Recent Advances in Drug Design and Drug Discovery for Androgen-Dependent Diseases

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Abstract: This article summarizes the importance of different targets such as 5α-reductase, 17β-HSD, CYP17A, androgen receptor and protein kinase A for the treatment of prostate cancer and benign prostatic hyperplasia. It is a well known fact that dihydrotestosterone (DHT) is associated with the development of androgen-dependent afflictions. At the present time, several research groups are attempting to develop new steroidal and non-steroidal molecules with the purpose of inhibiting the synthesis and biological response of DHT. This review also discusses the most recent studies reported in the literature that describe the therapeutic potential of novel compounds, as well as the new drugs, principally inhibitors of 5α-reductase.

Keywords: Benign prostatic hyperplasia, prostate cancer, 5-alpha reductase, 17-beta hydroxysteroid dehydrogenase, protein kinase A, androgen receptor, dehydroepiandrosterone derivatives, non-permeable testosterone conjugates, G proteins, PKA.

INTRODUCTION

Study of Different Androgen-Dependent Targets for Anti-Androgenic Drug Design

Testosterone (T) is the hormone that is responsible for male secondary sexual characteristics. This hormone is mainly produced by the testicles and can be found in the general circulation. Testosterone is converted to the more active metabolite 5α-dihydrotestosterone (DHT) in androgen-dependent tissues [1]. This reduction of T is catalyzed by the 5α-reductase enzyme (EC 1.3.99.5) (5α-R) present in these tissues. Three different isozymes of 5α-R have been reported, which are each encoded by different genes and can be identified as types 1, 2 [1] and 3 [2]. These isozymes have been described in androgen-dependent tissues of several species; [1, 3] types 1 and 2 isozymes are expressed in the human prostate gland, with type 2 highly expressed in this tissue [4]. A type 3 isozyme (5α-R3) has recently been identified in the brain, pancreas, skin, and adipose tissue but also in prostate cancer cell lines and in a human sebaceous gland cell line [2, 5].

Various evidences reported in the literature support the fact that DHT is necessary for prostate development and growth [6]. For example, males with a genetic deficiency of 5α-R type 2 enzyme (5α-R2) have smaller prostates than normal individuals due to the low level of DHT [7]. Furthermore, these persons show imperfect development of this gland and of the external genitalia because they resemble those of females. In addition, they do not develop baldness or acne; however, in these individuals, the epididymis, seminal vesicles, and vas deferens remain normal [7]. Other findings suggest that DHT stimulates the expression of several androgen-response genes in recurrent prostate cancer [7, 8]. This expression is strongly related to prostate preservation because these genes have the ability to induce apoptosis of the prostate cells and are less involved in cell proliferation throughout prostate development [8, 9]. Furthermore, the increased levels of intracellular DHT cause higher cellular propagation and faulty differentiation [3-5]. These facts could have relevance because an overabundance of DHT has been implicated in the pathogenesis of benign prostatic hyperplasia [6-10]. 5α-R2 plays an important biological role in pros-
tate gland development because it is responsible for intraprostatic DHT levels when physiological serum T concentration is low [9].

Various experiments have been carried out with the aim of clarifying these findings; the results demonstrated that human prostate 5α-R decreases its efficiency for DHT production in the presence of a high concentration of T, while a lower concentration of T increases the activity of this enzyme, thus producing more DHT [11].

5α-R is an allostERIC enzyme whose catalytic efficiency is higher in the presence of its own products of reaction, DHT or 5α-androstanedione (5α-dione) [7, 8]. This could explain the fact that in older men whose intraprostatic and serum levels of T are low, a higher level of intraprostatic DHT is produced [1, 9, 12-14]. As a consequence of this hormonal imbalance, a decrease in the T-response genes is observed, thus preserving the capacity of the prostatic cells to undergo apoptosis [8]. Furthermore, the increase of intracellular DHT induces cellular proliferation and inadequate cellular differentiation [2]. These findings could have an important clinical bearing because an overabundance of DHT has been implicated in the pathogenesis of benign prostatic hyperplasia [4, 6].

The fact that DHT could be implicated in the development of tumor growth in the prostate (benign or malignant), as well as other androgen-dependent afflictions such as acne, hirsutism, androgenic alopecia and male pattern baldness, has stimulated the development of new inhibitors for the 5α-R enzyme [2, 9, 11].

Unfortunately, until recently, researchers have not been able to isolate 5α-R in its crystalline form; this fact has made the development of more specific inhibitors difficult [8]. It is anticipated that the structural modifications described in this paper will facilitate the design and synthesis of new, more specific inhibitors with better pharmacodynamic and pharmacokinetic properties. As a result of this, it is believed that several new inhibitors that have fewer side effects will be developed in the future [1].

The mechanism of the reduction of T (Fig. 1) is consistent with the known stereochemistry of reduction and involves a direct hydride donation from NADPH to the C-5 position of testosterone, leading to enolate formation at C-3 [8, 9]. The enolate would be stabilized by some electrophilic residue (E+) at the active site. This process may be viewed alternatively as an activation of the enone by (E+) leading to a positively polarized species that accepts a hydride from NADPH at C-5. Enzyme-mediated tautomerism then leads to the product DHT with the release of NADP+. The DHT-dependent pathologies are briefly described below [1, 13].

**Metabolic Pathways of Androgen Production**

T is an important hormone for spermatozoa maturation as well as for muscle function and development [3]. As we indicated above, DHT is also imperative for the growth, function, and pathology of the prostate [3]. The biological action of T and DHT is mediated by the androgen receptor (AR), a specific ligand-dependent transcription factor present in the prostate [15]. Immunocytochemistry studies have shown stain accumulation in the nuclei of prostate stroma secretory cells (luminal cells) as well as in several nuclei of endothelial cells, whereas basal cells from the epithelium show no reactivity [4].

The complex formed by AR-DHT shows higher affinity than that formed with T. These AR-complexes interact with coregulator proteins (coactivators or corepressors) to adapt the transcription of androgen target genes using specific sequences in the DNA [15].

In addition to T, the weak adrenal androgen dehydroepiandrosterone (DHEA) present in the general circulation is taken up by the prostate tissue [1]. It is then metabolized by 3β-HSD to 4-dione and later transformed into 5α-androstanedione (5α-dione) by 5α-R2; (Fig. 2) [4]. In addition, 5α-dione is converted to DHT by 17β-HSD5 or to androsterone by 3α-HSD, which is further converted to DHT [4].

4-Dione is also converted to T by the action of 17β-HSD5 in the prostate. 17β-HSD5 has been detected by immunocytochemistry, principally in the epithelium basal cells but also in the stroma and blood vessels from the human prostate [4, 16].

*In situ* hybridization studies have shown the presence of 17β-HSD5 in secretory cells (luminal cells) of the alveoli and in stroma cells of this gland [12]. These histological data have suggested that after castration, adrenal DHEA from the general circulation is transformed by 3β-HSD present in the basall cells from the prostate epithelium into 4-dione, and then into T by 17β-HSD5 (AKR1C3), whereas T is converted to DHT by 5αR2 from prostate tissue (Fig. 2) [4, 14-16].

**5α-Reductase Enzyme (5α-R) and Prostate Cancer**

5α-R types 1 and 2 play an important role in the pathologies of prostate cancer and benign prostatic hy-
**Fig. (1).** Mechanism of testosterone reduction to dihydrotestosterone.
This figure shows the hydride donation from NADPH to the C-5 position of testosterone, with the formation of an enolate at C-3. This enolate is stabilized by the 5α-reductase enzyme. Enzyme-mediated tautomerism leads to the product DHT and consequently the release of NADP⁺.

**Fig. (2).** Biosynthesis of androgens in prostate gland [12].
perplasia because they are present in this gland and show different locations and biochemical characteristics [1, 2, 6, 17]. Type 1 has been identified in prostate epithelial cells, and type 2 is principally found in the stromal compartment. 5α-R1 has also been identified in the liver and skin; it is active at neutral or basic pH [1].
A different pH of activity has been reported for the type 2 enzyme; it is active in acidic media. Weisser and Krieg reported that stromal cells from benign prostatic hyperplasia produce more 5α-reduced metabolites (DHT and 5α-dione, Fig. 2) than normal cells [14]. Furthermore, the development and progression of prostate cancer (PCa) are also related to alterations in the 5α-R enzyme [17]. Immunostaining techniques for 5α-R1 and 5α-R2 using specific antibodies on different human PCa specimens have demonstrated that 5α-R1 is increased and 5α-R2 is decreased during the course of PCa [17]. However, the expression of both 5α-R isozymes increases in recurrent and metastatic cancers, thus suggesting that both isozymes may be important in the development and progression of PCa [17]. Moreover, the fact that finasteride, a 5α-R2 inhibitor, reduced the prevalence of PCa [17] also suggests that both isozymes could be required for the development and progression of PCa. Therefore, type 1 and 2 5α-R isozymes could be important therapeutic targets for this disease.

It is a well-known fact that adrenal androgen androstenedione (4-dione) is also a potential substrate for 5α-R [16, 9]. Kinetic parameters (Km and Vmax) measured for prostatic 4-dione 5α-R2 were two- to sixfold higher than values found for T 5α-R [10]. Km for 4-dione 5α-R2 in the stroma and epithelium was 211 and 120 nM, respectively, and Vmax for the stroma and epithelium was 130 and 56 pmol/mg/h [9]. Prostatic kinetic parameters for T 5α-R2 were 78.4 and 14.3 nM for Km of the stroma and epithelium, respectively, and 68.3 and 23.8 pmol/mg/h for Vmax of the stroma and epithelium [10]. Thus, this nuclear membrane enzyme (5α-R2) has higher affinity for T (lower Km value) than 4-dione [10].

However, a high level of 4-dione (1.22 ng/mL) measured by radioimmunoassay [18] was found in the general circulation. This indicates that the activity of the prostate 5α-R2 enzyme takes place prior to that of 17β-HSD5 [16], according to the 4-dione →5α-dione →DHT pathway (Fig. 2). On this basis, it is important to consider this source of DHT in benign prostatic hyperplasia and prostate cancer [17-19], although experimental evidence indicated that human prostate 5α-R2 converts T to DHT more efficiently than 4-dione to 5α-dione (Fig. 2) [11, 14].

**Prostatic 17β-Hydroxysteroid Dehydrogenase 5(17β-HSD5)**

The 17β-hydroxysteroid dehydrogenases/ketosteroid reductases (17β-HSDs/KSRS) are an NADPH oxidoreductase family that catalyzes different steps in steroid synthesis and degradation [4, 21].

17β-hydroxysteroid dehydrogenase enzyme type 3 (17β-HSD3) converts 4-dione to T, and its deficiency causes human intersex disorders known as pseudohemaphroditism in young boys, but it is asymptomatic in girls. At puberty, ambiguous genitalia and virilization take place, which is related to 17β-HSD type 3 deficiencies [20]. The 17β-HSD types 1 and 3 have been reported to be responsible for the biosynthesis of sex hormones, while 17β-HSD type 5 converts 4-dione to T in the cell layer in women and in the prostate in men [21-27].

The principal isozymes from this 17β-HSDs/KSRS family that are present in the prostate are type 2 and type 5 [22]. Testosterone and estradiol are the substrate options for type 2 17β-HSD, whereas for type 5 17β-HSD, it is androstenedione (4-dione).

Human type 5 17β-HSD is exclusive to the 17β-HSDs because it belongs to the aldo-keto reductase family, whereas the others are members of the short chain alcohol dehydrogenases. Type 5 17β-HSD mRNA is present in high levels in human prostate, and this enzyme is responsible for the conversion of 4-dione to T in this gland [5, 6] (Fig. 1).

Pelletier et al. demonstrated that 17β-HSD5 (Fig. 2) catalyzes the transformation of 5α-dione to DHT (Fig. 2) [21]; apparently, this enzyme has an important role in the biosynthesis of DHT and prostate gland pathologies [20-27].

