formed by 259 amino acids. Its molecular weight is 29,462 and is located in the microsomal fraction of target cells [6]. Type 1 isoenzyme is mainly located in non-genital skin, sebaceous glands, hair follicles and liver, whereas type 2 isoenzyme has been found in prostate in men, and in genital skin and dermal papilla in both sexes. An acidic pH of 5.5 is optimal for type 2 activity,

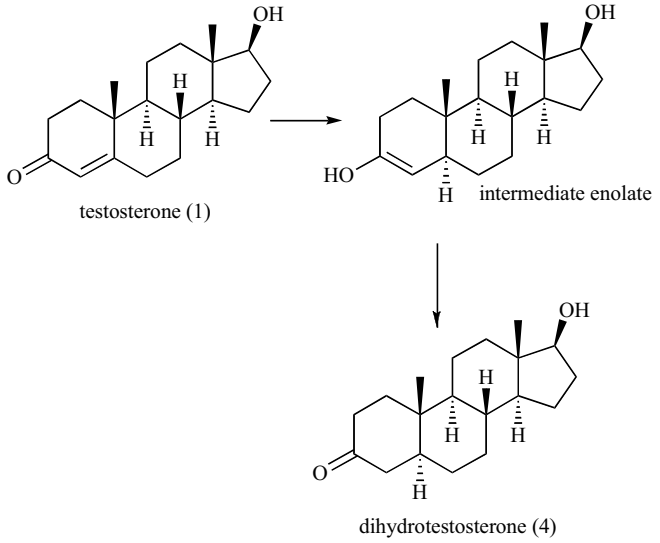

Fig. (2). Conversion of testosterone to dihydrotestosterone

whereas a pH between 6.0 and 8.5 is favourable for type 1 isoenzyme [7].

Since testosterone and dihydrotestosterone share a common receptor, it seems surprising that the effect of dihydrotestosterone is not mimicked by testosterone. The differences between dihydrotestosterone and testosterone have been studied in animal models as well as in men with 5α-reductase deficiency [8]. Dihydrotestosterone has several fold higher affinity for the androgen receptor, furthermore, the dihyrotestosterone-receptor complex may have a higher affinity for the acceptor site in the nuclear chromatin than does the testosterone-receptor complex [9]. Therefore, local conversion of testosterone to dihydrotestosterone by the 5α-reductase enzyme probably serves as an androgen amplification mechanism. However, in some cases this amplification may become pathological such as in hirsutism or in androgenic alopecia in women.

II. ANDROGEN RECEPTOR

The androgen receptor (AR), a ligand-regulated transcription factor is a member of the nuclear receptor superfamily. AR is widely distributed in tissues such as the genitalia, skin, ovary, cartilage, sebaceous glands, hair follicles, sweat glands, cardiac muscle, smooth muscle, gastrointestinal vesicular cells, thyroid follicular cells, adrenal cortex, liver, pineal gland and brain. Tissue selectivity is dependent on the regulation of AR expression, differential DNA binding at the promoter of regulated genes, and tissue-specific protein-protein interactions [10].

The gene coding for AR is mapped to the X-chromosome at Xq11.2-q12 and is over 90 kb. long. It consists of 8 exons encoding the 919 amino acid AR protein. Exon 1 in the N-terminal domain (NTD) of the AR gene contains two polymorphic trinucleotide repeats, GGN and CAG. These polymorphic repeats appear to influence the function of the receptor and the plasma testosterone levels [11-13]. In women, associations between AR polymorphisms and hirsutism, acne, androgenic alopecia, bone mineral density, and breast

cancer have been documented [14-16]. In addition, it has been shown that premenopausal women with relatively few CAG repeats displayed higher androgen levels, but lower LH as compared to women with longer CAG repeats [17].

The unliganded AR, primarily localized in the cytoplasm in a relatively unfolded state, is associated with heat-shock proteins. Like other members of the nuclear receptor superfamily, AR is modular and includes an amino-terminal region containing a ligand-independent transcriptional activation domain (activation function-1), a central DNA-binding domain that specifically recognizes response elements upstream of target genes, and a carboxy-terminal ligand binding domain (LBD). While activation function-1 (AF-1) domain present in NTD is not conserved, activation function-2 domain (AF-2) located in LBD is highly conserved and consists of amino acids that form a co-activator binding pocket on the surface of LBD [18]. According to crystallographic data the LBD consists of 12 helices with two antiparellel β-sheets. The LBD is a multifunctional domain, capable of ligand binding, dimerization and interaction with transcriptional co-regulators that enhance (co-activators) or decrease (co-repressors) the transcriptional activity of the receptor. Recent studies indicate that AR can adopt two distinct structural folds in the LBD with either an "agonist" conformation that binds co-activators such as SRC-1 (steroid receptor co-activator 1) and TIF2 (transcriptional intermediary factor 2), or an "antagonist" fold that binds co-repressors [19]. AR AF-2 binds short α-helical peptides with consensus FXXLF and the more common nuclear receptor consensus LXXLL. The receptor uses the same general coactivator binding mechanisms as other nuclear receptors, by providing a dimorphic cleft that facilitates interaction with aromatic amino acids in addition to leucines. The ability of the AR surface to rearrange for the interaction with FXXLF motifs is unique among transcription factors and represents a gain of function relative to other structurally defined interactions in the family [20]. X-ray structures of AR LBD with representative peptides revealed that AF-2 amino acid side chains move to create a deep pocket that accommodates the bulky aromatic amino acid side chains and forms an attractive target for small molecules. Upon agonist binding to AR a conformation change to active form occurs, in which NTD and LBD come into close association [21]. Furthermore, AR is translocated into the nucleus, moves to different subnuclear sites, binds to specific DNA sequences, and activates androgen-specific genes through protein-protein interactions with coregulatory proteins and general transcription factors. In the nucleus, AR dimerization is required for AR transcription activity [22]. Upon agonist binding, NR LBDs undergo conformational changes in such a way that some residues belonging to helices H3, H4, and H11 are clustered to form a predominantly hydrophobic surface onto which the apolar side of the highly mobile C-terminal LBD helix H12, encompassing the core of the activation function-2 (AF-2), binds in a stable conformation (Fig. (**3a**)). Binding of antagonists prevents the formation of this specific surface by interfering directly or indirectly with the active conformation of H12 [23].

