Part I
Biopharmaceuticals

1
Analogs and Antagonists of Male Sex Hormones

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Keywords

Androgens
Steroid hormones responsible for the primary and secondary sex characteristics of the male, including the development of the vas deferens, prostate, seminal vesicles, and penis.

Testosterone
The C_{19} steroid hormone that is the predominant circulating androgen in the bloodstream and is produced mainly by the testis in males.

Dihydrotestosterone
The C_{19} steroid hormone that is the 5α-reduced metabolite of testosterone. It is produced in certain androgen target tissues and is the most potent endogenous androgen.

Anabolics
Compounds that demonstrate a marked retention of nitrogen through an increase of protein synthesis and a decrease in protein catabolism in the body.

Antiandrogens
Agents that compete with endogenous androgens for the hormone-binding site on the androgen receptor and thus block androgen action.

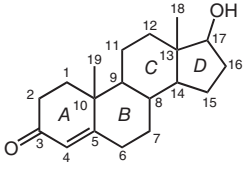
Selective androgen receptor modulators
Agents that may act as an androgen antagonist or weak agonist in one tissue, but as a strong androgen agonist in another tissue type.

5α-Reductase inhibitors
Compounds that inhibit the conversion of testosterone to its more active metabolite, dihydrotestosterone.

The steroid testosterone is the major circulating sex hormone in males and is the prototype for the androgens, the anabolic agents, and androgen antagonists. Endogenous androgens are biosynthesized from cholesterol; the majority of the circulating androgens are produced in the testes under the stimulation of luteinizing hormone (LH). The reduction of testosterone to dihydrotestosterone is necessary for androgenic actions of testosterone in many androgen target tissues such as the prostate; the oxidation of testosterone by the enzyme aromatase produces estradiol. The androgenic actions of testosterone and dihydrotestosterone are due to their binding to the androgen receptor, followed by nuclear localization, dimerization of the receptor complex, and binding to specific DNA sequences. This binding of the homodimer to the androgen response element leads to gene expression, stimulation, or repression of new mRNA synthesis, and subsequent protein biosynthesis. The synthetic androgens and anabolics were prepared to impart oral activity to the androgen molecule, to separate the androgenic effects of testosterone from its anabolic effects, and to improve on its biological activities. Novel nonsteroidal androgens, termed selective androgen receptor modulators, were developed to impart agonist activity in selective tissues. Drug preparations are used for the treatment of various androgen-deficient diseases and for the therapy of diseases characterized by muscle wasting and protein catabolism. Androgen antagonists include antiandrogens, which block interactions of androgens with the androgen receptor, and inhibitors of androgen biosynthesis and metabolism. Such compounds have therapeutic potential in the treatment of acne, virilization in women, hyperplasia and neoplasia of the prostate, and baldness.

1 Introduction

Androgens are a class of steroids responsible for the primary and secondary sex characteristics of the male. In addition, these steroids possess potent anabolic or growth-promoting properties. The general chemical structure of androgens is based on the androstane C_{19} steroid, which consists of the fused four-ring steroid nucleus (17 carbons atoms, rings A–D) and the two axial methyl groups (carbons 18 and 19) at the A/B and C/D ring junctions. The hormone testosterone (**1**) is the predominant circulating androgen and is produced mainly by the testis in males. 5α-Dihydrotestosterone (**2**) is a 5α-reduced metabolite of testosterone produced in certain target tissues and is the most potent endogenous androgen. Other endogenous androgens are produced by the adrenal gland in both males and females.



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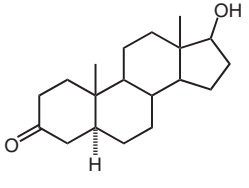


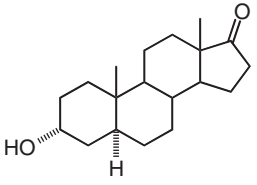
(**2**)

These two steroids and other endogenous androgens influence not only the development and maturation of the male genitalia and sex glands, but also affect other tissues such as kidney, liver, and brain. In this chapter, the endogenous androgens, synthetic analogs, various anabolic agents, and the androgen antagonists employed in clinical practice or animal husbandry in the United States and elsewhere will be discussed. Modified androgens that have found use as biochemical or pharmacological tools also are included. More extensive presentations of the topic of androgens, anabolics, and androgen antagonists have appeared in several treatises published over the past four decades [1–11].

2 Historical

The role of the testes in the development and maintenance of the male sex characteristics, and the dramatic physiological effects of male castration, have been recognized since early times. Berthold [12] was the first to publish (in 1849) a report that gonadal transplantation prevented the effects of castration in roosters, suggesting that the testis produced internal secretions exhibiting androgenic effects. However, the elucidation of the molecules of testicular origin responsible for these actions took almost another century. The first report of the isolation of a substance with androgenic activity was made by Butenandt [13, 14], in 1931. The material, isolated in very small quantities from human male urine [15], was named androsterone (**3**) [16]. A second weakly androgenic steroid hormone was isolated from male urine in 1934; this substance was named dehydroepiandrosterone (**4**) because of its ready chemical transformation and structural similarity to androsterone [17]. A year later, Laqueur [18, 19] reported the isolation of the testicular androgenic hormone, testosterone (**1**), which was 10-fold more potent than androsterone in promoting capon comb growth. Shortly after this discovery, the first chemical synthesis of testosterone was

reported by Butenandt and Hanisch [20] and confirmed by Ruzicka [21, 22].



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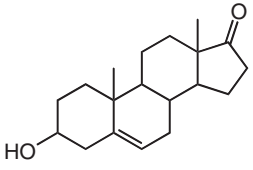
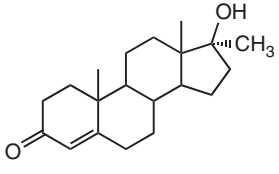


(**4**)

For many years, it was believed that testosterone was the active androgenic hormone in man. In 1968, however, research in two laboratories demonstrated that 5α-dihydrotestosterone (DHT, **2**), also referred to as stanolone, was the active androgen in certain target tissues such as the prostate and seminal vesicles, and was formed from testosterone by a reductase present in these tissues [23, 24]. Shortly thereafter a soluble receptor protein was isolated and shown to have a greater specificity for DHT and related structures [25, 26]. In general, DHT is thought to be the active androgen in tissues that express 5α-reductase (e.g., the prostate), whereas testosterone appears to directly mediate these effects in muscle and bone.

The anabolic action of the androgens was first documented by Kochakian and Murlin in 1935 [27]. In their experiments, extracts of male urine caused a marked retention of nitrogen when injected into dogs fed a constant diet. Soon afterwards, testosterone propionate was observed to produce a similar nitrogen-sparing effect in humans [28]. Subsequent clinical studies demonstrated that testosterone was capable of causing a major acceleration of skeletal growth and a marked increase in muscle mass [29–31]. This action on muscle tissue has been referred to more specifically as the myotrophic effect.