It has been previously detected that serum T levels in castrated men in the blood decrease by 90–95%; however, intra-prostatic DHT levels are reduced by only 50% [13]. This suggests that DHT synthesis could have additional pathways for T in the prostate. Furthermore, the presence of 3β-HSD type 1, 17β-HSD5 and 5α-R (type 1) in prostate epithelium has also been described. Therefore, in tissues in which these enzymes are present, such as the prostate gland, 4-dione is converted to 5α-dione, which is subsequently transformed to DHT; this metabolite is further converted by 3α-hydroxysteroid-dehydrogenase (3α-HSD) to 3α-androstandiol (Fig. 2) [13, 26-27].

The predominant isozyme in normal stromal cells from the prostate is 5α-R2; however, in prostate cancer, a decrease in 5α-R2 and an increase in 5α-R1 levels (5α-R1) take place [28]. These data could explain the mechanism by which castration-resistant prostate cancer can proliferate in the presence of low levels of serum T. These findings suggest that 5α-R and 17β-HSD
inhibition could be related to a recurrent prostate tumor [29-30].

**CYP17 (17α-HYDROXYLASE/17,20 LYASE)**

CYP17 (17α-hydroxylase/17,20 lyase) belongs to the cytochrome P450 family, whose members are involved in the prostate and adrenal steroidogenesis process (Figs. 2 and 14) [31]. In this process, progesterone (P) is converted to 17α-hydroxyprogesterone (17α-OHP) and subsequently to 4-dione in prostate and adrenal tissues. Pregnenolone is transformed to dehydroepiandrosterone (DHEA). Moreover, 3β-HSD converts DHEA to 4-dione (Fig. 2). The first two reactions are carried out in two steps, known as 17α-hydroxylation, which is followed by C17,20-lyase activity (Fig. 2) [31]. Both reactions are catalyzed by mitochondrial CYP17 (17α-hydroxylase/17,20 lyase), a protein that shows a marked preference for 5-ene substrate (Fig. 2). This enzyme is codified in men from only one gene and is present in testes and adrenals, whereas in women, CYP17 is present in the ovary, placenta and adrenals. Cytochrome CYP450scc catalyzes an analogous reaction in the side chain of cholesterol and in some circumstances depends on the concentration of the electron carrier CYP17 reductase (Fig. 2, 14). This enzyme catalyzes 17α-hydroxylation of pregnenolone, and after this reaction, the formation of cortisol by adrenal glands has been observed. (Fig. 14) Surprisingly, in the testes, a greater concentration of CYP17 reductase is required for an increase of lyase activity than in the adrenals (Fig. 2). As a consequence, lyase activity in the adrenals is less than in the male gonads. Lyase activity of CYP17 in the testes is responsible for the synthesis of androgens [31].

Androgen deprivation therapy (ADT) using CYP17 inhibitors as a treatment for prostate cancer could improve existing therapies for this disease [32]. However, these anti-hormonal agents induce a decrease in the production of cortisol by the adrenals, thus inducing strong metabolic and immunological disturbances (Fig. 14) [31].

On the basis of the molecular structure of the enzymes 5α-R (Types 1 and 2), 17β-HSD5 and CYP17, various inhibitors have been identified, which will be reviewed in this study.

**Androgen Mechanism Regulated by Androgen Receptor (AR) and Protein-Kinase A (PKA)**

AR is a transcriptional factor that belongs to the nuclear receptor (NR) superfamily composed of 48 members [15]. The NR superfamily regulates gene networks and biological effects such as growth, development, differentiation, reproduction, and apoptosis, as well as the metabolism of carbohydrates, lipids and various drugs [33]. T or DHT complex formation with AR induces a conformational change that enhances the binding of this complex to specific DNA sequences known as androgen response elements (AREs), inducing transcription of their target genes [15]. The AR gene is located on the proximal long arm of the X chromosome and regulates androgen-responsive genes by binding to AREs located within the promoter genes [15]. As a consequence of this pathway, a change in cell function is observed when the AREs join the coregulator proteins (coactivators or corepressors) present in the cell nucleus [15].

AR consists of four functional domains: C-terminal-ligand N-terminal domain (NTD), the core DNA-binding domain (DBD), the binding domain (LBD) and the region linking the LBD domains with DBD. Different mutations in the exons encoding these domains have been reported, mainly in the binding with androgens [34-36]. These mutations deliver different alterations in response to androgens, whose clinical manifestations are varied and are presented depending on the degree of dysfunctionality of AR. These mutations are transmitted as a chromosome X-linked recessive genetic disorder [34-36]. In male syndrome pseudohermaphroditism for example, a partial gene mutation occurs, in which case, despite the XY genotype, the phenotype presents as partially masculinized external genitalia. This can range from the presentation of female genitalia to genitalia that resemble the male phenotype, in addition to many other disorders. In the case of androgen insensitivity syndrome, a mutation prevents the AR from functioning correctly, showing clinical alterations as a female phenotype with an XY karyotype, as well as multiple other metabolic disorders. Besides these mutations, the presence of low intensity mutations in the AR can be clinically observed in an XY karyotype, displaying a male phenotype; but with the development of gynecomastia and infertility [34-36].

An understanding of these signaling pathways and their alterations prompted investigation of the carcinogenic process; some studies have demonstrated that the initiation and progression of prostate tumors is due to the accumulation of mutations of the tumor cell genome [34-36]. However, initial prostate cancers show few mutations in the AR, whereas in metastatic prostate cancer, these mutations are more common [36].

In addition to the accumulation of mutations in cancerous tumors of the prostate, androgens play a princi-
pal role in their growth, and their increased levels could effectively be suppressed by blocking androgen synthesis through orchidectomy or by using luteinizing hormone-releasing hormone agonists [15, 37]. Nevertheless, in some patients receiving ADT, the signaling pathway of prostate tumor cells is eventually reactivated and an enlargement in the tumor is observed [12, 17, 28, 30]. This prostate cell development, which could metastasize to local lymph nodes, is detected in 30% of tumors [36]. A study carried out with the mitochondrial fraction of the LNCaP cancer cell line displayed a mutated AR in this organelle [38]. This indicates that some changes in mitochondrial function in these cells have occurred. Therefore, castration-resistant tumors could grow with the additional energy provided by the new functional mitochondria [38].

It has been previously shown that there are regulatory transcription molecules that are associated with the AR–complex ligand in the prostate cell nucleus. These transcription factors bind to the NTD of this complex and induce gene activation. These molecules may be coactivators such as SRC1 (steroid receptor coactivator 1) or corepressors SMRT (silencing mediator for retinoic acid), which are present in the prostate [40]. It has also been shown that these coregulatory proteins act jointly to synchronize the androgen-mediated response. Therefore point mutations of these coactivators could be also a cause of androgen-resistant prostate cancer. Interestingly, however, the cellular imbalance of corepressors appears to modify the agonist response promoted by its ligand, suggesting that the potency of an antiandrogen may also be altered by these corepressors. This has been previously shown for the antiandrogen cyproterone acetate [41].

On the other hand, there is evidence to suggest that cAMP analogs are capable of inhibiting the binding between SMRT and NTD of the AR. These data indicate that there is communication between SMRT and NTD of the AR. These data indicate that there is communication between SMRT and NTD of the AR. The regulatory subunit RIα is one of two regulatory subunits that make up subtype I of PKA (PKA RIα); this heterotetrameric enzyme also shows two catalytic subunits [43]. PKA RIα is overexpressed in several tumor types, including colorectal, breast, prostate and lung cancers, in which it has been associated with a poor prognosis [43].

Another effector of PKA in the signaling pathway is the p21-activated kinase PAK4 [46, 47]. This kinase (PAK4) belongs to group II of the serine/threonine kinases and has been demonstrated to contribute to tumorigenesis of the prostate [45]. Experiments using immunoblotting techniques have shown that the PC-3 and DU145 cancer cell lines show activity of the PKA signal expressed the R1α subunit of PKA as well as AR. Furthermore, their data showed the downstream expression of the effector molecules PSA and vasodilator-stimulated phosphoprotein (pVASP) [43]. The expression of these effector molecules was inhibited after treatment of these cells with siRNA against AR. Moreover, treatment of these cancer cell lines with siPKA downregulated the expression of AR. These authors concluded that the siRNAs against AR were able to decrease PKA R1α level expression in LNCaP and VCaP cells, suggesting potential communication between AR and PKA pathways at the protein expression levels.

The regulatory subunit R1α is one of two regulatory subunits that make up subtype I of PKA (PKA RIα); this heterotetrameric enzyme also shows two catalytic subunits [43]. PKA RIα is overexpressed in several tumor types, including colorectal, breast, prostate and lung cancers, in which it has been associated with a poor prognosis [43].

Previous reports have also demonstrated that members of the CBP/p300 coactivator family (Fig. 3) are over-expressed in tumor cells and are responsible for the increased rate of gene transcription [43, 44]. The role of CBP/p300 protein in gene transcription was demonstrated by the fact that AMP-dependent kinase A (PKA) signaling activated by forskoline resulted in an enhanced androgen-induced expression of the prostate-specific antigen (PSA) gene [43, 44]. This mechanism was inhibited by the antiandrogen bicalutamide; however, when CBP/p300 is overexpressed in cancer cells, activation of PKA stimulates PSA transcription in the absence of androgens; this mechanism is not inhibited by bicalutamide [42]. PKA was shown to be capable of phosphorylating the cAMP-responsive element-binding protein (CREB) in the presence of androgens [39]. The mammalian two-hybrid assay demonstrated that CBP/p300 could be the contact between AR and CREB (Fig. 3) [42, 43].
ence of an active PKA. The phosphorylation of PAK4 was completely blocked [45]. Prostate tumorigenesis studies demonstrated that when PAK4 knockdown-PC3 (shPAK4) and DU145 cell lines were seeded in athymic mice, the growth of PC-3-shPAK4 decreased in comparison to controls. Furthermore, DU145-shPAK4 showed a complete blockade of tumor growth. These data suggest that PAK4 could be a potential therapeutic target for prostate cancer.

PAK4 could also regulate the expression of CREB because PAK4 depletion reduced the expression of CREB [45-48]. Moreover, PAK4 also reduces the expression of the gene Bcl-2, an antiapoptotic member of the Bcl family of proteins that is regulated by CREB. These data could explain the increase in apoptosis (1–6%) observed in PC-3-ShPAK4 compared to controls and the 5–15% increase when DU145-ShPAK4 was used in the experiments [45].

Finally, we must consider the fact that the signaling pathway turns on in the cell membrane. A messenger from the endocrine system is necessary to activate this membrane binding site or receptor in order to regulate the expression of different effector molecules to induce cell function. It has been reported that rapid androgen actions are produced through non-genomic signaling pathways, as well as stimulation of membrane androgen-binding sites or receptors (mAR) [46]. These signaling pathways have been described in different in vitro and in vivo models [46]; however, the molecular identity of these binding sites remains unknown. Because the cascade involving PKA R1α and PAK 4 activation is related to the expression of AR-dependent effectors, there has been special interest in the role of mARs in these mechanisms in prostate tumors [47]. The fact that mAR activation by non-permeable testosterone conjugates induced potent anti-tumorigenic responses in prostate, breast, colon, and glial tumors suggest the presence of a receptor that induces an intracellular signaling cascade [47].

The presence of a class of membrane-anchored receptors linked to guanine-binding protein-coupled receptors (GPCRs) has been previously reported in neoplastic prostate growth [48]. Prostate cancer cells displayed higher expression of these receptors than normal cells [48]. In vitro stimulation of GPCRs induced mitogenic signaling, and as a result of this, prostate cancer cell growth was observed, suggesting that the GPCR system could be activated. This mitogenic signal is transduced by G-proteins; Gα-GTP or Gβγ subunits, which could promote mitogenic signals via the activation of multiple effectors as described in the above paragraphs [48].