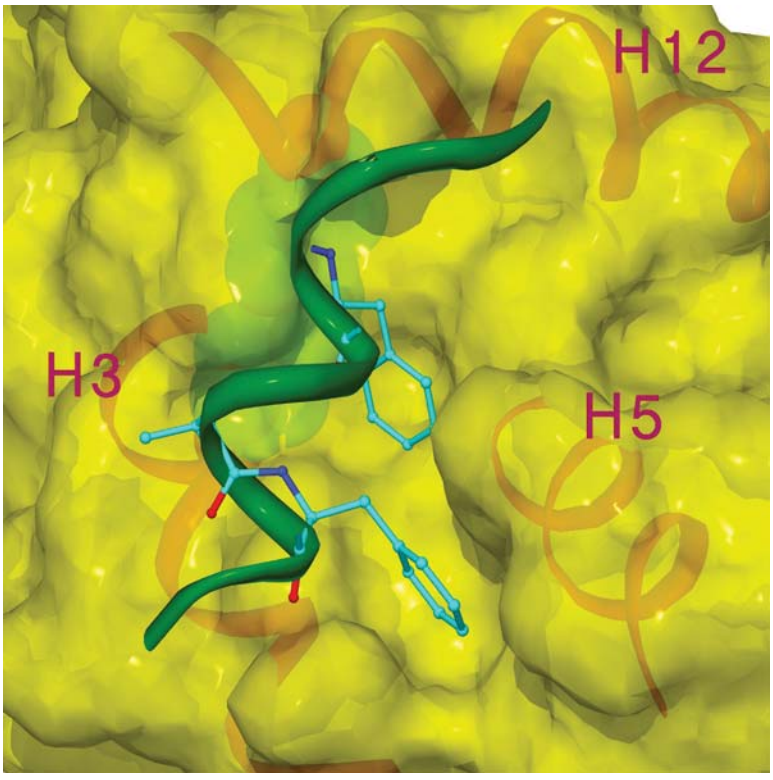

Fig. (3a). Schematic view of the AR LBD, with coactivator peptide (green ribbon). The FXXLF motif is highlighted. (ball and stick representation) The molecular surface of the AR LBD shown in yellow demonstrates the unique dimorphic cleft. Key AF2 helices H3, H5 and H12 are illustrated as orange ribbons. DHT is represented in space filling colored green. The figure was produced with Swiss-PdbViewer in combination with POV-Ray.

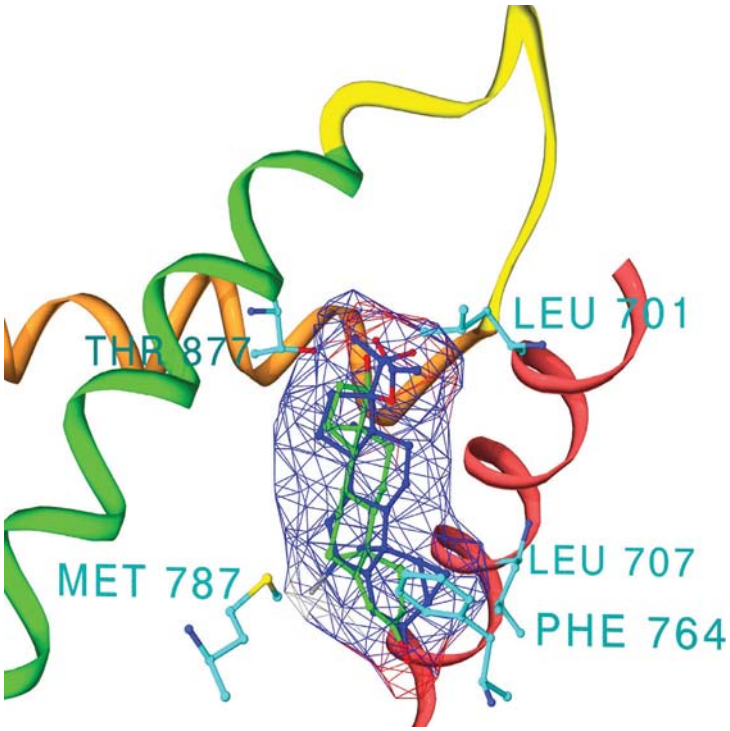

Fig. (3b). Schematic view of the ligand binding pocket of the AR with DHT (green ball and stick representation) (PDB ID: LI37) and with superimposed cyproterone acetate (blue ball and stick, and molecular surface representation) (PDB ID: 2OZ7). Helices are represented with ribbons (H3, mauve; H11, green; H12, gold; H11-H12 loop, yellow) Amino acids in steric constrain with cyproterone acetate are highlighted.

III. PHARMACOLOGICAL INHIBITION OF ANDROGEN EXCESS

There are several pharmacological approaches to suppress exaggerated androgen action in human (Fig. (**4**)). Inhibition of androgen activity can be carried out at different levels such as modulation of the hypothalamic–pituitary axis, inhibition of ovarian and adrenocortical enzymes involved in androgen biosynthesis and blockade of the AR. In this review we discuss different groups of drugs that can decrease androgen excess in women as well as recent data on new potential agents that may be available for treatment in the near future.

5α-Reductase Inhibitors

Studies on 5α-reductase inhibitors as potent antiandrogen drugs date back to more than 25 years. There are three types of 5α-reductase inhibitors which interact with different enzyme complexes. Type A inhibitors interact with the free enzyme and compete with the NADPH cofactor and the substrate testosterone. Type B and C inhibitors interact with the enzyme-NADPH cofactor complex. While type B inhibitors are competitive with the substrate, type C inhibitors do not generally compete with the substrate [7].

Steroidal 5α-Reductase Inhibitors

It has been shown that natural steroids have some antiandrogenic action. For example, progesterone (**5**) inhibits the formation of DHT by competing with the Δ^4–3-keto site of the testosterone molecule for 5α-reductase [24].

The structure of steroidal 5α-reductase inhibitors was based on the testosterone skeleton modified by the introduction of a nitrogen atom in the A ring (4-azasteroids) (**6**), in the B ring (6-azasteroids) (**7**) and at position 10 (10-azasteroids) (**8**), and by the introduction of a double bond. In steroidal 5α-reductase inhibitors A-ring lactam is a key element that acts as a transition state of the intermediate enolate [25] (Fig. (**5**)).

The first potent inhibitor of the 5α-reductase enzyme was **finasteride** (1S,3aS,3bS,5aR,9aR,9bS,11aS)-N-tert-butyl-9a,11a-dimethyl-7-oxo-1,2,3,3a,3b,4,5,5a,6,9b,10,11-dodecahydroindeno[5,4-f]quinoline-1-carboxamide) (**9**) (Type B), a 4-azasteroid compound. Finasteride is an irreversible inhibitor of the enzyme. A reduction of the Δ1 A-ring of finasteride to an enolate and a subsequent alkylation by $NADP^+$ create a stable and potent dihydrofinasteride-NAPD-enzyme complex. Finasteride is mainly a type 2 inhibitor with a weak potency for type 1 reductase. It can exist in two different polymorphic forms, 1 and 2, of which the polymorphic 1 is the usual form marketed. Clinical studies with finasteride treatment showed 60-80% reduction of DHT level in benign prostate hyperplasia in men and 30-60% reduction of hirsutism scores in women [3, 25]. A daily dose of 5 mg of finasteride is usually recommended, however, some data suggest that 7.5 mg is more effective [3]. It has been shown that treatment with finasteride for 9-12 months significantly reduced Ferriman-Gallwey score in women with hirsutism, but amelioration of hirsutism was reversed after cessation of treatment. Side-effects such as headache and depressive