The first androgenic-like steroid used for its anabolic properties in humans was testosterone. Unfortunately, its use for this purpose was limited by the inherent androgenicity and the need for parenteral administration. 17α-Methyltestosterone (**5**) was the first androgen discovered to possess oral activity, but it too failed to show any apparent separation of androgenic and anabolic activity. The promise of finding a useful, orally effective, anabolic agent free from androgenic side effects prompted numerous clinical and biological studies.



(**5**)

3
Endogenous Male Sex Hormones

3.1
Occurrence and Physiological Roles

The hormone testosterone affects many organs in the body, its most dramatic effects being observed on the primary and secondary sex characteristics of the male. These actions are first manifested in the developing male fetus, when the embryonic testis begins to secrete testosterone. Differentiation of the Wolffian ducts into the vas deferens, seminal vesicles, and epididymis occurs under this early androgen

influence, as does the development of external genitalia and the prostate [32]. The reductive metabolism of testosterone to DHT is critical for virilization during this period of fetal development, as demonstrated dramatically in patients with a 5α-reductase deficiency [33].

At puberty, further development of the sex organs (prostate, penis, seminal vesicles, and vas deferens) is again evident and under the control of androgens. Additionally, the testes now begin to produce mature spermatozoa. Other effects of testosterone, particularly on the secondary sex characteristics, are observed; hair growth on the face, arms, legs, and chest is stimulated by this hormone during younger years. In later years, however, DHT is responsible for a thinning of the hair and recession of the hairline. At puberty, the larynx develops and a deepening of the voice occurs, the male's skin thickens, the sebaceous glands proliferate, and the fructose content in human semen increases. Testosterone influences sexual behavior, mood, and aggressiveness of the male at the time of puberty.

In addition to these androgenic properties, testosterone also exhibits anabolic (myotropic) characteristics. A general body growth is initiated, including increased muscle mass and protein synthesis, a loss of subcutaneous fat, and increased skeletal maturation and mineralization. This anabolic action is associated with a marked retention of nitrogen brought about by an increase in protein synthesis and a decrease in protein catabolism. The increase in nitrogen retention is manifested primarily by a decrease in urinary rather than fecal nitrogen excretion, and results in a more positive nitrogen balance. For example, the intramuscular administration of 25 mg testosterone propionate twice daily causes nitrogen retention to appear within 1–3 days, reaching a maximum in about 5–8 days. This reduced level of nitrogen excretion may be maintained for at least a month, and depends on the patient's nutritional status and diet [34].

Androgens influence skeletal maturation and mineralization, which is reflected in an increase in skeletal calcium and phosphorus [35]. In various forms of osteoporosis, androgens decrease urinary calcium loss and improve the calcium balance in patients; this effect is less noticeable in normal patients. Moreover, the various androgen analogs differ markedly in their effects on calcium and phosphorus balance in man [35]. Androgens and their 5β-metabolites (e.g., etiocholanolone) markedly stimulate erythropoiesis, presumably by increasing the production of erythropoietin and by enhancing the responsiveness of erythropoietic tissue to erythropoietin [36]. The effects of androgens on carbohydrate metabolism appear to be minor, and secondary to their primary protein anabolic property, but the effects on lipid metabolism seem unrelated to this anabolic property. Weakly androgenic metabolites such as androsterone have been found to lower serum cholesterol levels when administered parenterally.

3.2 Biosynthesis

The androgens are secreted not only by the testis in males, but also by the adrenal cortex in males and females, and the ovary in females. Testosterone is the principal circulating androgen and is formed by the Leydig cells of the testes. Other tissues, such as liver and human prostate, form testosterone from precursors, but this contribution to the circulating androgen pool is minimal. Since dehydroepiandrosterone and androstenedione are secreted by the

adrenal cortex and ovary, they indirectly augment the circulating testosterone pool because they can be rapidly converted to testosterone by peripheral tissues. This local production of testosterone from circulating adrenal androgens can significantly contribute to local androgen concentrations in certain tissues, such as prostate.

Plasma testosterone levels for men usually range between 6 and 11 ng ml^{-1}, and are between fivefold and 100-fold the values in females [37]. The circulating level of DHT in normal adult men is about one-tenth the testosterone level [38]. Daily testosterone production rates have been estimated at 4–12 mg for young men and 0.5–2.9 mg for young women [39]. Although attempts have been made to estimate the secretion rates for testosterone, these studies have been hampered by the number of tissues capable of secreting androgens and the considerable interconversion of the steroids concerned [40, 41].

The synthesis of androgens in the Leydig cells of the testes is regulated by the gonadotropic hormone, luteinizing hormone (LH). The other pituitary gonadotropin, follicle-stimulating hormone (FSH), acts primarily on the germinal epithelium and is important for sperm development. Both of these pituitary gonadotropins are under the regulation of a decapeptide hormone produced by the hypothalamus. This hypothalamic hormone is luteinizing hormone-releasing hormone (LHRH), also referred to as gonadotropin-releasing hormone (GnRH). In adult males, a pulsatile secretion of LHRH, and subsequently of LH and FSH, occurs at a frequency of 8–14 pulses in 24 h [42]. The secretions of these hypothalamic and pituitary hormones are, in turn, regulated by circulating testosterone and estradiol levels in a negative feedback mechanism. Testosterone will decrease the frequency and amplitude of pulsatile LH secretion [43], whereas both testosterone and a gonadal peptide, inhibin, are both involved in suppressing the release of FSH [44].

The present understanding of steroidogenesis in the endocrine organs has advanced considerably during the past four decades, based largely on initial investigations with the adrenal cortex and subsequent studies also of the testis and ovary [45]. Figure 1 outlines the following sequence of events known to be involved with steroidogenesis in the Leydig cells. LH binds to its receptor located on the surface of the Leydig cell and, via a G protein-mediated process, activates adenylyl cyclase to result in an increase in intracellular concentrations of cyclic AMP (cAMP). cAMP activates a cAMP-dependent protein kinase, which subsequently phosphorylates and activates several enzymes involved in the steroidogenic pathway, including cholesterol esterase and cholesterol side-chain cleavage [46]. Cholesterol esters (present in the cell as a storage form) are converted to free cholesterol by cholesterol esterase, and free cholesterol is translocated to the mitochondria where a cytochrome P450 mixed-function oxidase system, termed cholesterol side-chain cleavage, converts cholesterol to pregnenolone. Several non-mitochondrial enzymatic transformations then convert pregnenolone to testosterone, which is secreted.