Various experiments using LNCaP cells with the AR-response element regulated luciferase reporters, and a DHT-activated Ga-subunit has been used to elucidate the role of androgens in the activation of G proteins.

The results of these studies have shown that DHT activates Gαq and Gas subunits from G proteins. This activation increased AR activity by 4 times (Gαq) to 10 times (Gas) [48]. This indicates that androgen stimulation of GPCR signaling in LNCaP increases the expression of Gas, and therefore the expression of AR. Stimulation of LNCaP cells with isoproterenol (a β-adrenergic agonist) increased the intracellular concentration of cAMP, while cAMP depletion using cAMP-degrading enzyme (phosphodiesterase) completely suppressed AR activation. These data taken together indicate that there could be crosstalk between Gas and AR in LNCaP cells. A simplified summary of this crosstalk is shown in Fig. (3).

RECENT ADVANCES IN NEW STEROIDAL AND NON-STEROIDAL INHIBITORS FOR THE TREATMENT OF ANDROGEN-DEPENDENT DISEASES

Steroidal Inhibitors of the 5α-Reductase Enzyme

4-Aza Steroids

Recent research suggests that the 4-azasteroidal derivatives are commonly used as 5α-R inhibitors (Fig. 4). These molecules were proposed to imitate the biosynthetic pathway in the reduction of T to DHT (Fig. 1) [49-54]. On the basis of this mechanism, a carbon atom (C-4) was replaced with a nitrogen atom, thus facilitating the bond between the ligand and the active site of the enzyme. In addition, it was observed that the incorporation of a lipophilic group at C-17 improves the biological activity. In view of the fact that these steroids form a strong bond with the enzyme, they can act as more efficient competitive inhibitors [50-54]. Considering that these structural modifications could increase inhibitory activity, several derivatives were prepared in order to find more specific 5α-R inhibitors.

17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA 2, Fig. 4) is a dual inhibitor of human 5α-R1 and 5α-R2, having IC50 values of 1.9 nM and 1.7 nM, respectively. Unfortunately, due to its hepatotoxicity and poor 3β-HSD selectivity, further studies of this azasteroid were discontinued [50-52]. In
Fig. (3). Nongenomic (rapid) mechanism of action of testosterone receptor (TR) complex. Nongenomic signaling requires a membrane androgen receptor that stimulates a cascade which induces CREB, (also called CBP) phosphorylation and stimulation of coactivators involved in the genomic mechanism.

Fig. (4). 4-aza steroids as type 1 and 2 5α-R inhibitors.

the last fifteen years, Merck Inc. synthesized several new azasteroidal derivatives; however, only N-(1,1-dimetiletil)-3-oxo- 4-aza-5α-androst-1-en-17β-carboxamida (finasteride 1, Fig. 4) was selected for the treatment of androgen-dependent diseases [52]. This compound is a better 5α-R2 than 5α- R1 inhibitor, having IC50 values of 9.4 and 410 nM, respectively [1, 52]. Finasteride is already marketed as Proscar for the treatment of BPH. At a clinical dose of 5 mg daily, this steroid decreases DHT plasma levels by 65–80%; nevertheless, it has been observed that DHT levels remain troublesome due to 5αR-1 action [36, 53].

17β-N-(2,5-bis(trifluoromethyl) phenyl-carbamoyl-4-aza-5α-androst-1-en-3-one (Dutasteride 3, Avodart, Fig. 4) is a dual inhibitor of the 5α-R en-
zyme (types 1 and 2) of the 4-azasteroid group that has been approved for the treatment of BPH [54-60]. This derivative is considered to be a competitive inhibitor of 5α-R1 and 5α-R2 enzymes; clinical reports indicated that DHT levels decreased by 90% after one year of oral treatment with dutasteride [55, 56]. Dutasteride and finasteride exhibit the same biological mechanism for inhibition of the 5α-R enzyme. Moreover, dutasteride does not bind to the androgen receptor. As a result of this inhibitory activity, this steroid reduces the size of the enlarged prostate gland and improves urinary flow rate [55-60].

Several studies have shown that compound 3 (Fig. 4) is well tolerated with daily use for up to 2 years [56, 59]. In studies, it did not significantly impact bone metabolism markers, bone mineral density, or lipid levels. Moreover, dutasteride at a low concentration was able to promote cell death in a LNCaP cancer cell line, thus expressing only an excess of 5α-R1 [56]. The up-regulation of 5α-R1 enhances the cellular response to low concentrations of T [57].

1-(4-methyl-3-oxo-4-aza-5α-androstane-17β-carbonyl)-1,3-diisopropylurea (Turosteride 5, Fig. 4) is an analog of 4-MA that appears to be a specific inhibitor of 5α-R2 because it does not inhibit 5α-R1 activity (Fig. 4). Another advantage of turosteride is that it shows no binding to the AR [61-63]. In view of these results; Di Salle et al. synthesized a series of 17-acylurea-substituents of 4-azasteroids to improve on this activity (Fig. 4). These authors observed that derivatives containing an N-4 methyl group, a saturated A ring, and an acyl urea moiety at C-17 increased the inhibitory activity of 5α-R2 (see compound 6, Fig. 5). Steroid 6 showed a higher inhibitory activity (IC50=40 nM) of this enzyme than turosteride (IC50=154 nM); however, more studies are necessary to determine whether this steroidal derivative could be used for the treatment of androgen-dependent afflictions [62, 63].

In view of the fact that 17β-carboxamide derivatives enhanced 5α-R1 inhibitory activity, steroids 7, 8 and 9, which belong to this group, were synthesized (Fig. 5). These compounds showed high 5α-R1 inhibitory activity (IC50= 23.9, 0.9 and 11.5 nM, respectively) [64]. Theoretical studies indicated that the enzyme 5α-R1 showed high recognition of compounds having a 4-5 carbon chain linked to the N atom. Although compounds 7, 1 and 9 exhibited considerable activity for 5α-R1, their poor selectivity for 5α-R2 (IC50= > 100nM) prevented them from being used in the treatment of BPH [62-65].

### 6-Azasteroids

Glaxo was the pioneer for the development of several 6-azasteroidal derivatives (Fig. 5) with 5α-R dual inhibitory activity, and analyzed the molecular mechanism. This mechanism explains the function of these compounds as a substrate for the inhibitory process very well; however, they showed a slow irreversible inhibition as exhibited by 4-azasteroids. Out of this group, only compound 10 (Fig. 5) has the same efficacy as finasteride (Fig. 4) in a castrated rat prostate growth model [66]. On the basis of the fact that lipophilic groups attached to C-17 improve selectivity for 5α-R1, a variety of C-17 amide-substituted 6-azasteroids were developed [66, 67]. Several compounds showed high activity against both isozymes. Since Proscar (finasteride) [52-53] and later Avodart (dutasteride) [56, 57] were very successful for the treatment of BPH, the 6-aza steroids could not compete and remained a laboratory curiosity [65-66].

### Phytopharmaceuticals

It has been known for a long time that drugs obtained from plants have been used successfully for the treatment of a variety of diseases. At the present time, a considerable number of Americans use herbal therapies combined with conventional treatments for BPH. Reviews of saw palmetto berry and Pygeum africanum have all concluded that analysis of the effectiveness of these preparations is limited by the short duration of the trials. Nevertheless, it seems likely that these agents do improve symptoms and peak urinary flow. Long-term trials of the effects of these phytopharmaceuticals as BPH treatments are still needed [70-78].

### Saw Palmetto Berry

Recently, the saw palmetto berry (Serenoa repens) has gained wide acceptance for the treatment of BPH. This berry contains β-sitosterol, which inhibits the binding of DHT to the AR. Unfortunately treatment of BPH with this herb does not decrease prostate-specific antigen (PSA) level or prostate volume. On the other hand, it helps decrease the prostate epithelium, which is related to low levels of DHT in the prostate. Clinical evaluation of this plant showed that the extract could reduce DHT concentration; however, it does not have any effect on PSA level [70-78].
β-Sitosterol improves the symptoms of BPH assessed by both the Boyarski system and the International Prostate System Score (IPSS). This compound is associated with an improvement in peak urine flow rate and residual urinary volume; it also causes a considerable reduction in the size of the prostate gland [79-81].

To determine the effect of β-sitosterol on 5α-R activity in the prostate, a study was carried out using this compound. The results showed that β-sitosterol inhibits 5α-R2 at a very high concentration (2.7 mM), but decreases the weight of the prostate in gonadectomized hamsters treated with T. Thus, the pharmacological effect produced by β-sitosterol is a consequence of its binding to the AR [82].

**Ganoderma Lucidum**

It has been shown that the ethanolic extract of the mushroom known as *Ganoderma lucidum* inhibits the activity of the enzyme 5α-R1. This extract contains triterpenoid compounds with a carbonyl group on C-3 and a C-26-α,β-unsaturated carbonyl group, which was characteristic of almost all inhibitors isolated [83].

*Ganoderma lucidum* is also used as blood pressure stabilizer, antioxidant, analgesic, diuretic and nerve tonic. It is also used as an antitumor agent for prostate cancer, producing apoptosis of cancer cells [83].

**Pygeum Africanum**

Extracts of *Pygeum africanum* (Tadenan) are currently used in patients with benign prostatic hyperplasia (BPH) and prostate cancer. This extract contains terpenoids, sterols, and some alcohols, in addition to N-butylbenzenesulfonamide and atraric acid (Fig. 17) which have been identified as the active principles of the extract. These compounds are antagonists of the AR, since they inhibit its translocation to the nucleus of human prostate cells [84-85]. The N-butylbenzenesulfamide also binds to the progesterone receptor, but not to glucocorticoid or to estrogen receptors [84]. Atraric acid is also capable of binding to the AR which is expressed in prostate cancer cell lines. Clinical studies show that this plant eliminates some of the symptoms of BPH in addition to reducing blood prostate antigen levels [85].

**Azulene Derivatives**

Kotobuki Seiyaku evaluated a series of novel azulene derivatives. The most potent inhibitor was compound 11 (Fig. 5), with an IC50 value of 2.8 nM for 5α-R2 [86].

**Advances in Drug Discovery for 5α-R Inhibitors**

In previous studies, our research group found a new mechanism based on the structure–activity relationship for inactivation of the enzyme 5α-R. The first step in this mechanism involves the formation of an enzyme-steroid complex. Subsequently, the enzyme with its nucleophilic part (cysteine SH, lysine NH2 and serine OH) is added to the double bond of the steroid in a 1-4 addition, forming an irreversible adduct. As a result of this, the activity of the enzyme 5α-R is inhibited and as a consequence, the DHT level is reduced (Fig. 6). On the basis of this mechanism, we have synthesized and evaluated several compounds with an electrophilic site that reacts with the nucleophilic part of the enzyme. The purpose of this study was to develop molecules more active than finasteride with fewer of the side effects associated with commercially available drugs. We found that several new pregnenolone, dehydroepiandrosterone and progesterone derivatives with an electrophilic center can interact more efficiently with the 5α-R enzyme [87].

**Pregnenolone Derivatives**

Compounds 12, 14 and 15 (Fig. 7) showed lower IC50 values than that observed for finasteride; IC50 9.4 nM (5α-R2). These results suggested that compounds having a fluorine atom in ortho and meta position in the phenyl group of the ester moiety (14 and 15) as well as an expanded 6-member D-ring improve the activity of the steroidal derivatives (Fig. 7). In vivo experiments showed that compound 14 and finasteride significantly decreased prostate weight. These results indicate that the in vivo activity of compound 14 depends on its pharmacokinetic properties [87-91]. Esterases present in the bloodstream could hydrolyze C-17 ester function more easily in 14 than in 12 or 13 (Fig. 7) due its spatial configuration. Consequently, this hydrolyzed compound could be (14) active in vivo.