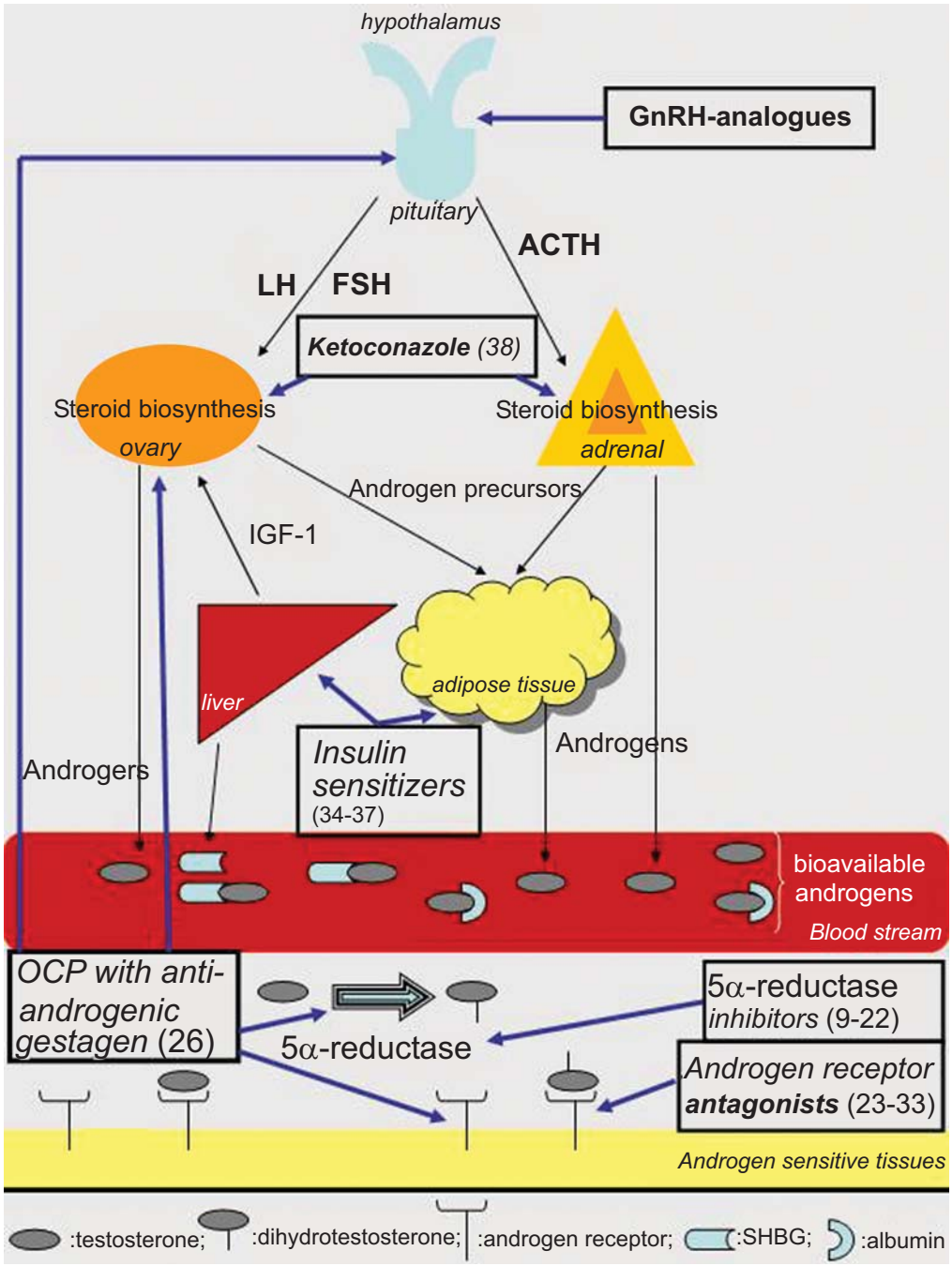

Fig. (4). Schematic view of the targets of different drugs used in the therapy of hyperandrogenism. FSH: follicle stimulating hormone, IGF-1: insulin-like growth factor 1., OCP: oral contraceptive pills, the numbers in brackets refer to the compounds mentioned in the text and showed in Figs. **5**-**8**.

mood were transient, whereas loss of libido was not observed [26, 27]. A few studies involving a small number of patients indicated that finasteride may improve androgenic alopecia in women [28]. Topical use of finasteride may be a good treatment option in hirsutism and androgenic alopecia. Current studies are in progress to develop an effective finasteride-containing formulation for local application [29]. Because finasteride is considered as a teratogenic drug causing feminization of male fetuses during pregnancy, an adequate contraception should be used during treatment.

In some hyperandrogenic disorders, such as hirsutism in women both types of reductases are probably involved [3]. Therefore, the use of dual inhibitors may have a potential advantage over type specific inhibitors. A potent dual inhibitor, **dutasteride** ((1S,3aS,3bS,5aR,9aR,11aS)-N-[2,5-bis(trifluoromethyl)phenyl]-9a,11a-dimethyl-7-oxo-1,2,3,3a,3b,4,5,5a,6,9b,10,11-dodecahydroindeno[5,4-f]quinoline-1-carboxamide) (**10**) (Type B) was approved for the treatment of benign prostate hyperplasia [30]. Until now no randomized studies have been published on the antiandrogenic effect of dutasteride in hyperandrogenic women, although some case