The conversion of cholesterol (**6**) to pregnenolone (**7**) has been termed the rate-limiting step in steroid hormone biosynthesis. The reaction requires NADPH and molecular oxygen, and is catalyzed by the cholesterol side-chain cleavage complex. The latter enzyme complex is comprised of three proteins: cytochrome $P450_{SCC}$ (also called cytochrome P450 11A1); adrenodoxin; and adrenodoxin reductase. Three moles of NADPH and

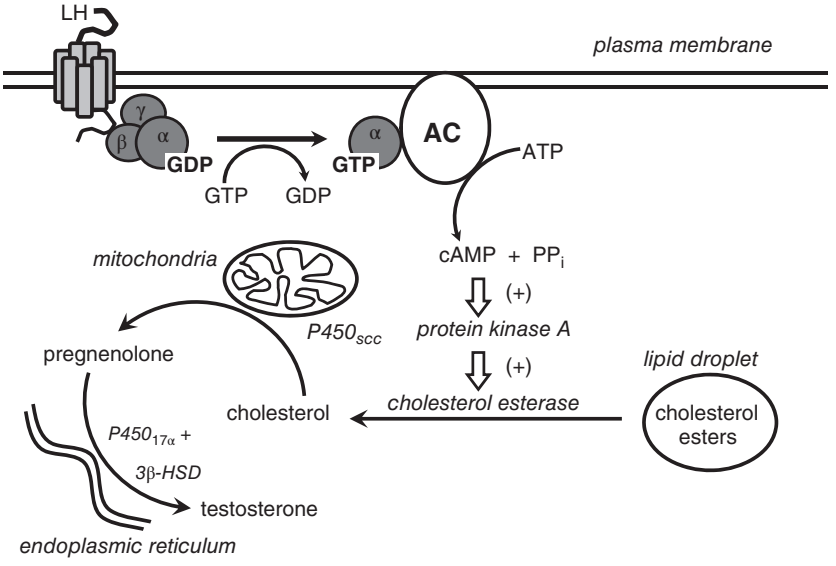


Fig. 1 Cellular events in steroidogenesis in the Leydig cell.

oxygen are required to convert one mole of cholesterol into pregnenolone (Fig. 2).

Tracer studies have shown that two major pathways known as the "4-ene" and "5-ene" pathways are involved in the conversion of pregnenolone to testosterone. Both of these pathways and their requisite enzymes are shown in Fig. 2. Earlier studies tended to favor the "4-ene" pathway, but more recent studies have disputed this view and suggest that the "5-ene" pathway is quantitatively more important in man. When Vihko and Ruokonen [47] analyzed the spermatic venous plasma for free and conjugated steroids, all intermediates of the "5-ene" pathway were identified but progesterone (**8**), an important intermediate of the "4-ene" pathway, was not found. In addition, sulfate conjugates were present in significant quantities, especially androst-5-ene-3β,17β-diol 3-monosulfate. These data strongly suggest that this intermediate and its unconjugated form constitute an important precursor of testosterone in man. This view, however, was not supported by a kinetic analysis of the metabolism of androst-5-ene-3β,17β-diol (**12**) in man [48]. Further evidence that the predominant pathway appears to be the "5-ene" pathway was provided by *in-vitro* studies in human testicular tissues [49].

Another important step is the conversion of the C-21 steroids to the C-19 androstene derivatives. Whereas, the enzymes for side-chain cleavage are localized in the mitochondria, those responsible for cleavage of the C_{17}-C_{20} bond (C_{17}-C_{20} lyase) reside in the endoplasmic reticulum of the cell. Early studies implicated 17α-hydroxypregnenolone (**9**) or 17α-hydroxyprogesterone (**10**) as obligatory intermediates in testosterone biosynthesis [50], and the C_{17}-C_{20} bond was subsequently cleaved by a second enzymatic process to produce the C-19 androstene molecule. This view of the involvement of two separate enzymes in the conversion of C-21 to C-19 steroids existed until purification of the proteins during the 1980s. The 17α-hydroxylase/17,20-lyase cytochrome

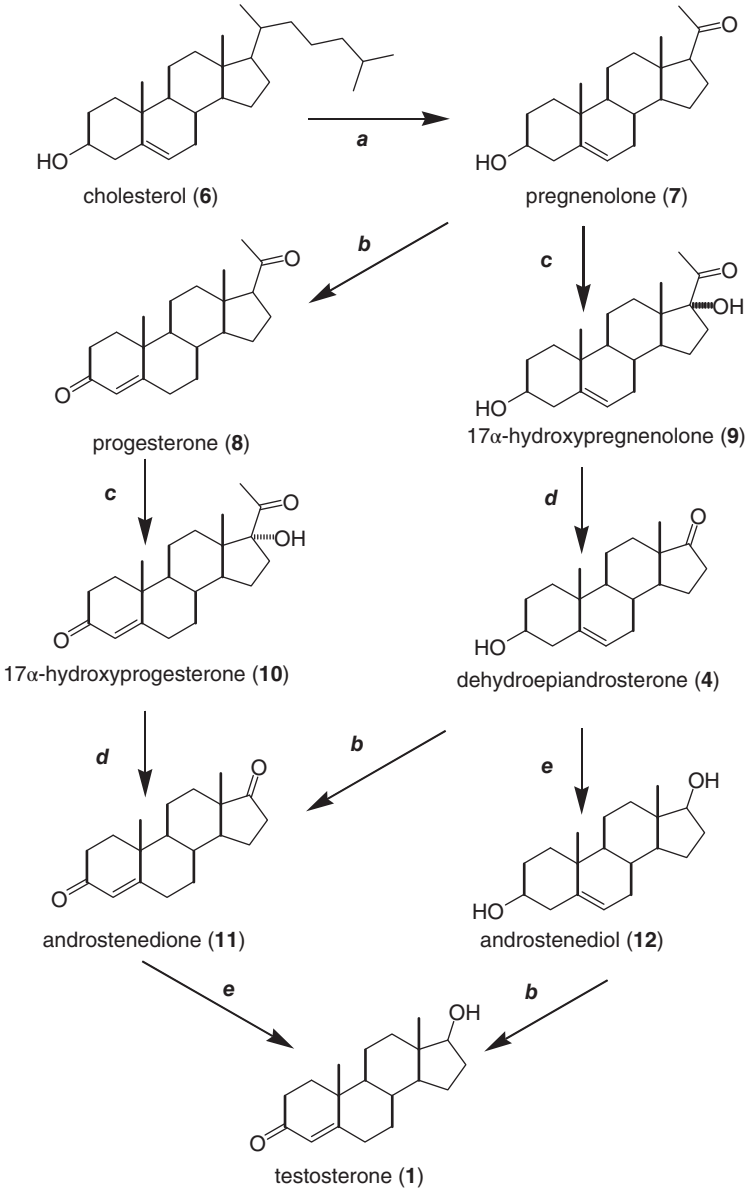


Fig. 2 Enzymatic conversion of cholesterol to testosterone. Enzymes are denoted as: (*a*) side chain cleavage; (*b*) 3β-hydroxysteroid dehydrogenase; (*c*) 17α-hydroxylase; (*d*) 17,20-lyase; (*e*) 17β-hydroxysteroid dehydrogenase.

P450 (abbreviated cytochrome P450 17 or cytochrome $P450_{17\alpha}$) was first isolated from neonatal pig testis microsomes by Nakajin and Hall [51]. Cytochrome $P450_{17\alpha}$ possessed both 17α-hydroxylase and 17,20-lyase activity when reconstituted with cytochrome P450 reductase and phospholipid. Identical full-length human cytochrome $P450_{17\alpha}$ complementary DNA (cDNA) sequences were independently isolated and reported in 1987 [52, 53]. Extensive reviews of the molecular biology, gene regulation, and enzyme deficiency syndromes have been published [46, 54].