In another study, several 6-oxo pregnane derivatives were evaluated as inhibitors of the 5α-R enzyme (Fig. 8). These results showed that compounds 16-20 and 22 are much better 5α-R inhibitors than finasteride; compound 21 exhibited an IC50 value comparable to that of the commercial drug (Fig. 8). The two electrophilic centers in the molecule (compound 16 has an epoxy ring as the electrophilic site) explains the higher activity of these steroidal derivatives (16-22) compared to that of finasteride [87-89].
Fig. (5). Some new active type 1 and 2 5α-R inhibitors.

Fig. (6). Mechanism proposed for the inhibition of 5α-R enzyme.

Fig. (7). 4,6-pregnadiene derivatives containing an ester function at C-17 as inhibitors of 5α-R2.
Progesterone Derivatives

Based on the proposed mechanism for inhibition of the 5α-R enzyme, we synthesized several new progesterone derivatives; 24, 26, 33 and 35 (Fig. 9). These compounds have a conjugated carbonyl function (except steroid 35). All derivatives showed 5α-R2 inhibitory activity comparable to that of finasteride. As an example, compound 35, with an epoxy group as the electrophilic center, exhibited an IC50 value of 4.8 nM, two times better than finasteride. Apparently, the epoxy group has an enhanced electrophilic character (increased ability to react with the nucleophilic part of the amino acid) thus showing improved biological activity [89-91]. Furthermore the epoxide group might also be involved in H-bond interaction which is not possible in finasteride, which could explain its better potency.

Androsterone Derivatives

Recently, we reported the synthesis and 5α-R inhibitory activity of compounds 35-46 (Fig. 10). Steroids 35-37 are androsterone derivatives having only an ester function at C-3. Compounds 38 and 39 have two bromine atoms at C-5 and C-6 and an expanded lactone D-ring. Steroids 40-43 retain the lactone and ester moieties; however, they have a double bond at C-5. The steroidal derivatives 44-46 are based on the 5,16-androstadiene skeleton having a chlorine atom at C-17, a formyl group at C-16 and an ester moiety at C-3.

Compounds 35-43 exhibited higher 5α-R2 inhibitory activity than that of finasteride. These steroidal...
derivatives have an electrophilic carbonyl functional group which could react with the nucleophilic part of the amino acid; this phenomenon could explain their biological activity [94]. The lack of 5α-R2 inhibitory activity of 44-46 could be attributed to the low solubility of these Vilsmeier reaction products [97].

Based on the results of our studies, in this paper we propose several structural modifications in the steroidal
scaffold in order to enhance 5α-R inhibitory activity: [92 87-97]

(a) An α,β-unsaturated carbonyl function in ring A or a 4-ene-3,6-dione moiety enhances 5α-R inhibitory activity.

(b) The presence of an ester moiety at C-3 or C-17 improves inhibitory activity on 5α-R.

(c) An electrophilic epoxy or a lactone function on the steroidal skeleton increases inhibitory activity on 5α-R (both types).

Although some of the newly synthesized derivatives have an activity similar to that of 4-aza steroids, at the present time, only finasteride and dutasteride are used for treating androgen-dependent afflictions. Since the 4-aza steroids are associated with several troublesome side effects because of the presence of two nitrogen atoms at C-4 and C-20 positions, this prompted us to consider synthesizing several new similar steroidal derivatives without the nitrogen atoms.

STEROIDAL AND NON-STEROIDAL INHIBITORS OF THE 17β-HSD

Since it has been proposed that the dual 5α-R and 17β-HSD inhibition (Fig. 2) could slow down the progression or even cause regression of early prostate cancer, this has inspired us to develop several different molecules as inhibitors of 17β-HSD [92-94]. Following this idea, several steroidal and non-steroidal molecules have been prepared. Recently, it has been found that the non-steroidal molecules 47 and 48 (Fig. 11) are selective HSD3 inhibitors [98-99]. These molecules decreased plasma T levels and inhibited the in vivo growth of an androgen-stimulated LNCaPwt (HSD3) xenograft in castrated mice. This fact indicated that HSD3 inhibitors may have applications in the treatment of prostate cancer [99-100].

Other non-steroidal molecules whose structures are based on oxazolidinone and thiazolidinone skeletons (Fig. 12) have been identified as potent inhibitors of type 3 17β-HSD (17β-HSD3) [98]. These compounds exhibited a promising activity profile and demonstrated significant selectivity for HSD3 over the related 17β-HSD isoenzymes and nuclear receptors [98].

Fig. (11) also shows a benzimidazole derivative 49 (Fig. 11), which shows high HSD3 selectivity and biological potency (IC50 40 nM). In addition, (Fig. 13) displays several steroidal derivatives (54, 55, Fig. 13) that inhibit HSD3 and HSD5 [92, 97-100].

The type 5 17β-HSD enzyme (17β-HSD5) is well characterized in human prostate tissues, and also contributes to local androgen formation [98]. During the conversion of 4-dione to T (Fig. 2), conformational changes of 17β−HSD5 have been observed by X-ray analysis [99]. Several reports have indicated that the inhibition of 17β-HSD5 could activate the peroxisome proliferator-activator, which is a nuclear receptor that functions as a transcriptional factor for encoding gene expression. As a consequence, cell differentiation and apoptosis of various different cell types and prostate cancers have been observed [99-100].

The gene that encodes 17β-HSD5 has been identified in bands 10 p15 and p14 of human chromosome 13 from prostate cells and its mRNA is over-expressed in prostate cancer tissue (77%). Evidence indicates that the activity of 17β-HSD5 is related to the elevated concentration of intraprostatic T observed in prostate cancer [23]. Studies carried out with the prostate cancer cell line LNCaP showed both activities for 17β-HSD5 in this cell line; reductive activity increased and oxidative action decreased [99-104].

As shown in Fig. (13), several different steroidal lactones exhibited 17β-HSD3 inhibitory activity. However, the most potent lactone (57) is an inhibitor of 17β-HSD5; this has an IC50 value of 2.9 nM in HEK293 cells, which over-express human 17β-HSD5 enzyme; this lactone (57) was identified by Schuster et al. in 2007 [100-111].

A third class of non-steroidal 17β-HSD5 inhibitors (sulfonylindoles) has recently been reported (Fig. 11). Compound 50 displayed the highest activity, with an IC50 value of 69 nM, and is orally active [100].
Recently, we synthesized new potent dehydroepiandrosterone derivatives (58-60, Fig. 13) that showed 17β-HSD5 inhibition. The in vitro effect of these steroidal compounds was evaluated in human prostate membrane fractions. The IC₅₀ values of these steroids indicated that the substituent at C-3 determines the activity of these compounds. Furthermore, the capacity of compounds 58-60 to form hydrogen bonds and hydrophobic interactions with the target molecules seems to be important. This fact could probably be applied to the steroidal lactones described in Fig. (13). Qiu et al. demonstrated that the binding pocket of 17β-HSD5 enzyme is lined with hydrophobic residues Leu-54, Trp-86, Trp-227, Phe-306, and Phe-311, having a cavity where the compound (the ligand) with the appropriate conformation can enter and form a stable complex [111].

**Inhibitors of CYP17 (17α-Hydroxylase/17,20 Lyase)**

It is well known that 90% of prostate cancer is androgen-dependent; therefore treatments based on reducing the concentration of androgen could be applied to inhibit cancer cell proliferation [111]. In order to block the conversion of pregnenolone to dehydroepiandrosterone (DHEA) and androstenedione (Fig. 2, 14) in gonadal and adrenal glands, the CYP17 enzyme could be a suitable target [111-114].

In addition to the mutations described above, the AR occasionally shows specific mutations in androgen-resistant cancer cell lines. This mutated AR can be activated by glucocorticoids, especially cortisol in cancer patients suffering an overproduction of glucocorticoids [111]. Therefore inhibition of CYP11B1 (Fig. 14) could be a possible therapeutic target for these individuals [111].

The antifungal ketoconazole (Fig. 15) has been used clinically for prostate cancer therapy as a CYP17 inhibitor (Fig. 2). This compound showed an IC₅₀ value of 1100 nM for the inhibition of CYP17. Ketoconazole is a non-specific CYP17 inhibitor, and has exhibited high liver toxicity, thus causing several side effects; nevertheless, in some cases, it is still being used for the treatment of refractory prostate cancer [113].

In April 2011, (3β)-17-(pyridin-3-yl)androsta-5,16-dien-3-ol (abiraterone, Fig. 15) was approved by the U.S. Food and Drug Administration (FDA) for the treatment of refractory prostate cancer [114]. This drug is a selective CYP (CYP17A) inhibitor (Figs. 2 and 14) that prolongs patient survival and seems to improve quality of life. In view of its poor bioavailability, abiraterone is commercially available as the prodrug abiraterone acetate (Zytiga, Fig. 15) which is rapidly deacetylated in the body to its active metabolite, abiraterone [115].

Abiraterone exhibits minimal side effects due to the fact that this compound is a specific CYP (CYP17A1) inhibitor; however, it can cause hypertension triggered by a corticosterone increase (Fig. 14) [114, 116]. Recently, it has been demonstrated that abiraterone given in large amounts tends to block glucocorticoid synthesis by inhibiting the 3β-HSD enzyme (Fig. 14), thus producing several side effects; for this reason, it must be administered with prednisone (1000 mg abiraterone and 10 mg of prednisone daily) [116].

On the basis of these results, the principal aim of some research groups has been to develop CYP17 lyase instead of hydroxylase inhibitors. Fig. (15) shows different CYP17 inhibitors, such as 17-(1H-benzimidazol-1-yl)androsta-5,16-dien-3β-ol (galetterone, Fig. 15) with an IC₅₀ value of 300 nM for CYP17 lyase. This compound exhibited higher CYP17 inhibitory activity than abiraterone (IC₅₀= 800 nM). Moreover, the data reported in the Vasaitis et al. review indicated that galetterone prevents the formation of LAPC-4 tumors [3]. In view of the fact that this compound is also an androgen receptor blocker, its therapeutic value for the treatment of prostate cancer increases in the presence of a mutated AR [113-115].

The non steroidal compound 6-(7-Hydroxy-6,7-dihydro-5H-pyrrolo[1,2-c]imidazol-7-yl)-N-methyl-naphthalene-2-carboxamide (TAK-700, Orteronel, Fig. 15) is a selective inhibitor for CYP17 (Figs. 2 and 14). This compound was capable of reducing the weight of prostate and seminal vesicles in rats, and it also decreased serum T levels in castrated monkeys treated with an oral dose of 1 mg/kg after 8 h [115, 117].

Fig. (15) also shows VN/85-1, an inhibitor of CYP17 having an IC₅₀ value of 50 nM, thus indicating a 6-fold higher level of activity than galetterone (Fig. 15) [115-117].

**Androgen Receptor Antagonists Non Steroidal Antagonists**

AR could be another important target for the design of new antagonists; there are several reports in the literature describing a new class of non-steroidal antagonists [83, 84, 115-123]. Compound 61 (IC₅₀=16 nM) is a synthetic thiohydantoin derivative which binds with higher specificity and greater affinity to the AR than compound 62 (IC₅₀= 160 nM) (Fig. 16). The latter an-
Fig. (12). Non-steroidal thiozolidinediones (51) and oxazolidinediones (52, 53); potent 17β-HSD3 inhibitors [95-96].

Fig. (13). Steroidal inhibitors of type 3 and 5 17β-HSD.

Fig. (14). Biosynthesis of steroids from cholesterol in the adrenal glands cortex.
Fig. (15). Structure and IC50 values of steroidal and non-steroidal CYP17A1 inhibitors.

tagagonist was approved by the FDA for clinical use as antiandrogen [110]. Compound 61 inhibits the growth and androgen-mediated gene transcription in AR-overexpressing prostate cancer cell lines and castration-resistant LNCaP/AR (Fig. 16) [116].