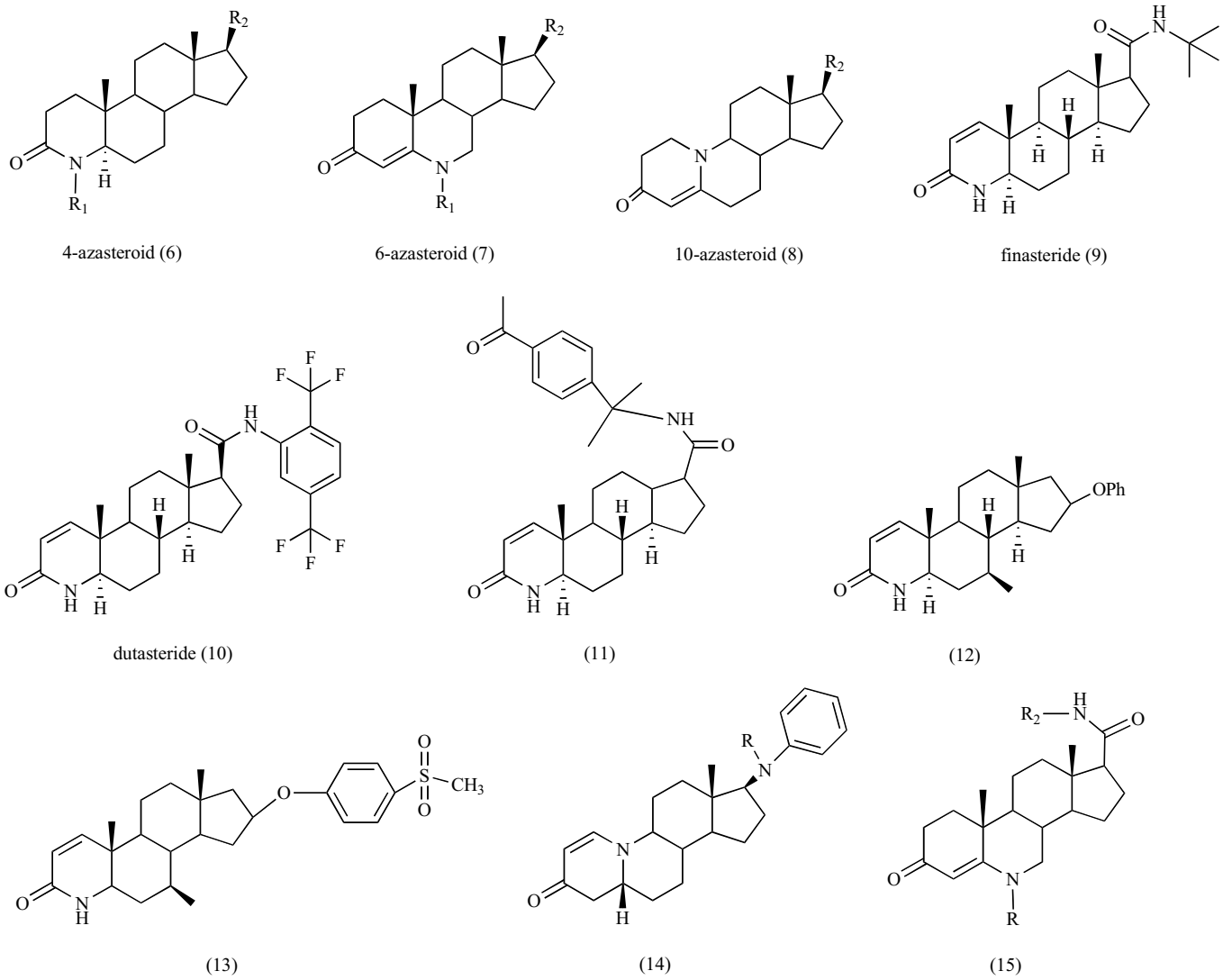

Fig. (5). Steroidal 5α-reductase inhibitors.

reports have been published [28]. Another finasteride-like compound (**11**) (Type B) was claimed to be effective in the treatment of baldness, female hirsutism and seborrhoea [31]. A series of 7,16 disubstituted-4-azasteroids (**12,13**) (Type B) containing an aryloxy group at C16 has been developed by Merck (Merck & Co., Inc. Whitehouse Station, NJ) as a potent inhibitor of the 5α-reductase type 1 enzyme (IC_{50} < 50 nM). According to the manufacturer's report this compound appears to be very active in the treatment of PCOS [31]. In addition, there are some new 10- and 6-azasteroid compounds under development, of which the 17β-(N-*tert*-butyl)carbamoyl derivative (**14**) (Type B) in the 10-azasteroid series exhibited the most potent *in vitro* inhibition of human 5α-reductases (IC_{50} = 127 and 37 nM toward type I and type II reductase, respectively) [32]. The design of 6-azasteroids was based on the 3-keto-4-ene-6-amine functionality thus mimicking the structural and charge polarization features of the transition state of the enzyme catalyzed transfer of a hydride from NADPH to testosterone (**15**) (Type B) [31]. However, there are no clinical data on the antiandrogenic affect of these compounds in hyperandrogenic women.

Undesired adverse effect may occur during treatment with steroidal 5α-reductase inhibitors due to cross-reactivity with other steroid hormone receptors, including progesterone receptor (PR) [33]. Moreover, steroidal compounds are rapidly modified by several steroid-metabolizing enzymes [34]. Therefore, development of 5α-reductase inhibitors with a non-steroidal skeleton is expected to be more effective with less adverse effects.

Non-Steroidal 5α-Reductase Inhibitors

During the last decade several non-steroidal inhibitors have been discovered. They can be classified according to their structure as benzo[*c*]quinolizinones, benzo[*f*]quinolinones, piperidones and carboxylic acids. These compounds are generally thought to act all as competitive inhibitor versus testosterone with exception of epristeride. The structures have been derived from the (aza) steroid structure by removing one or two rings and/or by replacing a ring with an aromatic one (Fig. (**6**)). Benzo[*c*]quinolizinones (**16**) have been obtained from 10-azasteroids by removing the D-ring, and by substitution of the C ring with an aromatic one. The most

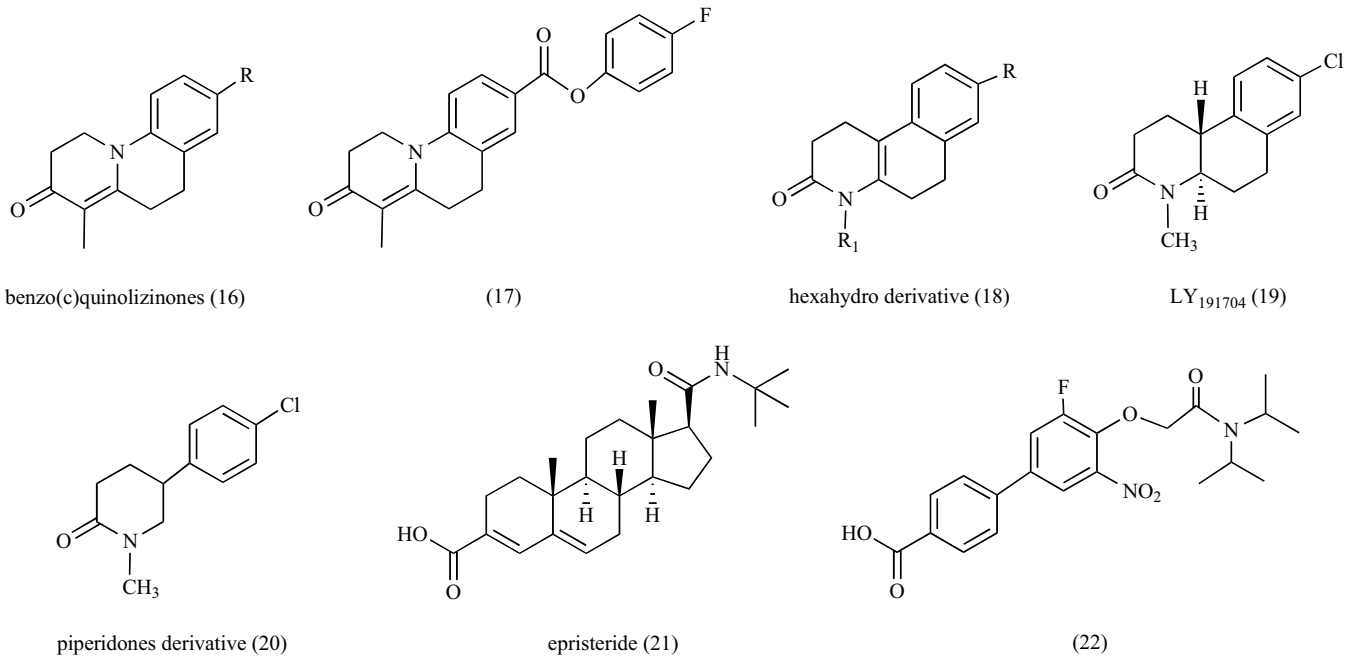

Fig. (6). Non-steroidal 5α-reductase inhibitors.

potent compound of this structure class (**17**) harbors the F atom in the *para* position of the phenol moiety (IC_{50} = 93-119 nM). A structure-activity relationship study indicated that the presence of F atom in the ester moiety at position 8 was the most important for the inhibitory activity [35]. Benzo(*f*)quinolinones derivates have been obtained from 4-azasteroids by removal of the D-ring and by substitution of the C-ring with an aromatic one. A detailed review about these derivates has been published [36]. This group of derivates can be divided in two subgroups: *hexahydro derivatives* (**18**) which have an unsaturation at position 4a-10a, and *octahydro derivatives*. Structure-activity relationship studies revealed that octahydro derivatives are more potent than hexahydro compounds. The most active compound from the octahydro group (LY191704) (IC_{50} = 8 nM) (**19**) (Type C) with Cl atom at position 8 and a methyl group at position 4 has progressed to human clinical trials [7].