Two additional enzymes are necessary for the formation of testosterone from dehydroepiandrosterone. The first is the 3β-hydroxysteroid dehydrogenase/$\Delta^{4,5}$-isomerase complex, which catalyzes the oxidation of the 3β-hydroxyl group to the 3-ketone and isomerization of the double bond from $C_5{=}C_6$ to $C_4{=}C_5$. Again, these processes were originally thought to involve two different enzymes, but purification of the enzymatic activity demonstrated that a single enzyme catalyzes both reactions [55]. The final enzyme in the pathway is the 17β-hydroxysteroid dehydrogenase, which catalyzes the reduction of the 17-ketone to the 17β-alcohol.

3.3 Absorption and Distribution

Although considerable research has been devoted to the biochemical mechanism of the action of natural hormones and the synthesis of modified androgens, little is known about the absorption of these substances. It is well recognized that a steroid hormone might have a high intrinsic activity but exerts little or no biological effect because its physico-chemical characteristics prevent it from reaching the site of action. This is particularly true in humans, where slow oral absorption or rapid inactivation may greatly reduce the efficacy of a drug. Even though steroids are commonly given by mouth, little is known of their intestinal absorption. One study in rats showed that androstenedione (**11**) was absorbed better than testosterone or 17α-methyltestosterone, and conversion of testosterone to its acetate enhanced absorption [56]. Results with other steroids have indicated that lipid solubility is an important factor for intestinal absorption, and this may explain the oral activity of certain ethers and esters of testosterone.

Once in the circulatory system, either by secretion from the testis or absorption of the administered drug, testosterone and other androgens will reversibly associate with certain plasma proteins, the unbound steroid being the biologically active form. The extent of this binding is dependent on the nature of the proteins and the structural features of the androgen.

The first protein to be studied was albumin, which exhibited a low association constant for testosterone and bound less-polar androgens such as androstenedione to a greater extent [57–59]. α-Acid glycoprotein (AAG) was shown to bind testosterone with a higher affinity than albumin [60, 61]. A third plasma protein to bind testosterone is corticosteroid-binding α-globulin (CBG) [62]. However, under normal physiological conditions these plasma proteins are not responsible for an extensive binding of androgens in plasma.

A specific protein termed sex hormone binding β-globulin (SHBG) or testosterone-estradiol binding globulin (TEBG) was found in plasma that bound testosterone with a very high affinity [63, 64]. The SHBG–sex hormone complex serves several functions, such as a transport or carrier system in the bloodstream, a storage site or reservoir for the hormones, and protection

of the hormone against metabolic transformations [65]. SHBG has been purified and contains high-affinity, low-capacity binding sites for the sex hormones [66]; the protein has subsequently been cloned and crystallized [66]. Dissociation constants of approximately 1×10^{-9} M have been reported for the binding of testosterone and estradiol to SHBG, and are two orders of magnitude less than values reported for the binding of the hormone to the cytosolic receptor protein [67–69]. The plasma levels of SHBG are regulated by the thyroid hormones [70] and remain fairly constant throughout adult life in both males and females [71]. SHBG is not present in the plasma of all animals [65, 72]; for example, SHBG-like activity is notably absent in the rat, and testosterone may be bound in the rat plasma to CBG.

Numerous studies have been performed on the specificity of the binding of steroids to human SHBG [65, 71–77]. The presence of a 17β-hydroxyl group is essential for binding to SHBG. In addition to testosterone, DHT, 5α-androstane-3β,17β-diol (**20**), and 5α-androstane-3α,17β-diol (**21**) bind with high affinity, and these steroids compete for a common binding site. Binding to SHBG is decreased by 17α-substituents such as 17α-methyl and 17α-ethinyl moieties and by unsaturation at C-1 or C-6. Also, 19-nortestosterone derivatives have lower affinity. The steroid-binding site and the dimerization domain of SHBG, referred to as the amino-terminal laminin G-like domain, has been crystallized and demonstrated important hydrogen bonding of the C_3 and C_{17} moieties of steroidal ligands with Ser^{42} and Asp^{65} of SHBG [78].

Another extracellular carrier protein which exhibits a high affinity for testosterone, is found in seminiferous fluid and the epididymis and originates in the testis, is called androgen binding protein (ABP) [79–81]. This protein is produced by the Sertoli cells on stimulation by FSH [82, 83], and has very similar characteristics to those of plasma SHBG produced in the liver [82].

The absorption of androgens and other steroids from the blood by target cells was usually assumed to occur by a passive diffusion of the molecule through the cell membrane. However, studies conducted during the early 1970s, using tissue cultures or tissue slices, suggested entry mechanisms for the steroids. Estrogens [84, 85], glucocorticoids [86, 87] and androgens [88–91] exhibit a temperature-dependent uptake into intact target cells, suggesting a protein-mediated process. Among the androgens, DHT exhibited a greater uptake than testosterone in human prostate tissue slices [92], and it was found that estradiol or androstenedione interfered with this uptake mechanism [93, 94]. In addition, cyproterone competitively inhibited androstenedione, testosterone and DHT entry, whereas cyproterone acetate enhanced the uptake of these androgens [91]. Little is known regarding the exit of steroids from target cells; the only reported studies have investigated the active transport of glucocorticoids out of cells [92, 93].

3.4 Metabolism

For decades, the primary function of metabolism was thought to be an inactivation of testosterone, an increase in hydrophilicity, and a mechanism to facilitate excretion of the steroid into the urine. However, the identification of metabolites of testosterone formed in peripheral tissues, as well as the potent and sometimes different biological activities of these products, has emphasized the importance of

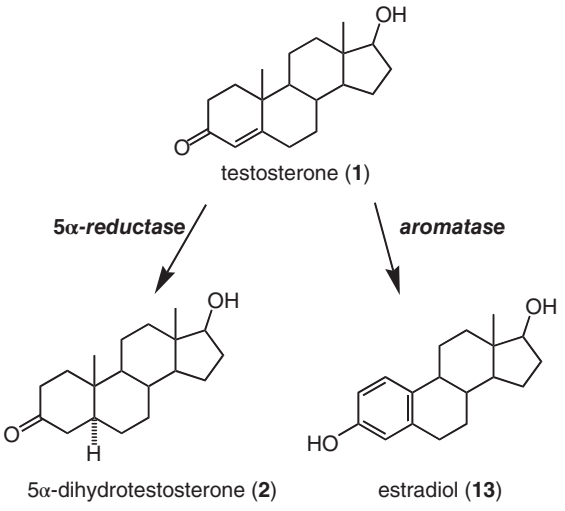


Fig. 3 Enzymatic conversion of testosterone to biologically active metabolites, 5α-dihydrotestosterone and estradiol.

metabolic transformations of androgens in endocrinology. Two active metabolites of testosterone have received considerable attention, namely the reductive metabolite 5α-dihydrotestosterone (**2**) and the oxidative metabolite estradiol (**13**) (Fig. 3).