The non-steroidal compounds 64 and 66 (Fig. 16) are both thiohydantoin derivatives similar to the antiandrogen 61 (nilutamide) [116-118]. These derivatives did not show agonism to the AR or any interrelation with the FXXLF peptide sequence of co-activators. Apparently, 64 (IC50= 30.9 nM) is a more potent antagonist than 62 for the AR; this non-steroidal compound showed in vivo anti-tumor effects in prostate cancer xenografts. This biological activity was also observed for compound 66 (Enzalutamide or MV 3100 IC50= 21.4 nM) (Fig. 16) in tumors that express high AR as well as androgen-resistant levels [117-118]. Preclinical studies have demonstrated that 64 is an orally active and very potent antagonist of AR. These preclinical research protocols have also demonstrated the efficacy of 64 in preventing the growth of prostate cancer xenografts in mice. This antiandrogen is distributed by Orion Pharmaceuticals [117, 120].

Studies carried out using 66 with 140 patients suffering from metastatic cancer demonstrated that a dose of 30–600 mg/day induced an anti-tumor effect and a decrease in PSA level. However, three patients receiving 360, 480 and 600 mg daily showed seizures as a principal side effect; as a result of this study, the dose currently used is 240 mg [118]. The pharmaceutical company Medivation developed derivative 66, which is currently distributed by Orion Pharmaceuticals for the treatment of metastatic castration-resistant prostate cancer; this drug was approved by the FDA in August 2012 [118].

Clinical studies using 61 have shown that this compound is safe, well tolerated and displays dose-proportional pharmacokinetics. The compound showed antitumor activity across all dosage levels tested. Based on both preclinical and clinical data, the maximum effective dose of this compound is 240 mg daily and it has been selected for phase II evaluation [116, 119].

Transactivation assays showed that the imide derivative 63 (BMS-641988, IC50= 56 nM, Fig. 16) is a more potent antagonist for the AR than 62 (IC50= 160 nM) [120]. This compound shows 20-fold higher binding affinity to the AR and 3 to 7-fold higher antagonistic activity than bicalutamide (62). Compound 63 also exhibited an in vivo effect and decreased prostate tumor xenografts as well as demonstrating higher potency than 62 [120]. On the basis of these preclinical results, a phase I study was undertaken; it was carried out with 61 metastatic cancer patients at doses of 5 and 100 mg daily [121]. The results from this test were that in one patient only was tumor reduction observed; however, in all patients the PSA level decreased 16–30%. The low anti-tumor activity of compound 63 and the occurrence of seizures prompted a discontinuation of this study [120-121].

In order to determine the side effects of the antiandrogens displayed in Figs. (15 and 16), several preclinical studies have been carried out. These results demonstrated antiandrogenic activity for compounds 63 and 65 (BMS 779333 IC50=148 nM) and an-
agonistic effect on GABA receptors [120]. The epileptic seizures exhibited in the in vivo experiments could be explained by the high concentrations of these non-steroidal antagonists in the brain [120-121]. Compound 67 has also been identified as an antiandrogen since it demonstrated antitumor activity in LNCaP-BC2 and VCaP-CRPC xenograft models, thus suggesting that 67 could be a suitable candidate for CRPC treatment [122].

Steroidal Antagonists

In 2012 Gauthier et al. reported the synthesis and biological effect of two estrogen derivatives, 68 and 69, both with electronegative groups at C-3 and C-4 (Fig. 17) [123]. Compound 68 (RBA= 0.66%) was observed to exhibit a higher binding affinity for human AR (3.7-fold) than bicalutamide (62, RBA= 0.18%, Fig. 16). Moreover, derivative 69 (RBA= 17%) showed a much higher binding affinity for human AR (94-fold) than 62 (Figs. 16 and 17). Furthermore in the cancer cell line LNCaP, compounds 68 and 69 (66%) were 16 times more potent for the inhibition of androgen-stimulated secretion of PSA than 62 (6%). However, the thiohydantoin derivative 66 (Fig. 16) showed the same potency in this in vitro test as bicalutamide (62). Compound 68 (36%) showed 6-fold higher antagonistic potency to human AR than 62 [123].

Furthermore, in vivo experiments in rats demonstrated that compound 66 exhibited lower activity for decreasing the growth of the ventral prostate than derivative 62 [124].

Studies using the androgen-sensitive Shionogi carcinoma cell model showed that 68 (Ki= 2 nM) and 69 (Ki= 0.77 nM) have 40-fold and 105-fold greater potencies respectively for cell proliferation inhibition than 62 (Ki= 81 nM) [123].

Fig. (16). Novel non-steroidal AR antagonists [114-115].

Natural compounds

Fig. (17) also shows the novel antagonist 70 for the AR; this androstane derivative exhibited potent antian drogenic activity for wild and mutated AR present in LNCaP and 22Rv1 cancer cell lines compared to that of bicalutamide (62). Furthermore, this steroid has a higher affinity for the AR than 62 [124].

We found that steroids 71-73 (Fig. 17) showed a very low binding affinity for the AR; nevertheless, in the in vivo experiments they decreased the weight of prostate and seminal vesicles of gonadectomized and T-treated hamsters, suggesting that these compounds could function as prodrugs [93].
Investigating other aspects and knowing that the 17β-hydroxy group is the key to DHT interaction with the amino acid residues of both wild and mutated AR, the group of Fousteris in 2010 [125] developed and identified two aminosteroids with a lactam moiety (Fig. 17). Both molecules showed efficacy in antagonizing the activity of both wild and mutant AR (T877A).

Inhibitors of Protein-Kinase A (PKA)

Since PKA activation has been related to enhanced gene transcription, inhibition of this enzyme could be a target for improving metastatic prostate cancer treatment [38, 45, 120-121].

The role of a specific p21-activated kinase 4 (PAK4) in prostate cancer progression has been described. As we mentioned above, several studies have showed that PAK4 signaling increases the transcriptional activity of CREB (Fig. 3) [46]. When the activity of PAK4 enzyme was inhibited by compound 77 (Fig. 19), which is a specific inhibitor of the activity of PKA in PC-3 and DU145 cells, no tumor formation was observed in nude mice [47]. Reduction of this tumor was related to a decrease in the expression of CREB protein. Therefore, PAK4 could modulate the progression of hormone-independent prostate cancer that does not respond to chemotherapy treatment, suggesting a new signaling pathway linked to the AR [47, 124].

An enhanced antiproliferative effect was observed on LNCaP, DU145 and PC3 cell lines treated with PD153035, which is a tyrosine kinase inhibitor, a specific and potent antagonist for the epidermal growth factor receptor (EGFR); (Fig. 3) [127]. The same activity is also exhibited by compound 75 (Fig. 18), which is a specific inhibitor for PKA activity [128]. These facts indicate that a positive crosstalk relationship between EGFR and PKA intracellular pathways could exist (Fig. 3). This phenomenon can be demonstrated using 75 (Fig. 18) and PD153035 for the inhibition of EGRF and PKA signaling pathways. When both signaling cascades were synergistically blocked, an apoptotic effect was observed in these cell lines [127].

Some compounds (74, 76 and 77, Fig. 17) have been shown to be inhibitors of the activity of types I and II PKA isozymes. These derivatives could reduce the signaling pathway induced by cAMP in wild cells in certain cellular processes such as gene expression, shape changes, apoptosis, DNA replication, and protein phosphorylation (Fig. 3). The activity of 74, 76 and 77 depends on their capacity to stabilize the holoenzyme and to dissociate the cAMP molecule from the complex formed by types I or II cAMP-PKA [126].

Compounds 74 and 76 (Fig. 18) were the most potent blockers for cAMP function in cells that express PAK type I. In experiments carried out using cells that express this enzyme, compounds 74 and 76 can com-
pate with the cAMP molecule to predominantly inhibit PKA type 1 activity [126].

Fig. (18). Inhibitors of the activity of protein kinase A (PKA).

CONCLUSION

Recent advances in the identification, characterization, and isolation of new targets enable us to ascertain the molecular bases for the study of prostate cancer and BPH. This understanding can help us propose and design new steroidal and non-steroidal inhibitors. In the future, we anticipate that the bases described in this paper could stimulate the synthesis of new molecules with higher inhibitory activity for a specific enzyme or receptor. In view of the fact that the drugs currently used show a variety of side effects, it is believed that the guidelines described in this review could contribute to the design and synthesis of new drugs for the treatment of prostate cancer and BPH with fewer side effects.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Russell, D.W.; Wilson, J.D. Steroid 5-alpha-reductase: two genes/two enzymes. Ann. Rev. Biochem., 1994, 63(1), 25-61.

[2] Godoy, A.; Kawinski, E.; Li, Y.; Oka, D.; Alexiev, B.; Az zoumi, F.; Titus, M.A.; Mohler, J.L. 5a-Reductase type 3 expression in human benign and malignant tissues: A com- parative analysis during prostate cancer progression. Prostate, 2011, 71(10), 1033-1046.

[3] Liang, T.E.H.M.; Masciari, M.A.; Cheung, A.H.; Reynolds, G.F.; Rasmusson, G.H. Species differences in prostatic steroid 5α-reductases of rat, dog, and human. Endocrinology, 1985, 117(2), 571-579.

[4] Pelletier, G. Expression of steroidogenic enzymes and sex-steroid receptors in human prostate. Best Pract. Res. Clin. Endocrinol. Metab., 2008, 22(2), 223-228.

[5] Uemura, M.; Tamura, K.; Chung, S.; Homma, S.; Okuyama, A.; Nakamura, Y.; Nakagawa, H. Novel α-steroid reductase (SRD5A3, type-3) is overexpressed in hormone-refractory prostate cancer. Cancer Sci., 2008, 99(1), 81-86.

[6] Dadras, S. S.; Cai, X.; Abasolo, I.; Wang, Z. Inhibition of 5a-reductase in rat prostate reveals differential regulation of androgen-response gene expression by testosterone and dihydrotestosterone. Gene Expr., 2001, 9(4-5), 183-194.

[7] Imperato-McGinley, J.; Zhu, Y.S. Androgens and male physiology the syndrome of 5α-Reductase-2 deficiency. Mol. Cell Endocrinol., 2002, 198(1-2), 51-59.

[8] Walsh, P.C.; Madden, J.D.; Harrod, M.J.; Goldstein, J.L.; MacDonald, P.C.; Wilson, J.D. Familial incomplete male pseudohermaphroditism, type 2. N. Eng. J. Med., 1974, 291(18), 944-949.

[9] Raynaud, J.-P. Prostate cancer risk in testosterone-treated men. J. Steroid Biochem. Mol. Biol., 2006, 102(1-5), 261-266.

[10] Wright, A.S.; Douglas, R.C.; Thomas, L.N.; Lazier, C.B.; Rittmaster, R.S. Androgen-induced regrowth in the castrated rat ventral prostate: role of 5α-reductase. Endocrinology, 1999, 140(10), 4509-4515.

[11] Bratoeff, E.; Sánchez, A.; Arelano, Y.; Heuze, Y.; Soriano, J.; Cabeza, M. In vivo and in vitro effect of androstene derivatives as 5α-Reductase type 1 enzyme inhibitors. J. Enzyme Inhib. Med. Chem., 2013, 28(6), 1247-1254.

[12] Bosland, M.C. The role of steroid hormones in prostate carcinogenesis. J. Natl. Cancer Inst. Monogr., 2000, 2000(27), 39-66.

[13] Bull, H.G.; Garcia-Calvo, M.; Andersson, S.; Baginsky, W.F.; Chan, H.K.; Ellsworth, D.E.; Miller, R.R.; Stearns, R.A.; Bakshi, R.K.; Rasmusson, G.H.; Tolman, R.L.; Myers, R.W.; Kozarich, J.W.; Harris, G.S. Mechanism-based inhibition of human steroid 5α-reductase by finasteride-enzyme-catalyzed formation of nadph-dihydrofinasteride, a potent bisubstrate analog inhibitor. J. Am. Chem. Soc., 1996, 118(10), 2359-2365.

[14] Weissner, H.; Krieg, M. Kinetic analysis of androstenedione 5α-Reductase in epithelium and stroma of human prostate. Steroids, 1997, 62(8-9), 589-594.