Compounds derived from 4-azasteroids are bicyclic lactams. Removal of B and D rings from 4-azasteroids resulted in piperidone derivatives. *In vitro* studies suggest that these derivatives have higher potency towards type 1 5α-reductase. The most potent inhibition was achieved by the presence of a chloride atom on the aromatic ring (IC_{50} = 1690 nM) (**20**).

Epristeride (**21**) (Type C) is a carboxyl acid that demonstrated significant reduction of DHT in animal studies. It has a high potency toward type 2 reductase. It acts as a noncompetitive inhibitor and its steroidal structure mimics the enolate intermediate [7]. A new subfamily of non-steroidal 5α-reductase inhibitors has been obtained by replacing the steroid skeleton to estrone with a biphenyl moiety (**22**) [37]. These derivatives display reductase enzyme inhibition in the nanomolar range. The inhibitory effect can be increased by the introduction of a fluorine and a nitro group in the ortho position of the phenyl ring (IC_{50} = 9.8 nM).

There are several other new classes of nonsteroidal inhibitors of 5α-reductases published in last few years [38-41]. However, much research is yet to be undertaken to find optimal selective or dual inhibitors which can be used for the treatment of human diseases. Non-steroidal inhibitors have been investigated mainly in *in vitro* and animal studies, and only very few data in humans are available. Currently no data regarding the effect of non-steroidal inhibitors in hyperandrogenic women are known.

In clinical practice only finasteride has been extensively studied in androgen dependent diseases in women. These studies indicated that finasteride treatment may in part ameliorate hirsutism and androgenic alopecia [3, 25-28, 42], and it may improve insulin resistance in women with PCOS [43]. However, finasteride acts first of all on type 2 5α-reductase with a week potency for type 1 reductase found in the skin. For this reason finasteride is not the ideal 5α-reductase inhibitor for the treatment of hyperandrogenic skin manifestations.

Androgen Receptor Antagonists

Since binding of testosterone and DHT to the AR is an essential step in the action of androgens in target cells, a logical approach for neutralizing the effect of androgens is the use of AR antagonists which prevent the interaction of testosterone and DHT with the AR. There are two classes of AR antagonists; the *steroidal derivatives*, all of which possess mixed agonistic and antagonistic androgenic activities, and the *non-steroidal derivatives* or pure antiandrogens which block the AR without exerting any agonistic or any other hormonal activities.

Steroidal Antiandrogens

The most studied steroidal antiandrogens used in the treatment of hyperandrogenic disorders are spironolactone (**23**) and cyproterone acetate (**24**) (Fig. (**7**)). Both drugs have

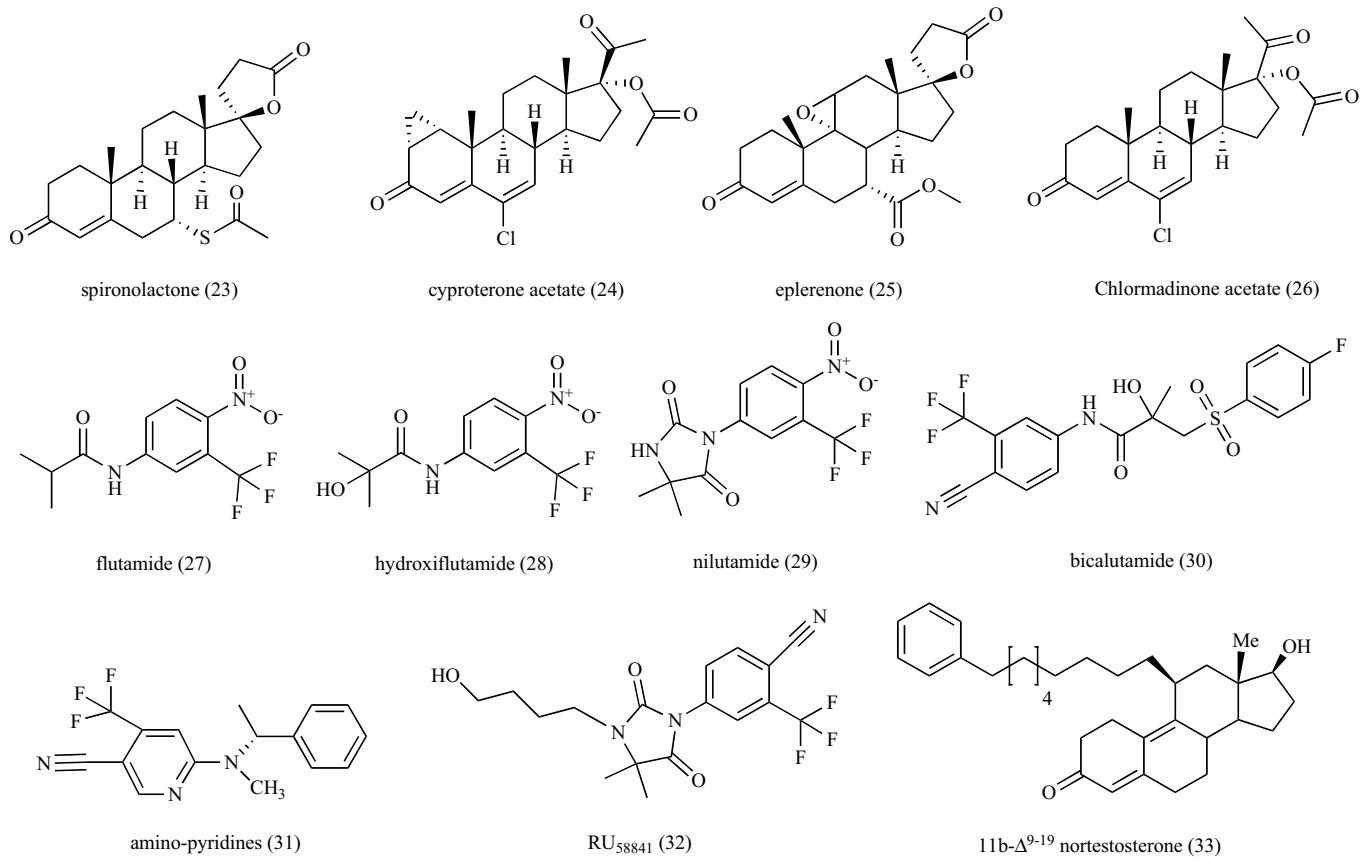

Fig. (7). Steroidal and non-steroidal AR antagonists.

been developed originally for the treatment of disorders other than androgen excess.

It has been discovered in the 1950s that progesterone antagonizes mineralocorticoid activity [44, 45]. After several modifications of the compound **spironolactone** (S-(10,13-dimethyl-3,5'-dioxospiro[2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthrene-17,2'-oxolane]-7-yl) ethanethioate) has been invented [46]. Spirolactones are synthetic molecules with a 17γ-lactone and with various C7-substituents (canrenone is an active metabolite of spironolactone without a C7-substituent). Clinical observations showed that the use of spironolactone, particularly at higher doses or for a longer period of time, was associated with endocrine side effects such as loss of libido and menstrual irregularities in women and gynecomastia and impotence in men [47].