3.4.1 Reductive Metabolism

The metabolism of testosterone in a variety of *in-vitro* and *in-vivo* systems has been reviewed [50, 94–96]. The principal pathways for the reductive metabolism of testosterone in man are shown in Fig. 4. Human liver produces a number of metabolites, including androstenedione (**11**), 3β-hydroxy-5α-androstan-17-one (**17**), 5α-androstane-3β,17β-diol (**20**), and 5α-andro-stane-3α,17β-diol (**21**) [97, 98]. In addition, cirrhotic liver was shown to produce more 17-keto-steroids than normal liver [99]. Human adrenal preparations, on the other hand, produced 11β-hydroxytestosterone as the major metabolite [100]. The intestinal metabolism of testosterone is similar to transformations in the liver [95], while the major metabolite in lung is androstenedione [101].

Studies on testosterone metabolism conducted since the late 1960s have centered on steroid transformations by prostatic tissues. Normal prostate, benign prostatic hypertrophy (BPH), and prostatic carcinoma all contain 3α-, 3β-, and 17β-hydroxysteroid dehydrogenases, and 5α- and 5β-reductases, capable of converting testosterone to various metabolites. Prostatic carcinoma metabolizes testosterone more slowly than does BPH or normal prostate [102]. On the other hand, recent studies have shown that adrenal androgens can be converted into testosterone and dihydrotestosterone in prostate cancer cells [103, 104]. K. D. Voigt *et al.* [105, 106] have performed extensive studies of *in-vivo* metabolic patterns of androgens in patients with BPH by injecting them (intravenously) with tritiated androgens 30 min before prostatectomy. Tissues from the prostate and surrounding skeletal muscle, as well as blood plasma, were then analyzed for

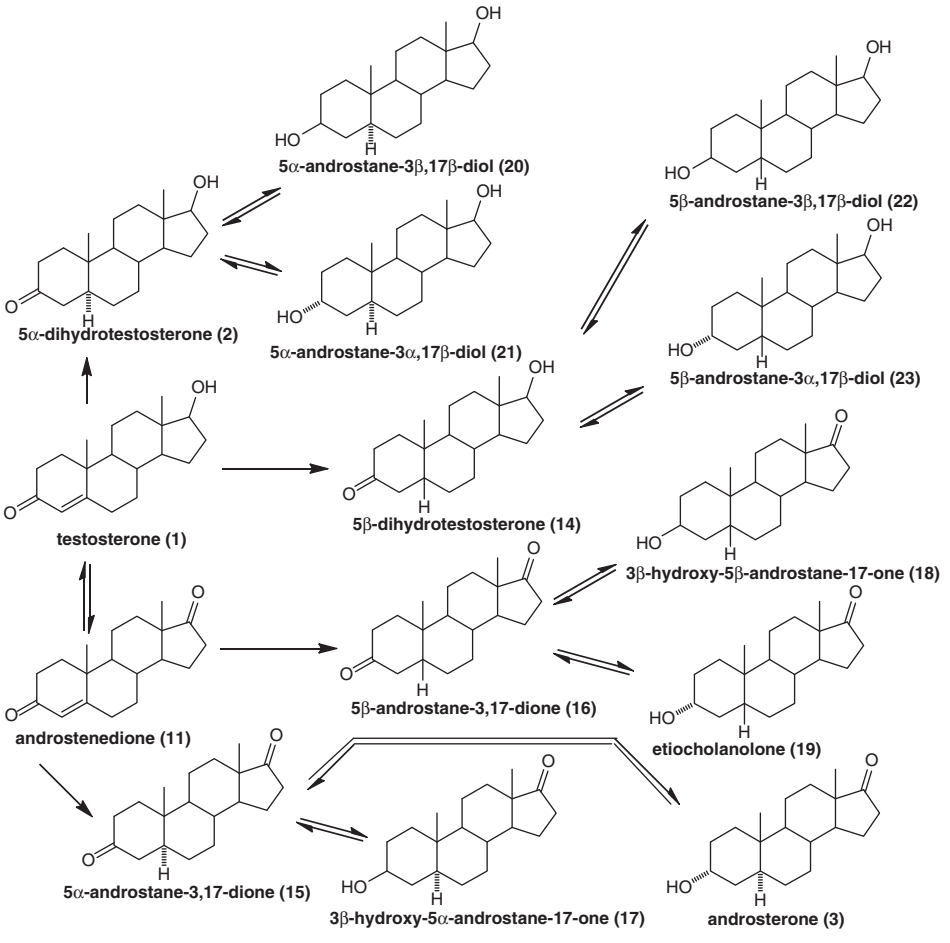


Fig. 4 Reductive metabolites of testosterone.

metabolites. The major metabolite of testosterone found in BPH tissues was DHT, with minor amounts of diols isolated. Skeletal muscle and plasma contained primarily unchanged testosterone.

Androsterone (**3**) and etiocholanolone (**19**), the major urinary metabolites, are excreted predominantly as glucuronides, and only about 10% as sulfates [37, 107]. These conjugates are capable of undergoing further metabolism. Testosterone glucuronide, for example, is metabolized differently from testosterone in man, giving rise mainly to 5β-metabolites [108]. Only a relatively small amount of the urinary 17-ketosteroids is derived from testosterone metabolism. In men, at least 67% and in women about 80% or more, of the urinary 17-ketosteroids are metabolites of adrenocortical steroids [39]. This explains why a significant increase in testosterone secretion associated with various androgenic syndromes does not usually lead to elevated levels of 17-ketosteroid excretion.

Although androsterone and etiocholanolone are the major excretory

products, the exact sequence whereby these 17-ketosteroids arise is still not clear. Studies with radiolabeled androst-4-ene-3β,17β-diol and the epimeric 3α-diol in humans showed that oxidation to testosterone was necessary before reduction of the A-ring [109]. Moreover, 5β-androstane-3α,17β-diol (**23**) was the major initial liver metabolite in rats, but this decreased with time with a simultaneous increase of etiocholanolone [110]. This formation of saturated diols agrees with studies using human liver [97] and provided evidence that the initial step in testosterone metabolism is a reduction of the α,β-unsaturated ketone to a mixture of diols, followed by oxidation to the 17-ketosteroids.

Until 1968, it was generally thought that the excretory metabolites of testosterone were physiologically inert, but subsequent studies have shown that etiocholanolone has thermogenic effects when administered to man [111]. Hypocholesterolemic effects of parenterally administered androsterone have also been described [112].

The conversion of testosterone to DHT by 5α-reductase is of major importance in the mechanism of action of the hormone, as this enzyme has been found active in the endoplasmic reticulum [113, 114] and the nuclear membrane [23, 115–120] of androgen-sensitive cells. In addition, levels of 5α-reductase are under the control of testosterone and DHT [120]; 5α-reductase activity decreases after castration and can be restored to normal levels of activity with testosterone or DHT administration [121].

Early biochemical studies of 5α-reductase were performed using a microsomal fraction from rat ventral prostate. The irreversible enzymatic reaction catalyzed by 5α-reductase requires NADPH as a cofactor, which provides the hydrogen for carbon-5 [122]. The 5α-reductase from rat ventral prostate tissues exhibited a broad range of substrate specificity for various C_{19} and C_{21} steroids [99]; this broad specificity was also observed in inhibition studies [123]. However, more detailed studies of the enzyme were limited due to the extreme hydrophobic nature of the protein, its instability upon isolation, and its low concentrations in androgen-dependent tissues [96].