[15] Lee, H. J.; Chang, C. Recent advances in androgen receptor action. Cell. Mol. Life Sci., 2003, 60(8), 1613-1622.

[16] Luu-The, V.; Belanger, A.; Labrie, F. Androgen biosynthetic pathways in the human prostate. Endocrinol. Prostate., 2008, 22(7), 207-221.

[17] Thomas, L.N.; Lazier, C.B.; Gupta, R.; Norman, R.W.; Troyer, D.A.; O'Brien, S.P.; Rittmaster, R.S. Differential alterations in 5α-reductase type 1 and type 2 levels during development and progression of prostate cancer. Prostate, 2005, 63(3), 231-239.

[18] Stanczyk, F.Z.; Azen, C.G.; Pike, M.C. Effect of finasteride on serum levels of androstenedione, testosterone and their 5α-reduced metabolites in men at risk for prostate cancer. J. Steroid Biochem. Mol. Biol., 2013, 138, 10-16.

[19] Clark, R.V.; Hermann, D.J.; Cunningham, G.R.; Wilson, T.H.; Morrill, B.B.; Hobbs, S. Marked suppression of dihydrotestosterone in men with benign prostatic hyperplasia by...
dutasteride, a dual 5α-reductase inhibitor. *J. Clin. Endocrinol. Metab.*, 2004, 89(5), 2179-2184.

[20] Geissler, W.M.; Davis, D.L.; Wu, L.; Bradshaw, K.D.; Patel, S.; Mendonca, B.B.; Elliston, K.O.; Wilson, J.D.; Russell, D.W.; Andersson, S. Male pseudohermaphroditism caused by mutations of testicular 17[beta]-hydroxysteroid dehydrogenase 3. *Nat. Genet.*, 1994, 7(1), 34-39.

[21] Pelletier, G.; Luu-The, V.; Tétu, B.; Labrie, F. Immunocytochemical localization of type 5 17β-hydroxysteroid dehydrogenase in human reproductive tissues. *J. Histochem. Cytochem.*, 1999, 47(6), 731-737.

[22] Penning, T.M.; Burczynski, M.E.; Jez, J.M.; Hung, C.F.; Lin, H.K.; Ma, H.; Moore, M.; Palackal, N.; Ratnam, K. Human 3α-hydroxysteroid dehydrogenase isosforms (AKR1C1-AKR1C4) of thealdo-keto reductase superfamily: Functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *Biochim. Biophys. Acta*, 2000, 1511(1), 67-77.

[23] Dufort, I.; Rheault, P.; Huang, X.-F.; Soucy, P.; Luu-The, V. Characteristics of a highly labile human type 5 17β-hydroxysteroid dehydrogenase. *Endocrinology*, 1999, 140(2), 568-574.

[24] Luu-The, V.; Tremblay, P.; Labrie, F. Characterization of type 12 17β-hydroxysteroid dehydrogenase, an isofrom of type 3 17β-hydroxysteroid dehydrogenase responsible for estradiol formation in women. *Mol. Endocrinol.*, 2006, 20(2), 437-443.

[25] Peltohieto, H.; Isomaa, V.; Mänttäusta, O.; Vihko, R. Complete amino acid sequence of human placental 17β-hydroxysteroid dehydrogenase deduced from cDNA. *FEBS Lett.*, 1998, 439(1), 73-77.

[26] Li, K.X.Z.; Smith, R.E.; Krokowski, Z.S. Cloning and expression of a novel tissue specific 17β-hydroxysteroid dehydrogenase. *Endocrinology*, 1998, 274(3), 663-667.

[27] El-Alfy, M.; Luu-The, V.; Huang, X.F.; Berger, L.; Labrie, F.; Pelletier, G. Localization of type 5 17β-hydroxysteroid dehydrogenase, 3 β-hydroxysteroid dehydrogenase, and androgen receptor in the human prostate by *in situ* hybridization and immunocytochemistry. *Endocrinology*, 1999, 140(3), 1481-1491.

[28] Thomas, L.N.; Douglas, R.C.; Rittmaster, R.S.; Too, C.K.L. Overexpression of 5α-reductase type 1 increases sensitivity of prostate cancer cells to low concentrations of testosterone. *Prostate*, 2009, 69(6), 595-602.

[29] Bonkoff, H.; Stein, U.; Aumüller, G.; Remmerber, K. Differential expression of 5α-reductase isoenzymes in the human prostate and prostatic carcinomas. *Prostate*, 1996, 29(4), 261-267.

[30] Labrie, F.; Dupont, A.; Giguerre, M.; Borsani, J.P.; Belanger, A.; Lacourciere, Y.; Emond, J.; Monfette, G. Advantages of the combination therapy in previously untreated and treated patients with advanced prostate cancer. *J. Steroid Biochem. Mol. Biol.*, 1996, 55(3B), 877-883.

[31] Hall, P.F. Cytochrome P-450 21C1ssc: One enzyme with two actions: hydroxylase and lyase. *J. Steroid Biochem. Mol. Biol.*, 1991, 40, 527-532.

[32] Handratta, V.D.; Vassatis, T.S.; Njar, V.C.O.; Gedaiya, L.K.; Kataria, R.; Chopra, P.; Newman, D.; Farquhar, R.; Guo, Z.; Qiu, Y.; Brodie, A.M.H. Novel C-17- heteroaryl steroid CYP17 inhibitors/antiandrogens: Synthesis, *in vitro* biological activity, pharmacokinetics, and antitumor activity in the LAPC4 human prostate cancer xenograft model. *J. Med. Chem.*, 2005, 48(8), 2972-2984.

[33] Evans, R. A transcriptional basis for physiology. *Nat. Med.*, 2004, 10(10), 1022-1026.

[34] Sonoda, J.; Pei, L.; Evans, R.M. Nuclear receptors: Decoding metabolic disease. *Metab. Dis.*, 2008, 58(1), 2-9.

[35] Gottlieb, B.; Beitel, L.K.; Nadarajah, A.; Paliouras, M. The androgen receptor gene mutations data base: 2012 update. *Hum. Mutat.*, 2012, 33, 887-894.

[36] Rasmusson, G.H.; Reynolds, G.F.; Ute, T.; Jobson, R.B.; Primka, R.L.; Berman, C.; Brooks, J.R. Azasteroids as inhibitors of rat prostatic 5α-reductase. *J. Med. Chem.*, 1984, 27(12), 1690-1701.

[37] Lévesque, M.H.; El-Alfy, M.; Cusan, L.; Labrie, F. Androgen receptor as a potential sign of prostate cancer metastasis. *Prostate*, 2009, 69(15), 1704-1711.

[38] Knudsen, K.E.; Scher, H.I. Starving the addiction: New opportunities for durable suppression of AR signaling in prostate cancer. *Clin. Cancer Res.*, 2009, 15(15), 4792-4798.

[39] Solakidi, S.; Psarra, A.M.G.; Nikolapopoulos, S.; Sekeris, C.E. Estrogen receptors α and β (ERα and ERβ) and androgen receptor (AR) in human sperm: localization of ERα and AR in mitochondria of the midpiece. *Hum. Reprod.*, 2005, 20(12), 3481-3487.

[40] Liao, G.; Chen, L.-Y.; Zhang, A.; Godavarthi, A.; Xia, F.; Ghosh, J.C.; Li, H.; Chen, J.D. Regulation of androgen receptor activity by the nuclear receptor corepressor SMRT. *J. Biol. Chem.*, 2003, 278(7), 5052-5061.

[41] Dotzlaw, H.; Papaiannou, M.; Moeheur, U.; Claessens, F.; Baniahmad, A. Agonist–antagonist induced coactivator and corepressor interplay on the human androgen receptor. *Mol. Cell. Endocrinol.*, 2003, 213(1), 79-85.

[42] Dotzlaw, H.; Moeheur, U.; Mink, S.; Cato, A.C.; Iniguez Lluhi, J.A.; Baniahmad, A. The amino terminus of the human AR is target for corepressor action and antagonism. *Endocrinology*, 2004, 145(6), 2751-2757.

[43] Lang, F.; Alevizopoulos, K.; Stournaras, C. Targeting membrane androgen receptors in tumors. *Expert Opin. Ther. Targets*, 2013, 17(8), 951-963.

[44] Park, M.H.; Lee, H.S.; You, S.T.; Kim, D.J.; Park, B.H.; Kang, M.J.; Heo, W.D.; Shin, E.Y.; Schwartz, M.A.; Kim, E.G. p21-Aktivated kinase 4 promotes prostate cancer progression through CREB. *Oncogene*, 2013, 32(19), 2475-2482.

[45] Kasbohm, E.A.; Guo, R.; Yowell, C.W.; Bagchi, G.; Kelly, P.; Arora, P.; Casey, P.J.; Daaka, Y. Androgen receptor activation by Gs signaling in prostate cancer cells. *J. Med. Chem.*, 2005, 280(12), 11583-11589.
Cell Targets in Prostate Cancer Therapies

McConnell, J.D. The pathophysiology of benign prostatic hyperplasia. *J. Androl.*, 1991, 12(6), 356-363.

Faller, B.; Farley, D.; Nick, H. Finasteride: A slow-binding 5α-reductase inhibitor. *Biochemistry*, 1993, 32(21), 5705-5710.

Stuart, J.D.; Lee, F.W.; Noel, D.S.; Kadwell, S.H.; Overton, L.K.; Hoffman, C.R.; Kost, T.A.; Tippin, T.K.; Yeager, R.L.; Batchelor, K.W.; Bramson, H.N. Pharmacokinetic parameters and mechanisms of inhibition of rat type 1 and 2 steroid 5α-reductases: Determinants for different in vivo activities of GH198745 and finasteride in the rat. *Biochem. Pharmacol.*, 2001, 62(7), 933-942.

Bakshi, R.K.; Rasmusson, G.H.; Patel, G.F.; Mosley, R.T.; Chang, B.; Ellsworth, K.; Harris, G.S.; Tolman, R.L. Dramatic effect of N-aryl substituents on type 1 and type 2 5α-reductase inhibitory potency. *J. Med. Chem.*, 1995, 38(3190). *J. Med. Chem.*, 1996, 39(5), 1192.

Kaplan, S.A. Re: effect of dutasteride on clinical progression of benign prostatic hyperplasia in asymptomatic men with enlarged prostate: A post hoc analysis of the REDUCE study. *J. Urol.*, 2014, 191(2), 424.

Schulman, C.; Pommerville, P.; Hörner, K.; Wachs, B. Long-term therapy with the dual 5α-reductase inhibitor dutasteride is well tolerated in men with symptomatic benign prostatic hyperplasia. *BJU Int.*, 2006, 97(1), 73-80.

Djavan, B.; Milani, S.; Fong, Y.K. Dutasteride: a novel dual inhibitor of 5α- reductase for benign prostatic hyperplasia. *Expert Opin. Pharmacother.*, 2005, 6(2), 311-317.

Brown, C.T.; Nuttall, M.C. Dutasteride: a new 5α-reductase inhibitor for men with lower urinary tract symptoms secondary to benign prostatic hyperplasia. *Int. J. Clin. Pract.*, 2003, 57(8), 705-709.

Andriole, G.L.; Kirby, R. Safety and tolerability of the dual 5α-reductase inhibitor dutasteride in the treatment of benign prostatic hyperplasia. *Eur. Urol.*, 2003, 44(1), 82-88.

Lazzer, C.B.; Thomas, L.N.; Douglas, R.C.; Vessey, J.P.; Rittmaster, R.S. Dutasteride, the dual 5α-reductase inhibitor, inhibits androgen action and promotes cell death in the LNCaP prostate cancer cell line. *Prostate*, 2004, 58(2), 130-144.

Thomas, L.N.; Douglas, R.C.; Lazzer, C.B.; Too, C.K.L.; Rittmaster, R.S.; Tindall, D.J. Type 1 and type 2 5α-reductase expression in the development and progression of prostate cancer. *Eur. Urol.*, 2008, 53(2), 244-252.