Spironolactone with 7α-substitution but without any substituent at the 11β-position exhibits antiandrogen activity. The role of the 7α-substituent is not fully understood because canrenone, the active metabolite of spironolactone without any 7α–substitution exerts the same antiandrogen effect as spironolactone. However, it has been shown that structural modification of the steroid skeleton around the 3-keto, 11β-R_1, and 17-R_2 groups may be crucial for the stabilization of the conformation of the AR, and for the determination of the agonistic and antagonistic activity. The presence of a hydrophobic element at 11β-position of the spironolactone skeleton can induce an androgenic agonist activity, whereas spironolactone without any 11β–substituents exhibits an antiandrogenic activity [48].

In addition to the competitive inhibition of the AR, spironolactone can inhibit activation of the 5α-reductase enzyme as well as enzymes involved in androgen biosynthesis [42, 49].

Although no dose-response clinical studies have been conducted, the antiandrogenic effect of spironolactone appears to be dose dependent. In clinical practice a relatively high dose (around 100 mg/day) of spironolactone is needed for the antiandrogenic effect. Clinical studies which compared the effect of spironolactone with placebo treatment showed a greater reduction in hirsutism score in women treated with spironolactone compared to those receiving placebo [42]. A review of 5 clinical studies involving patients with PCOS showed an improvement of hirsutism but a limited effect was observed in menstrual irregularity and insulin resistance during spironolactone treatment [50].

Spironolactone is usually well tolerated, however, in higher doses it may cause menstrual irregularity, elevation of serum potassium, increased diuresis, dyspepsia, fatigue and headache [3]. Similar to other antiandrogens, it may cause pseudohermaphroditism in male fetuses when applied during pregnancy. In cases of severe renal insufficiency or elevated serum potassium level, or in pregnancy the therapy with spironolactone is contraindicated.

Eplerenone (**25**) is a highly selective mineralocorticoid receptor antagonist with a negligible AR antagonist activity (AR IC_{50} eplerenone, 4.82μM; spironolactone, 0.013μM). Eplerenone was 370-fold less potent than spironolactone in inhibiting DHT-induced activation of the AR. The critical feature of the molecule conferring selectivity is the presence of the 9,11-epoxide in the lactone ring [46].

Cyproterone acetate, an analogue of hydroxiprogesterone, acts as a competitive androgen receptor inhibitor and it inhibits 5α-reductase activity. In addition, it suppresses serum gonadotropin and androgen levels and increases hepatic clearance of testosterone [3].

Cyproterone acetate possesses an A-ring with 1, 2 cyclopropane moiety, a 6-chloride atom on the B-ring and a C17 substituent. X-ray crystal structure studies revealed that this molecule is seemingly too large to fit within the AR binding pocket due to its bulky substituent at the C17 position (Fig. (**3b**)). It has been proposed that cyproterone acetate interacts with the AR *via* "passive antagonism" and that the structural basis of antagonism does not rely on the presence of a bulky extension on the antagonist ligand but rather on the production of suboptimal side chain conformations of residues involved in the interaction with helix H12 in its active conformation. Structural studies revealed steric constraints between the cyclopropyl ring and Leu707 and between C6-Cl and Met787 and Phe764 [51]. Furthermore, the 17α-acetate group of cyproterone acetate induces movement of the Leu-701 side chain, which results in partial unfolding of the C-terminal end of helix 11 and displacement of the loop between helices 11 and 12 [52]. The proper hydrophobic binding surface for the holo-helix H12 is not generated and, therefore, the surface involved in the recruitment of coactivators and composed of helices H3, H4, and holo-H12 is destabilized [51]. It is of interest to note that in the presence of L701A and T877A mutations, cyproterone acetate exhibits an agonistic activity on the AR LBD whereas mutations at positions 708 and 709 of the AR LBD turn cyproterone acetate from a partial agonist/antagonist to a pure antagonist [52, 53].

Cyproterone acetate is widely used in Europe for the treatment of hyperandrogenic disorders in women. It has been shown that cyproterone acetate administered with ethinyl estradiol was more effective than placebo in women with hirsutism [54]. Cyproterone acetate has a long half-life and thereby, treatment with cyproterone acetate is usually sequential with doses ranging between 50 and 100 mg/day. CPA is also available as an oral contraceptive in a lower daily dose of 2 mg with ethinyl estradiol. Potential side effects such as loss of libido, liver toxicity, adrenal insufficiency or, in combination with ethinyl estradiol, increased risk for thromboembolism should be taken into account before use. In severe liver diseases, depression or in recent thromboembolism the treatment with cyproterone acetate is contraindicated. A lipid nanoparticle system containing cyproterone acetate is under development for topical acne treatment [55].

Chlormadinone acetate ([(8R,9S,10R,13S,14S,17R)-17-acetyl-6-chloro-10,13-dimethyl-3-oxo-2,8,9,11,12,14,15,16-octahydro-1H-cyclopenta[a]phenanthren-17-yl] acetate) (**26**) is a derivative of naturally secreted progesterone that shows high affinity and activity for the progesterone receptor. It has an anti-estrogenic effect and, in contrast to natural progesterone, shows moderate anti-androgenic properties. Chlormadinone acetate acts by blocking androgen receptors in target organs and by reducing the activity of skin 5α-reductase. It suppresses gonadotropin secretion and thereby reduces ovarian and adrenal androgen production [56].

Non-Steroidal Antiandrogens

Several structural classes of non-steroidal AR antagonist have been discovered. Non-steroidal antiandrogens possess structural features that appear to be important for achieving high binding affinity, including structural features which mimic the steroid plane and maintain hydrophobic interactions, and that mimic the 3-keto or 17α-OH groups and help form H bonds with AR LBD [10].

Toluidide derivates (Fig. (**7**)) are considered as pure antiandrogens since they posses little or no intrinsic androgenic activity when bound to AR. They do not have cross-reactivity with any other steroidal receptors. One of these compounds is **flutamide** (2-methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]propanamide) (**27**), a pure antiandrogen which undergoes a first pass metabolism to form its main metabolite, 2-hydroxiflutamide (**28**). 2-hydroxiflutamide is a more powerful antiandrogen with higher binding affinity to AR than flutamide [57]. In addition, flutamide inhibits the activity of the cytochrome P450 17,20-lyase [58]. For the treatment of hirsutism or other hyperandrogenic disorders, the average oral dose of flutamide varies between 250 and 750 mg/day. The efficacy of flutamide in female hyperandrogenic disorders appears to be similar to that observed with spironolactone, cyproterone acetate or finasteride [59-61].

There are some studies reporting beneficial effect of flutamide treatment on metabolic alterations in women with PCOS. Flutamide can reduce visceral fat, improve atherogenic lipid profile, attenuate insulin resistance and improve ovulation [62-64]. However, one study showed that flutamide can suppress adrenal androgen synthesis in obese women without an effect on metabolic disturbances [65]. Is has also been shown that flutamide can improve uterine perfusion in women with PCOS [66].

The major concern with flutamide is its propensity for hepatic toxicity. The hepatotoxicity can be dose-dependent, since in studies with small doses (<375 mg/day) no such adverse event has been reported. Because of its possible liver toxicity the Endocrine Society does not support the use of flutamide as a first-line therapy in hirsutism [3]. In severe liver diseases the treatment with flutamide is contraindicated.

A hydantoin analogue of flutamide, **nilutamide** (5,5-dimethyl-3-[4-nitro-3-(trifluoromethyl)phenyl]imidazolidine-2,4-dione) (**29**) is widely used in prostate cancer therapy. However, no studies with nilutamide in women with hyperandrogenic disorders have been conducted. Hepatic failure has been reported in patients treated with nilutamide, as well [67].