Investigations of the molecular biology of 5α-reductase resulted in the demonstration of two different genes and two different isozymes of the enzyme [124–126]. The first cDNA to be isolated and cloned that encoded 5α-reductase was designated Type 1, and the second Type 2. The gene encoding Type 1 is located on chromosome 5, while the gene encoding Type 2 is located on chromosome 2. The two human 5α-reductases have approximately 60% sequence homology. The two isozymes differ in their biochemical properties, tissue location, and function [126, 127]. For example, Type 1 5α-reductase exhibits an alkaline pH optimum (6–8.5) and has micromolar affinities for steroid substrates, whereas Type 2 5α-reductase has a sharp pH optimum at 4.7–5.5, a higher affinity (lower apparent K_m) for testosterone, and is more sensitive to inhibitors than the Type 2 isozyme. The latter isozyme is expressed primarily in androgen target tissues, the liver expresses both types, and Type 1 is expressed in various peripheral tissues. Type 2 5α-reductase appears to be essential for masculine development of the fetal urogenital tract and the external male phenotype, whereas the Type 1 isozyme is primarily a catabolic enzyme. In certain cases of human male pseudohermaphroditism, mutations in the Type 2 5α-reductase gene have been observed that resulted in significant decreases in DHT levels needed for virilization [128].

3.4.2 Oxidative Metabolism

Another metabolic transformation of androgens leading to hormonally active compounds involves their conversion to estrogens. Estrogens are biosynthesized in the ovaries and placenta and, to a lesser extent, in the testes, adrenals and certain regions of the brain. The enzyme complex that catalyzes this biosynthesis is referred to as aromatase, and the enzymatic activity was first identified by Ryan [129] in the microsomal fraction from human placental tissue. The mechanism of the aromatization reaction was first elucidated during the early 1960s and continues to be the subject of extensive studies. Aromatase is a cytochrome P450 enzyme complex [130] that requires 3 mol of NADPH and 3 mol of oxygen per mole of substrate [131]. Aromatization proceeds via three successive steps, the first two of which are hydroxylations. The observation by Meyer [132] that 19-hydroxyandrostenedione (**24**) was a more active precursor of estrone (**27**) than the substrate androstenedione led to its postulated role in estrogen biosynthesis. This report, as well as numerous subsequent studies, led to the currently accepted pathway for aromatization (as shown in Fig. 5).

The first two oxidations occur at the C_{19} position, producing the 19-alcohol (**24**) and then the 19-*gem*-diol (**25**), originally isolated as the 19-aldehyde (**26**) [133, 134]. The exact mechanism of the last oxidation remains to be fully determined. The final oxidation results in a stereospecific elimination of the 1β and 2β hydrogen atoms [135–137] and a concerted elimination of the oxidized C_{19} moiety as formic acid [134]. Hydroxylation at the 2β-position was suggested as an intermediate in this final oxidation, as this substance is spontaneously aromatized to estrone [138]. However, investigations using $^{18}O_2$ and isotopically labeled steroidal intermediates failed to show any incorporation of the 2β-hydroxyl group into formic acid under enzymatic or nonenzymatic conditions [139]; neither was it demonstrated that the oxygen atoms from the first and third oxidation steps were incorporated into formic acid [140–142]. These results led to the proposal that the last oxidation step

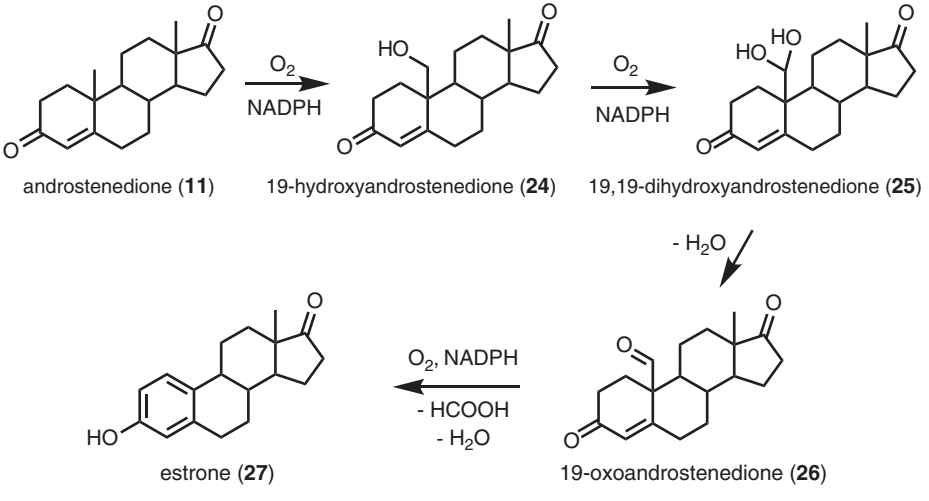


Fig. 5 Aromatization of androgens.

is a peroxidative attack at the C_{19} position [143–145]. However, recent computational chemistry studies have suggested the involvement of a cytochrome P450 oxene intermediate in the final catalytic step of aromatase, resulting in 1β-hydrogen atom abstraction and the release of formic acid [146].

The incubation of a large number of testosterone analogs with human placental tissue [147, 148] has provided some insight into the structural requirements for aromatization. Whereas, androstenedione was converted rapidly to estrone, the 1-dehydro and 19-nor analogs were metabolized slowly, and the 6-dehydro isomer and saturated 5α-androstane-3,17-dione remained unchanged. Hydroxyl and other substituents at lα, 2β, and 11β interfered with aromatization, whereas similar substituents at 9α and 11α seemingly had no effect. Among the stereoisomers of testosterone, only the 8β, 9β, 10β-isomer was aromatized, in addition to compounds having the normal configuration (8β, 9α, 10β). Thus, the substrate specificity of aromatase appears to be limited to C_{19} steroids with the 4-en-3-one system. Inhibition studies with various steroids have provided additional insights into the structural requirements for the enzyme [149–151]; steroidal aromatase inhibitors are described later in Sect. 6.3.3.

Recent investigations of aromatase have focused on the biochemistry, molecular biology and regulation of the aromatase protein. Aromatase is a membrane-bound cytochrome P450 monooxygenase consisting of two proteins: aromatase cytochrome P450 ($P450_{arom}$); and NADPH-cytochrome P450 reductase. Cytochrome $P450_{arom}$ is a heme protein which binds the steroid substrate and molecular oxygen and catalyzes the oxidations. The reductase is a flavoprotein, is found ubiquitously in endoplasmic reticulum, and is responsible for transferring reducing equivalents from NADPH to cytochrome $P450_{arom}$. The purification of cytochrome $P450_{arom}$ proved to be very difficult because of its membrane-bound nature, instability, and low tissue concentration. The reconstitution of a highly purified cytochrome $P450_{arom}$ with NADPH-cytochrome P450 reductase and phospholipid resulted in a complete conversion of androstenedione to estrone, thus, demonstrating that one cytochrome P450 protein catalyzes all three oxidation steps [152]. The first report of the three-dimensional (3-D) crystal structure of human aromatase, published over two decades later, provided a molecular understanding of androgen substrate specificity and the unique enzyme reaction [153]. Knowledge of the molecular biology of aromatase has advanced greatly during the past two decades. A full-length cDNA complementary to messenger RNA (mRNA) encoding cytochrome $P450_{arom}$ was sequenced, and the open reading frame (ORF) encodes a protein of 503 amino acids [154]. When this cDNA sequence was inserted into COS1 monkey kidney cells, aromatase mRNA and aromatase enzymatic activity were detected in the transfected cells. The entire human cytochrome $P450_{arom}$ gene is greater than 70 kb in size [155, 156] and is located on chromosome 15 [157]. Clones have been utilized to examine the regulation of aromatase in ovarian, adipose, and breast tissues [158–161].