Di Salle, E.; Briatico, G.; Giudici, D.; Ormati, G.; Nesi, M.; Panzeri, A. 17β-acylurea derivatives of 4-azasteroids as inhibitors of testosterone 5α-reductase. *J. Steroid Biochem. Mol. Biol.*, 1992, 41(3-8), 765-768.

Di Salle, E.; Briatico, G.; Giudici, D.; Ormati, G.; Panzeri, A. Endocrine properties of the testosterone 5α-reductase inhibitor turosteride (FCZ 26073). *J. Steroid Biochem. Mol. Biol.*, 1994, 48(2-3), 241-248.

Lourdasamy, M.; Côté, J.; Laplante, S.; Labrie, F.; Singh, S.M. Synthesis and in vitro study of 17β-[N-ureylene-N,N-B-disubstituted]-4-methyl-4-aza-α-androstan-3-ones as selective inhibitors of type I 5α-Reductase. *Bioorg. Med. Chem.*, 1997, 5(2), 305-310.

Ahmad, M.S.; Shaﬁullah; Mushfq, M. Azasteroids from 3α;5α;Cycolcholestan-6-one and 3β; bromo-5α-cholestan-6-one. *Aust. J. Chem.*, 1971, 24(1), 213-215.

Frey, S.V.; Hafner, C.D.; Maloney, P.R.; Hiner, R.N.; Dorsey, G.F.; Hoe, R.A.; Unwalla, R.J.; Batchelor, K.W.; Bramson, H.N.; Stuart, J.D.; Schweiker, S.L.; Van Arnold, J.; Bickett, D.M.; Moss, M.L.; Tian, G.; Lee, F.W.; Tippin, T.K.; James, M.K.; Grizzle, M.K.; Long, J.E.; Croom, D.K. Structure-activity relationships for inhibition of type 1 and 2 human 5α-reductase and human adrenal 3β-hydroxy-Δ4-steroid dehydrogenase/3-keto-Δ4-steroid isomerase by 6-azaandrost-4-ene-3-ones: Optimization of the C17 substituent. *J. Med. Chem.*, 1995, 38(14), 2621-2627.

Morzycki, J.W.; Sicinski, R.R. Synthesis of 6,7-diazacholesterol derivatives. *Acta Chim. Hung.*, 1985, 120(4), 239-246.

Rao, H.S.P.; Senthilkumar, S.P. Review on the synthesis of 8-azasteroids. *Curr. Org. Chem.*, 2004, 8(15), 1521-1528.

Akers, A.; Ammermann, E.; Buschmann, E.; Götz, N.; Himmele, W.; Lorenz, G.; Pommer, E.H.; Rentzea, C.; Röhl, F.; Siegel, H.; Zipperer, B.; Sauter, H.; Zippelis, M. Chemistry and biology of novel amine fungicides: Attempts to improve the antifungal activity of fenpropimorph. *Pestic. Sci.*, 1991, 31(4), 521-538.

Carraro, J.C.; Raynald, J.P.; Koch, G.; Chisholm, G.D.; Di Silverio, F.; Teillac, P.; Da Silva, F.C.; Cauquil, J.; Chopin, D.K.; Hamdy, F.C.; Hansu, M.; Hauri, D.; Kalinteris, A.; Marencak, J.; Perier, A.; Perrin, P. Comparison of phytotherapy (Permixon®) with finasteride in the treatment of benign prostate hyperplasia: A randomized international study of 1,098 patients. *Prostate*, 1996, 29(4), 231-240.

Bales, G.T.; Christiano, A.P.; Kirsh, E.J.; Gerber, G.S. Phytotherapeutic agents in the treatment of lower urinary tract symptoms: A demographic analysis of awareness and use at the University of Chicago. *Urology*, 1999, 54(1), 86-89.

Marks, L.S.; Partin, A.W.; Epstein, J.I.; Tyler, V.E.; Simon, I.N.P.A.; Macairan, M.L.; Chan, T.L.; Dorey, F.J.; Garris, J.B.; Veltri, R.W.; Santos, P.B.; Stonebrook, K.A.; deKernion, J.B. Effects of a saw palmetto herbal blend in men with symptomatic benign prostatic hyperplasia. *J. Urol.*, 2000, 163(5), 1451-1456.

Marks, L.S.; Hess, D.L.; Dorey, F.J.; Luz Macairan, M.; Cruz Santos, P.B.; Tyler, V.E. Tissue effects of saw palmetto and finasteride: use of biopsy cores for in situ quantification of prostatic androgens. *Urology*, 2001, 57(5), 999-1005.

Wilt Tj, I.A. Saw palmetto extracts for treatment of benign prostatic hyperplasia: A systematic review. *JAMA*, 1998, 280(18), 1604-1609.

Di Silverio, F.; Monti, S.; Sciarra, A.; Varasano, P.A.; Martini, C.; Lanza, S.; D’Eramo, G.; Di Nicola, S.; Toscano, V. Effects of long-term treatment with *Serenoa repens* (Permixon®) on the concentrations and regional distribution of androgens and epidermal growth factor in benign prostatic hyperplasia. *Prostate*, 1998, 37(2), 77-83.

Wilt, T.J.; Ishani, A.; Rutks, I.; MacDonald, R. Phytotherapy for 17β-estradiol deficiency: a review of the evidence. *Public Health Nutr.*, 2003, 6(Suppl. 4a), 459-472.

Bayne, C.W.; Donnelly, F.; Ross, M.; Habib, F. *K. Serenoa repens* (Permixon®): A 5α-reductase types I and II inhibitor, new evidence in a coculture model of BPH. *Prostate*, 1999, 40(4), 232-241.

Scholtyssek, C.; Krukiewicz, A.A.; Alonso, J.-L.; Sharma, K.P.; Sharma, P.C.; Goldmann, W.H. Characterizing components of the Saw Palmetto Berry Extract (SPBE) on prostate cancer cell growth and traction. *Biochem. Biophys. Res. Commun.*, 2009, 379(3), 795-798.

Berges, R.R.; Windeler, J.; Trampisch, H.J.; Senge, T. Randomised, placebo-controlled, double-blind clinical trial of β-sitosterol in patients with benign prostatic hyperplasia. *Lancet*, 1995, 345(8964), 1529-1532.

Cabeza, M.; Bratoff, E.; Heuze, I.; Ramirez, E.; Sánchez, M.; Flores, E. Effect of β-sitosterol as inhibitor of 5α-reductase in hamster prostate. *Proc. West Pharmacol. Soc.*, 2003, 46, 153-155.

Wilt, T.J.; Macdonald, R.; Ishani, A. β-sitosterol for the treatment of benign prostatic hyperplasia. *BJU Int.*, 1999, 83(9), 976-983.
Current Medicinal Chemistry, 2016, Vol. 23, No. 8

[82] Wehrberger, C.; Dreikorn, K.; Schmitz-Dräger, B.J.; Oelke, M.; Madersbacher, S. Phytotherapy bei benignem prostatasyndrom und prostatakarzinom. Urologe, 2012, 51(12), 1674-1682.

[83] Liu, J.; Kurashiki, K.; Shimizu, K.; Kondo, R. Structure–activity relationship for inhibition of 5α-reductase by triterpenoids isolated from Ganoderma lucidum. Bioorg. Med. Chem., 2006, 14(24), 8654-8660.

[84] Papaioannou, M.; Schleich, S.; Roell, D.; Schubert, U.; Tanner, T.; Claessens, F.; Matusch, R.; Baniamihd, A. NBBS isolated from Pygeum africanum bark exhibits androgen antagonistic activity, inhibits AR nuclear translocation and prostate cancer cell growth. Invest. N. Drugs, 2010, 28(6), 729-743.

[85] Hessenkemper, W.; Roediger, J.; Bartsch, S.; Houtsmuller, A.B.; van Royen, M.E.; Petersen, I.; Grimm, M.-O.; Baniamihd, A. A natural androgen receptor antagonist induces cellular senescence in prostate cancer cells. Mol. Endocrinol., 2014, 28(11), 1831-1840.

[86] Bratoeff, E.; Cabeza, M.; Ramírez, E.; Heuze, Y.; Flores, E. Recent advances in the chemistry and pharmacological activity of new steroidal antiandrogens and 5α-Reductase inhibitors. Curr. Med. Chem., 2005, 12(8), 927-943.

[87] Cabeza, M.; Bratoeff, E.; Heuze, I.; Rojas, A.; Terán, N.; Ochoa, M.; Ramírez-Apan, M.T.; Ramírez, E.; Pérez, V.; Gracía, I. New progesterone derivatives as inhibitors of 5α-reductase enzyme and prostate cancer cell growth. J. Enzyme Inhib. Med. Chem., 2006, 21(4), 371-378.

[88] Pérez-Oremelas, V.; Cabeza, M.; Bratoeff, E.; Heuze, I.; Sánchez, M.; Ramírez, E.; Naranjo-Rodriguez, E. New 5α-reductase inhibitors: In vitro and in vivo effects. Steroids, 2005, 70(3), 217-224.

[89] Cabeza, M.; Bratoeff, E.; Ramírez, E.; Heuze, I.; Recillas, S.; Berrios, H.; Cruz, A.; Cabrera, O.; Pérez, V. Biological activity of novel progesterone derivatives having a bulky ester side chains at C-3. Steroids, 2008, 73(8), 838-843.

[90] Bratoeff, E.; Cabeza, M.; Pérez-Oremelas, V.; Recillas, S.; Heuze, I. In vivo and in vitro effect of novel 4,16-pregnadiene-6,20-dione derivatives, as 5α-reductase inhibitors. J. Steroid Biochem. Mol. Biol., 2008, 11(3-5), 275-281.

[91] Cabeza, M.; Zambrano, A.; Heuze, I.; Carrizales, E.; Palacios, A.; Segura, T.; Valencia, N.; Bratoeff, E. Novel C-6 substituted and unsubstituted pregnane derivatives as 5α-reductase inhibitors and their effect on hamster flank organs diameter size. Steroids, 2009, 74(10-11), 793-802.

[92] Bratoeff, E.; Segura, T.; Recillas, S.; Carrizales, E.; Palacios, A.; Heuze, I.; Cabeza, M. Aromatic esters of progesterone as 5α-reductase and prostate growth inhibitors. J. Enzyme Inhib. Med. Chem., 2009, 24(3), 655-662.

[93] Bratoeff, E.; Zambrano, A.; Heuze, I.; Palacios, A.; Ramírez, D.; Cabeza, M. Synthesis and biological activity of progesterone derivatives as 5α-reductase inhibitors, and their effect on hamster prostate weight. J. Enzyme Inhib. Med. Chem., 2010, 25(3), 306-311.

[94] Collins, K.D.; Stark, G.R. Aspartame Transcarbamylase: Interaction with the transition state analogue N- (phosphonacetyl)-L-aspartate. J. Biol. Chem., 1971, 246(21), 6599-6605.

[95] Bratoeff, E.; Sainz, T.; Cabeza, M.; Heuze, I.; Recillas, S.; Pérez, V.; Rodríguez, C.; Segura, T.; Gonzáles, J.; Ramírez, E. Steroids with a carbamate function at C-17, a novel class of inhibitors for human and hamster steroid 5α-Reductase. J. Steroid Biochem. Mol. Biol., 2007, 107(1-2), 48-56.

[96] Garrido, M.; Bratoeff, E.; Bonilla, D.; Soriano, J.; Heuze, Y.; Cabeza, M. New steroidal lactones as 5α-reductase inhibitors and antagonists for the androgen receptor. J. Steroid Biochem. Mol. Biol., 2011, 127(3-5), 367-373.

[97] Arellano, Y.; Bratoeff, E.; Garrido, M.; Soriano, J.; Heuze, Y.; Cabeza, M. New ester derivatives of dehydroepiandrosterone as 5α-reductase inhibitors. Steroids, 2011, 76(12), 1241-1246.