A novel compound is **bicalutamide** (N-[4-cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenyl)sulfonyl-2-hydroxy-

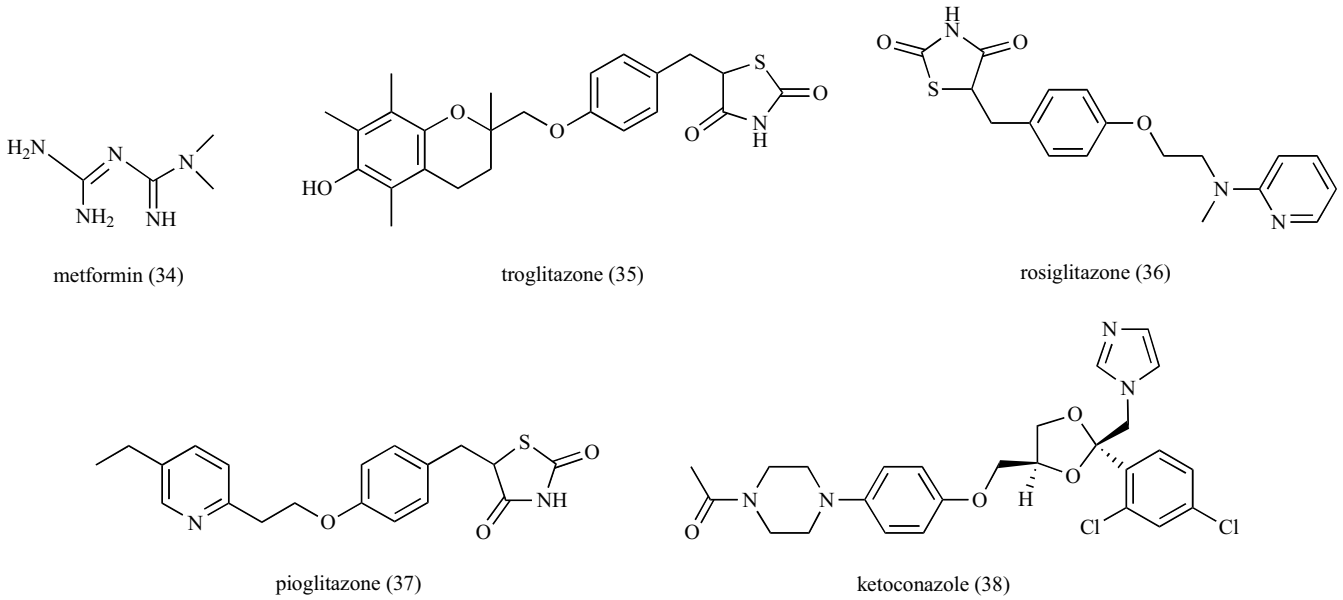

Fig. (8). Insulin sensitizers and ketoconazole.

2-methylpropanamide) (**30**) that has less hepatotoxic feature and a longer half-life than flutamide and nilutamide [68]. As a structural analogue, bicalutamide shares the amide bond structure with flutamide. However, replacement of the nitro group with a cyano group avoids the nitro reduction observed in the case of nilutamide or hydroxyflutamide. Bicalutamide – having a chiral carbon in its structure – is administered as a racemate. The *in vivo* antiandrogenic activity of bicalutamide arises almost entirely from its R-isomer that has approximately 30-fold greater binding affinity than the S-isomer [10]. Bicalutamide in a dose of 25 mg/day proved to be effective in women with severe hirsutism [69, 70].

A new series of androgen receptor antagonists may arise from amino-pyridines. Amino-pyridines are a series of hybrids of the structures of flutamide and bicalutamide with a nitrogen inserted into the phenyl ring. The pharmacokinetic characteristics of the most potent compound of this series (**31**) may be favourable for a topical agent. Ideally, topical drugs exert their desired effects locally but are rapidly inactivated *via* metabolism once they reach the systemic circulation, thereby reducing unwanted systemic effects. This compound proved to have *in vivo* activity for both stimulating hair growth and reducing sebum production [71]. Another hydantoin derivate, RU58841 (4-[3-(4-hydroxybutyl)-4,4-dimethyl-2,5-dioxoimidazolidin-1-yl]-2-(trifluoromethyl)benzonitrile) (**32**), that has a half life shorter than 1 hour, proved to be effective for topical treatment of acne and alopecia. [72, 73].

Insulin Sensitizers

The majority of women with clinical hyperandrogenism have PCOS. According to the Rotterdam criteria, the diagnosis of PCOS can be established if two of the three main features (polycystic ovarian morphology, oligo/amenorrhea with oligo or anovulation, and elevated androgen level) are present and other causes of hyperandrogenism are excluded [74]. The pathogenetic background of this syndrome involves an increased production of ovarian and adrenal androgens, disturbances of the hypothalamic-pituitary-ovarian axis, and hyperinsulinemia as a consequence of insulin resistance [75]. Clinical observations indicate that insulin sensitizers have beneficial effect on hyperandrogenism in women with PCOS [76].

Metformin (3-(diaminomethylidene)-1,1-dimethylguanidine) (**34**) (Fig. (**8**)), a biguanid compound used in the therapy of patients with type 2 diabetes lowers hepatic glucose production by reducing gluconeogenesis and glycogenolysis, increases peripheral glucose uptake in skeletal muscle and adipose tissue, and reduces intestinal glucose absorption [77]. One of the underlying mechanisms of these pleiotropic effects seems to be the activation of the 5'-AMP-activated protein kinase, which involves activation of a proximal kinase, the serine–threonine protein kinase 11 (previously termed LKB1) [78, 79].

In women with PCOS, metformin administered at daily doses up to 1500 mg inhibits ovarian and adrenal androgen secretion, decreases serum insulin, testosterone and LH levels, increases androgen binding to plasma proteins due to an enhanced SHBG synthesis, and it also appears to induce some weight loss [76]. Reduction of elevated 17-hydroxyprogesterone levels was also observed in women with PCOS during metformin treatment [58].

Randomized clinical studies indicated that metformin was superior to placebo, but not as effective as clomiphene citrate in ovulation induction and pregnancy rates in women with PCOS. Furthermore, a combination therapy with metformin and clomiphene citrate was more effective than clomiphene alone [80]. A recent metaanalysis showed that metformin in obese or overweight women produced a weak improvement of hirsutism [81].

Combination therapies with metformin and antiandrogens, most frequently with flutamide are under evaluation. Some studies showed a beneficial effect of the combined

therapy on androgen excess and metabolic disturbances in young non-obese women [82].

Metformin can cause tolerable gastrointestinal side effects and, in exceptionally rare cases lactate acidosis may also develop.

The insulin-sensitizing **thiazolidinediones** (Fig. (**8**)) are selective ligands of the nuclear transcription factor peroxisome-proliferator-activated receptor γ (PPARγ), which is expressed most abundantly in adipose tissue, but is also found in pancreatic beta cells, vascular endothelium and macrophages. The first approved thiazolidinedione, **troglitazone** (5-[[4-[(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl) methoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione) (35) has been withdrawn from the market due to hepatotoxicity. The two currently available PPARγ agonists are **rosiglitazone** (5-[[4-[2-(methyl-pyridin-2-ylamino)ethoxy]phenyl] methyl]-1,3-thiazolidine-2,4-dione) (**36**) and **pioglitazone** (5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione) (**37**).