The metabolism of androgens by the mammalian brain has also been investigated under *in-vitro* conditions. In 1966, Sholiton *et al.* [162] were the first to report the metabolism of testosterone in rat brain, while later studies demonstrated the conversion of testosterone to DHT, androstenedione, 5α-androstane-3,17-dione, and 5α-androstane3β,17β-diol

[163–168]. The aromatization of androgens to estrogens was also found to occur in the hypothalamus and the pituitary gland [169–174]. The full significance of these metabolites on various neuroendocrine functions, such as the regulation of gonadotropin secretion and sexual behavior, is not yet fully understood [175, 176].

3.5 Mechanism of Action

It would indeed be impossible to explain all the varied biological actions of testosterone by one biochemical mechanism. Androgens, as well as the other steroid hormones adrenocorticoids, estrogens and progestins, exert potent physiological effects on sensitive tissues, yet are present in the body in only extremely low concentrations (e.g., 0.1–1.0 nM). The majority of investigations to elucidate the mechanisms of action of androgens have dealt with actions in androgen-dependent tissues and, in particular, the rat ventral prostate. The results of these studies have indicated that androgens act primarily to regulate gene expression and protein biosynthesis by the formation of a hormone–receptor complex, analogous to the mechanisms of action of estrogens and progestins. Extensive studies directed at elucidating the general mechanism of steroid hormone action have been performed for over three decades, and several reviews have emerged on this subject [177–190].

Jensen and Jacobson [191], using radiolabeled 17β-estradiol, were the first to show that a steroid was selectively retained by its target tissues. Investigations of a selective uptake of androgens by target cells performed during the early 1960s were complicated by a low specific activity of the radiolabeled hormones and the rapid metabolic transformations. Nonetheless, it was noted that target cells retained primarily unconjugated metabolites, whereas conjugated metabolites were present in nontarget cells such as blood and liver [192, 193]. With the availability of steroids of high specific activity, later studies demonstrated the selective uptake and retention of androgens by target tissues [23, 24, 115, 194, 195]. In addition, DHT was found to be the steroidal form selectively retained in the nucleus of the rat ventral prostate [23, 115]. This discovery led to the current concept that testosterone is converted by 5α-reductase to DHT, which is the active form of cellular androgen in androgen-dependent tissues such as the prostate. In general, DHT is thought to be the active androgen in tissues that express 5α-reductase (e.g., the prostate), whereas testosterone appears to directly mediate these effects in muscle and bone where 5α-reductase is absent.

The rat prostate has been the most widely examined tissue, and current hypotheses on the mode of action of androgens are based largely on these studies (see Fig. 6). The lipophilic steroid hormones are carried in the bloodstream, with the majority of the hormones reversibly bound to serum carrier proteins and a small amount of free steroids. The androgens circulating in the bloodstream are the sources of steroid hormone for androgen action in target tissues. Testosterone, synthesized and secreted by the testis, is the major androgen in the bloodstream and the primary source of androgen for target tissues in men. Dehydroepiandrosterone (DHEA) and androstenedione also circulate in the bloodstream and are secreted by the adrenal gland under the regulation of adrenocorticotrophic hormone (ACTH). DHEA and androstenedione supplement the androgen sources in normal adult men, but these

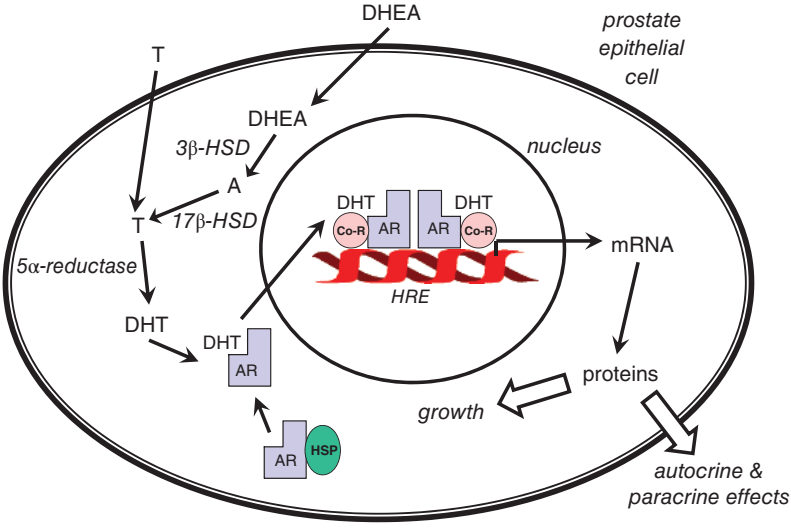


Fig. 6 Mechanism of action of 5α-dihydrotestosterone (DHT). T = testosterone; A = androstenedione; DHEA = dehydroepiandrosterone; AR = androgen receptor; HSPs = heat shock proteins; Co-R = coregulators/coactivators; HRE = hormone response element; 3β-HSD = 3β-hydroxysteroid dehydrogenase; and 17β-HSD = 17β-hydroxysteroid dehydrogenase.

steroids are the important circulating androgens in women. The free circulating androgens diffuse passively through the cell membrane and are converted to the active androgen 5α-DHT within the target tissues that express the enzyme.

The androgens act on target cells to regulate gene expression and protein biosynthesis via the formation of steroid–receptor complexes. Those cells sensitive to the particular steroid hormone (referred to as target cells) contain high-affinity steroid receptor proteins capable of interacting with the steroid [25, 196]. The binding of androgen with the receptor protein is a necessary step in the mechanism of action of the steroid in the prostate cell. The results of early studies suggested that the steroid receptor proteins were located in the cytosol of target cells [191] and, following formation of the steroid–receptor complex, the latter would be translocated into the nucleus of the cell. More recent investigations on androgen action have indicated that the unoccupied receptor proteins are present in the cytoplasm bound to various heat shock proteins (Hsps) and chaperones such as Hsp70 and Hsp90 to prevent degradation [197]. Binding of androgen with the androgen receptor results in a conformational change of the receptor complex, a disassociation of the Hsp proteins, and nuclear localization of the steroid–receptor complex.

In the nucleus, the steroid–androgen receptor complex is activated, resulting in the formation of a homodimer [186]. The homodimer then interacts with particular regions of the cellular DNA that are referred to as androgen-responsive elements (AREs), and also with various coactivator/coregulator proteins and other nuclear transcriptional factors [197].