[98] Marchais-Oberwinkler, S.; Henn, C.; Möller, G.; Klein, T.; Negri, M.; Oster, A.; Spadaro, A.; Werth, R.; Wetzel, M.; Xu, K.; Frotscher, M.; Hartmann, R.W.; Adamski, J. 17β-Hydroxysteroid dehydrogenases (17β-HSDs) as therapeutic targets: Protein structures, functions, and recent progress in inhibitor development. J. Steroid Biochem. Mol. Biol., 2011, 125(1-2), 66-82.

[99] Brandt, M.; Levy, M.A. 3β-Hydroxy-Δ5-stereoid dehydrogenase/3-keto- Δ5-steroid isomerase from bovine adrenals: mechanism of inhibition by 3-oxo-4-aza steroids and kinetic mechanism of the dehydrogenase. Biochemistry, 1989, 28(1), 140-148.

[100] Day, J.M.; Foster, P.A.; Tutill, H.J.; Schmidlin, F.; Sharrland, C.M.; Hargrave, J.D.; Vicker, N.; Potter, B. V.L.; Reed, M.J.; Purohit, A. STX2171, a 17β-hydroxysteroid dehydrogenase type 3 inhibitor, is efficacious in vivo in a novel hormone-dependent prostate cancer model. Endocr. Relat. Cancer, 2013, 20(1), 53-64.

[101] Harada, K.; Kubo, H.; Tanaka, A.; Nishioka, K. Identification of oxazolidinediones and thiazolidinediones as potent 17β-hydroxysteroid dehydrogenase type 3 inhibitors. Bioorg. Med. Chem. Lett., 2012, 22(1), 504-507.

[102] Spires, T.E.; Fink, B.E.; Kick, E.K.; You, D.; Rizzo, C.A.; Takenaka, I.; Lawrence, R.M.; Ruan, Z.; Salvati, M.E.; Vite, G.D.; Weinmann, R.; Attar, R.M.; Gottardis, M.M.; Lorenzi, M.V. Identification of novel functional inhibitors of 17β-hydroxysteroid dehydrogenase type III (17β-HSD3). Prostate, 2005, 65(2), 159-170.

[103] Bérubé, M.; Poirier, D. Chemical synthesis and in vitro biological evaluation of a phosphorylated bisubstrate inhibitor of type 3 17β-hydroxysteroid dehydrogenase. J. Enzyme Inhib. Med. Chem., 2007, 22(2), 201-211.

[104] Hong, Y.; Chen, S. Aromatase, estrone sulfatase, and 17β-hydroxysteroid dehydrogenase: Structure–function studies and inhibitor development. Mol. Cell. Endocrinol., 2011, 340(2), 120-126.

[105] Nakamura, Y.; Suzuki, T.; Nakabayashi, M.; Endoh, M.; Sakamoto, K.; Mikami, Y.; Moriya, T.; Ito, A.; Takahashi, S.; Yamada, S.; Arai, Y.; Sasano, H. 114 androgen producing enzymes in human prostate cancer. Endocr. Relat. Cancer, 2005, 12(1), 101-107.

[106] Duf, W.; Zhou, M.; Mazumdar, M.; Azzi, A.; Ghammi, D.; Lus-The, V.; Labrie, F.; Lin, S.X. Structure-based inhibitor design for an enzyme that binds different steroids: A potent inhibitor for human type 5 17β-hydroxysteroid dehydrogenase. J. Biol. Chem., 2007, 282(11), 8368-8379.

[107] Schuster, D.; Kowalik, D.; Kirchmair, J.; Laggner, C.; Markt, P.; Aebsicher-Guny, C.; Ströhle, F.; Möller, G.; Wolber, G.; Wiekens, T.; Langer, T.; Odermatt, A.; Adamski, J. Identification of chemically diverse, novel inhibitors of 17β-hydroxysteroid dehydrogenase type 3 and 5 by pharmacophore-based virtual screening. J. Steroid Biochem. Mol. Biol., 2011, 125(1-2), 148-161.

[108] Sharifi, N. Mechanisms of androgen receptor activation in castration-resistant prostate cancer. Endocrinology, 2013, 154(11), 4010-4017.

[109] Zhao, X.Y.; Malloy, P.J.; Krishnan, A.V.; Swami, S.; Navone, N.M.; Peehl, D.M. Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a mutated androgen receptor. Nat. Med., 2000, 6, 703-706.

[110] Moreira, V.M.A.; Vassatis, T.S.; Guo, Z.; Njar, V.C.O.; Salvador, J.A.R. Synthesis of novel C17 steroidal carboxylic acids. Studies on CYP17 action, androgen receptor binding
and function, and prostate cancer cell growth. *Steroids*, 2008, 73(12), 1217-1227.

[111] Hu, Q.; Jagusch, C.; Hille, U.E.; Haupenthal, J.; Hartmann, R.W. Replacement of Imidazolyl by Pyridyl in biphenylmethylenes results in selective Cyp17 and dual Cyp17/Cyp11b1 inhibitors for the treatment of prostate cancer. *J. Med. Chem.*, 2010, 53(15), 5749-5758.

[112] Francini, E.; Fiaschi, A.I.; Petroli, R.; Francini, F.; Bianco, V.; Perrella, A.; Paganini, G.; Laera, L.; Roviello, G. Abiraterone in heavily pretreated patients with metastatic castrate-resistant prostate cancer. *Anti-Cancer Drugs*, 2014, 25(4), 472-477.

[113] Pinto-Bazurco Mendieta, M.A.E.; Negri, M.; Jagusch, C.; Muüller-Vieira, U.; Lauterbach, T.; Hartmann, R.W. Synthesis, biological evaluation, and molecular modeling of abiraterone analogues: novel CYP17 inhibitors for the treatment of prostate cancer. *J. Med. Chem.*, 2008, 51(16), 5009-5018.

[114] O'Donnell, A.; Judson, I.; Dowsett, M.; Raynaud, F.; Dearnaley, D.; Mason, M.; Harland, S.; Robbins, A.; Halbert, G.; Nutley, B.; Jarman, M. Hormonal impact of the 17[alpha]-hydroxylase/C17,20-lyase inhibitor abiraterone acetate (CB7630) in patients with prostate cancer. *Br. J. Cancer*, 2004, 90(12), 2317-2325.

[115] Tran, C.; Ouk, S.; Clegg, N.J.; Chen, Y.; Watson, P.A.; Arora, V. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science*, 2009, 324, 787-790.

[116] Clegg, N.J.; Wongvipat, J.; Joseph, J.D.; Tran, C.; Ouk, S.; Dhillas, A.; Chen, Y.; Grillot, K.; Bischoff, E. D.; Cai, L.; Aparicio, A.; Dorow, S.; Arora, V.; Shao, G.; Qian, J.; Zhao, H.; Yang, G.; Cao, C.; Sensintaffar, J.; Wasielewska, T.; Herbert, M.R.; Bonnefous, C.; Darimont, B.; Scher, H.I.; Smith-Jones, P.; Klang, M.; Smith, N.D.; De Stanis, E.; Wu, N.; Ouerfelli, O.; Rix, P.J.; Heyman, R.A.; Jung, M.E.; Sawyer, C.L.; Hager, J.H. ARN-509: A novel antiandrogen for prostate cancer treatment. *Cancer Res.*, 2012, 72(6), 1494-1503.

[117] Haendler, B.; Cleve, A. Recent developments in antiandrogens and selective androgen receptor modulators. *Mol. Cell. Endocrinol.*, 2012, 352(1-2), 79-91.

[118] Scher, H.I.; Beer, T.M.; Higano, C.S.; Anand, A.; Taplin, M.E.; F Castaño, E.; Wu, N.; Ouerfelli, O.; Rix, P.J.; Heyman, R.A.; Rathkopf, D.; Liu, G.; Carducci, M.A.; Eisenberger, M.A.; Anand, A.; Morris, M.J.; Slovin, S.F.; Sasaki, Y.; Takahashi, S.; Ozono, S.; Fun, N.E.K.; Cheng, S.; Gan, J.; Gottardis, M.; Obermeier, M.T.; Reddy, J.; Zhang, S.; Vakalagasadda, B.J.; Alland, L.; Widing, G.; Scher, H.I. Phase I dose-escalation study of the novel antiandrogen BMS-641981 in patients with castration-resistant prostate cancer. *Clin. Cancer Res.*, 2011, 17(4), 880-887.

[119] Gavai, A.V.; Foster, W.R.; Balog, A.; Vite, G.D. Novel Androgen Receptor Antagonists for the Treatment of Prostate Cancer Accounts in Drug Discovery: Case Studies in Medicinal Chemistry. In: *Accounts in Drug Discovery: Case Studies in Medicinal Chemistry*; Barrish, J.C., Carter, P.H., Cheng, P.T.W., Zahler, R., Eds.; Royal Society of Chemistry; London, 2011.

[120] Attar, R.M.; Jure-Kunkel, M.; Balog, A.; Cvijic, M.E.; Delli-Donis, I.; Rizzo, C.A.; Schweizer, L.; Spires, T.E.; Platero, J.S.; Obermeier, M.; Shan, W.; Salvati, M.E.; Foster, W.R.; Dinchuk, J.; Chen, S.-J.; Vite, G.; Kramer, R.; Gottardis, M.M. Discovery of BMS-641988, a novel and potent inhibitor of androgen receptor signaling for the treatment of prostate cancer. *Cancer Res.*, 2009, 69(16), 6522-6530.

[121] Ruthkopf, D.; Liu, G.; Carducci, M.A.; Eisenberger, M.A.; Anand, A.; Morris, M.J.; Slovin, S.F.; Sasaki, Y.; Takahashi, S.; Ozono, S.; Fung, N.E.K.; Cheng, S.; Gan, J.; Gottardis, M.; Obermeier, M.T.; Reddy, J.; Zhang, S.; Vakalagasadda, B.J.; Alland, L.; Widing, G.; Scher, H.I. Phase I dose-escalation study of the novel antiandrogen BMS-641981 in patients with castration-resistant prostate cancer. *Prostate*, 2011, 71(12), 1344-1356.

[122] Gauthier, S.; Martel, C.; Labrie, F. Steroid derivatives as pure antagonists of the androgen receptor. *J. Steroid Biochem. Mol. Biol.*, 2012, 132(1-2), 93-104.

[123] Andrieu, T.; Bertoloni, R.; Nichols, S.E.; Setoud, R.; Frey, F.J.; Baker, M.E.; Frey, B.M. A novel steroidal antiandrogen targeting wild type and mutant androgen receptors. *Biochem. Pharmacol.*, 2011, 82(11), 1651-1662.

[124] Fousteris, M.A.; Schubert, U.; Roell, D.; Roediger, J.; Bailis, N.; Nikolaropoulos, S.S.; Banaihamad, A.; Giannis, A. 20-Aminosteroids as a novel class of selective and complete androgen receptor antagonists and inhibitors of prostate cancer cell growth. *Mol. Cancer*, 2010, 18(19), 6960-6969.

[125] Sarwar, M.; Sandberg, S.; Abrahamsson, P.A.; Persson, J.L. Protein kinase A (PKA) pathway is functionally linked to androgen receptor (AR) in the progression of prostate cancer. *Urol. Oncol.*, 2014, 32(1), 25.e1-12.e1.

[126] Mimeault, M.; Pommery, N.; Hénichart, J.P. Synergistic antiproliferative and apoptotic effects induced by epidermal growth factor receptor and protein kinase a inhibitors in human prostate cancer cell lines. *Int. J. Cancer*, 2003, 106 (1), 116-124.

[127] Gjertsen, B.; Mellgren, G.; Otten, A.; Maronde, E.; Genieser, H.G.; Jastorff, B.; Vintermyr, O.K.; McKnight, G.S.; Stein, O. Novel (Rp)-cAMPS analogs as tools for inhibition of cAMP-kinase in cell culture: BASAL cAMP-kinase activity modulates interleukin-1β action. *J. Biol. Chem.*, 1995, 270(35), 20599-20607.