Thiazolidinediones exert their insulin-sensitizing actions directly by promoting fatty acid uptake and storage in adipose tissue. In addition, thiazolidinediones increase the expression of adiponectin, an adipocytokine with an insulin sensitizing effect, and they possibly decrease the expression of the 11ß-hydroxysteroid dehydrogenase type 1, an enzyme which catalyzes the conversion of inactive cortisone to active cortisol [83]. Most clinical studies evaluating the effect of thiazolidinediones in women with PCOS demonstrated that thiazolidinedione administration was associated with significant improvements in insulin resistance, hyperinsulinemia, hyperandrogenemia, hirsutism and ovulatory function [76, 82, 84]. These beneficial effects on hyperandrogenism are not only due to an improvement of hyperinsulinemia but also a direct inhibition of ovarian steroid synthesis and secretion, as evidenced by the reduction of serum 17-hydroxyprogesterone and androstenedione responses to ACTH stimulation [43]. Cell culture studies revealed that thiazolidinediones reduce ovarian testosterone synthesis and abolish insulin-induced stimulation of testosterone production. In addition, thiazolidinediones enhance progesterone production that is commonly reduced in women with PCOS [85].

Glitazones may have direct effect on the AR. Experimental data showed that troglitazone and its PPARγ inactive derivatives mediate transcriptional repression of the AR by facilitating ubiquitin dependent proteosomal degradation of Sp1 [86].

A combination therapy containing pioglitazone, flutamide, oral contraceptive and metformin has been shown to result in a larger improvement of hirsutism score, androgen level and metabolic abnormalities than combination therapy with flutamide, metformin and oral contraceptive [87]. Glitazones are known to increase the risk of cardiac failure. Additionally, rosiglitazone may increase the risk of myocardial ischaemia and bone fractures. The main contraindications of glitazones are cardiac failure and myocardial infarction.

Other agents

Ketoconazole (1-[4-[4-[[2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]ethanone) (**38**) (Fig. (**8**)), an imidazole derivate is widely used as an antifungal agent. The first observations as a possible antiandrogenic compound came from clinical studies showing the development of gynecomastia in some men during treatment. Further studies revealed that ketoconazole inhibits the microsomal P450c17α enzyme that contains a heme prosthetic active group at its active site [88]. The enzyme has both C17,20-lyase and 17α-hydroxylase activities. The C17,20-lyase activity is more sensitive to ketoconazole than the 17α-hydroxylase activity and, therefore, testosterone secretion is more suppressed than cortisol secretion during treatment [89]. Molecular studies showed that ketoconazole binds to the P450c17α enzyme with two polar-polar interactions *via* its 4'-chlorophenyl and 1-acetil-piperazine groups and a Fe-N covalent bond [90]. In a small study of 37 women with hyperandrogenism a low dose of ketoconazole (400 mg/day) significantly improved hirsutism, acne, and androgen hormone levels [91]. The major concerns in connection with ketoconazole are the inhibition of cortisol biosynthesis and hepatotoxicity [88].

Since the discovery and synthesis of **gonadotropin-releasing hormone (GnRH)** in 1971, numerous long-acting agonistic and antagonistic analogues have been synthesized. Hypothalamic GnRH is a major factor orchestrating gonadal functioning *via* pituitary gonadotropin release. During long-term use agonistic analogues have been found to desensitize pituitary GnRH receptors resulting in a decreased gonadotropin secretion. These analogues can be used in the treatment of several conditions in which decreased gonadal steroid production is desired (e.g. breast cancer and androgen excess in women). They can be used in severely androgenised women who do not respond to conventional therapy. Studies with GnRH analogues showed an attenuation of ovarian androgen excess and hirsutism in women with PCOS [92-95]. However, a prolonged use of long-acting analogues can deplete calcium from bone, resulting in osteoporosis. Hot flushes and vaginal dryness may also occur during treatment. There are many limitations of GnRH therapy such as the relative high cost and the inconvenient application as a subcutaneous injection. Long-term safety studies are also lacking and in many countries GnRH agonists are not approved for the treatment of androgen excess in women.

Conclusion and Future Prospects

Since elevated androgen level can arise from different diseases, an extensive investigation of the origin of androgen excess is inevitable before launching an antiandrogen therapy. For example, androgen secreting ovarian or adrenal tumors should be operated; and antiandrogen therapy can be considered as an additional option when surgical therapy is not possible.

Steroidal and non-steroidal 5α-reductase inhibitors and androgen receptor antagonists are potent drugs for the treatment of androgen excess in women. The most frequently used drugs are finasteride, flutamide, spironolactone and cyproterone acetate. All these drugs possess a potential fetotoxicity and, therefore, an adequate contraception is required during treatment.

Despite a wide range of available antiandrogen agents, these drugs are not always the best first line therapy in

women with androgen excess. Reduction of body weight in obese women with PCOS and HAIRAN syndrome may result in a remarkable attenuation of androgen excess *via* an improvement of hyperinsulinemia and a decrease of peripheral androgen production [96]. According to the available evidence based data and the recommendation of the Endocrine Society, an estrogen-progestagen combination therapy with antiandrogenic properties such as oral contraceptive pills (OCP) containing antiandrogenic gestagens can be considered as a first choice of therapy in many cases of non-tumorous ovarian androgen overproduction. When the results are unsatisfactory, an OCP and antiandrogen drug combination may be the second alternative [3]. According to a recent metaanalysis, the antiandrogen (spironolactone or finasteride) adds-on therapy to OCP seems to be more effective than OCP therapy alone in women with hirsutism [42]. In women with PCOS or HAIRAN syndrome insulin sensitizers may also ameliorate elevated androgen levels. Although a large number of studies support the beneficial effect of insulin sensitizers in women with PCOS, none of them are approved for the therapy of PCOS.

Further development and application techniques are expected for local antiandrogen therapy options for women with androgen excess.

As the primary drive of the development of novel antiandrogenic drugs is mainly related to the treatment of prostate cancer, new compounds of reductase inhibitors and AR antagonists are under investigation. There are some very exciting data about the design of bifunctional antiandrogens, which bind as an agonist, but overall act as an antagonist by covering the binding site of coactivators on the AR surface and, therefore, by preventing activation of the receptor. One possibility was to design bulky antiandrogens that can recruit FK506-binding chaperone proteins (FKBPs) to the co-activator site of the AF-2 domain of the AR, as described by Singh *et al*. [97]. These recruited proteins can sterically prevent binding of any co-activator protein to the AR. The FKBPs, encoded by multiple genes and ubiquitously expressed in all mammalian cells [98] are ideal candidates for recruitment to protein surface. It has been already demonstrated that such a bifunctional approach based on the recruitment of FKBPs to the surface of cellular targets can be efficient in preventing protein-protein interactions [99, 100]. Structural studies revealed that a molecule such as the 11β-Δ^9-19-nortestosterone (**33**) (Fig. (**7**)) substituted with an aliphatic side chain at 11β-position may be an attractive candidate for incorporation into bifunctional antiandrogens [97].

In addition, other X-ray studies revealed that the binding to allosteric sites of the AF2, such as binding function 3 (BF3) may modulate the activity of the AR. Drugs interacting with the BF3 exert an indirect effect on AF-2 by inhibiting co-regulator binding [101].

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