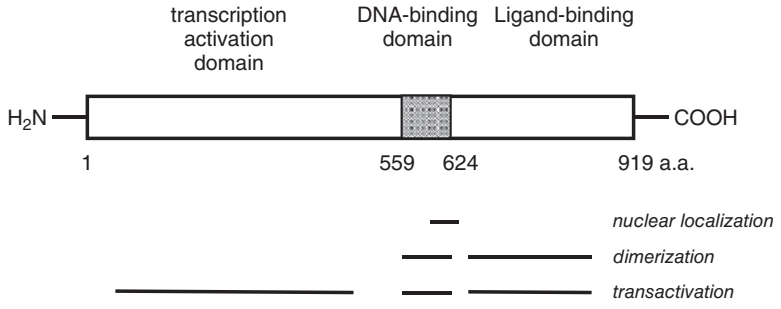


Fig. 7 Schematic diagram of the androgen receptor.

Binding of the nuclear steroid–receptor complex to DNA initiates a transcription of the DNA sequence to produce mRNA. Finally, the elevated levels of mRNA lead to an increased protein synthesis in the endoplasmic reticulum; the proteins synthesized include enzymes, receptors and/or secreted factors that subsequently result in the steroid hormonal response regulating cell function, growth, and differentiation.

Extensive structure–function studies on the androgen receptor (AR) have identified regions critical for hormone action. The AR is encoded by the *AR* gene located on the X chromosome, and the *AR* gene is comprised of eight exons. The human AR contains approximately 900–920 amino acids, and the exact length varies due to polymorphisms in the NH_2-terminal of the protein. The primary amino acid sequences of AR, as well as of the various steroid hormone receptors, were deduced from cloned cDNAs [186, 188]. The calculated molecular weight of AR is approximately 98 kDa, based on amino acid composition; however, the AR is a phosphoprotein and migrates higher at approximately 110 kDa in sodium dodecyl sulfate (SDS) gel electrophoresis. The steroid receptor proteins form part of a larger family of nuclear receptor proteins that also include receptors for vitamin D, thyroid hormones, and retinoids. The overall structural features of the AR have strong similarities to the other steroid hormone receptors (Fig. 7), with proteins containing regions that bind to the DNA and bind to the steroid hormone ligand [189, 198, 199]. A high degree of homology (sequence similarities) in the steroid receptors is found in the DNA-binding region that interacts with the hormone response elements (HREs). The DNA-binding region is rich in cysteine amino acids and chelate zinc ions, forming finger-like projections called zinc fingers that bind to the DNA. The hormone-binding domain (or ligand-binding domain; LBD) is located on the COOH-terminal of the protein. Structure–function studies of cloned receptor proteins have also identified regions of the molecules that are important for nuclear localization of the receptor, receptor dimerization, interactions with nuclear transcriptional factors, and the activation of gene transcription. Importantly, two regions of the AR protein are identified as transcriptional activation domains; the domain on the NH_2-terminal region may interact with both coactivators and corepressors, while the COOH-terminal domain initiates transcriptional activation only upon binding of an agonist such as 5α-DHT. The interactions necessary for formation

of the steroid–receptor complexes and subsequent activation of gene transcription are complicated, involve multiple protein partners referred to as coactivators and corepressors, and leave many unanswered questions.

Although the tertiary structure of the entire AR has not been determined, the crystallographic structure of the LBD has been reported [200, 201]. The AR LBD consists of an α-helical sandwich, similar to the LBDs reported for other nuclear receptors, and contains only 11 helices (no Helix 2) and four short β-strands. Minor differences in the two reported crystallographic structures are likely due to limits of experimental resolution, differences in data interpretation, and the use of different ligands for crystallization. The endogenous ligand DHT (**2**) interacts with helices 3, 5, and 11, and the DHT-bound AR LBD has a single, continuous helix 12. Similar interactions are observed with metribolone (methyltrienolone, **55**); however, helix 12 is split into two shorter helical segments. Overall, the binding of steroidal ligands to amino acid residues of the AR LBD involves two hydrogen bonds with the 3-ketone function, and two hydrogen bonds with the 17β-hydroxyl group. Hydrophobic interactions of several amino acid residues with the steroid scaffold are also observed. Investigations on selective androgen receptor modulator (SARM binding to the LBD have provided further insights into the molecular interactions of nonsteroidal agents with AR [202].

Additional information on receptor structure–function has been obtained by analyzing AR mutations in patients with various forms of androgen resistance and abnormal male sexual development [189, 199, 203–205]. Two polymorphic regions have been identified in the NH_2-terminal region, encoding a polyglycine repeat and a polyglutamate tract. Certain polymorphic regions have recently been shown to significantly alter AR levels, stability, or transactivation [199]. These repeats are useful in the pedigree analysis of patients [189]. Mutations in the AR have been identified in patients with either partial or full androgen insensitivity syndrome (AIS), with the majority of mutations identified in exons 4 through 8 encoding the DNA-binding domain and the hormone-binding domain. Finally, studies with the human LNCaP prostate cancer cell line have provided interesting results regarding receptor protein structure and ligand specificity. The LNCaP cells exhibited an enhanced proliferation in the presence of androgens, but these cells unexpectedly proliferated in the presence of estrogens, progestins, cortisol, or the antiandrogen flutamide [206, 207]. Analysis of the cDNA for the LNCaP AR revealed that a single base mutation in the LBD was present, and this resulted in the increased affinity for progesterone and estradiol [208]. The crystallographic structures of the LBD with the T877A mutation confirm that the mutated AR LBD can accommodate larger structures at the C-17 position [200, 201].

The ultimate action of androgens on target tissues is the stimulation of cellular growth and differentiation through the regulation of protein synthesis, and numerous androgen-inducible proteins have been identified [199]. One prominent androgen-inducible protein is prostate-specific antigen (PSA), a serine protease that is expressed by secretory prostate epithelial cells and utilized as blood test in screening for possible prostate diseases such as prostate cancer. Three AREs have been identified in the promoter regions of the *PSA* gene [209–211]. Another androgen-regulated gene which has been examined extensively in rats is the gene encoding

the protein probasin [212, 213], a 20-kDa secretory protein from the rat dorsolateral prostate that is structurally similar to serum globulins. Recently, a transmembrane serine protease called TMPRSS2 was identified in human prostate cells that may have a role in male reproduction and is overexpressed in poorly differentiated prostate cancer [214]. Other proteins induced by androgens include spermine-binding protein [215], keratinocyte growth factor (KGF or FGF-7) [216], androgen-induced growth factor (AIGF or FGF-8) [217, 218], nerve-growth factor [219], epidermal growth factor (EGF) [220], c-myc [221], protease D [222], β-glucuronidase [223], and α_{2u}-globulin [224, 225]. Studies of these proteins have suggested that androgens act by enhancing the transcription and/or translation of specific RNAs for the proteins. The AR also represses the gene expression of certain proteins such as glutathione *S*-transferase, TRPM-2 (which is involved in apoptosis), and cytokines such as interleukin (IL)-4, IL-5, and γ- interferon (IFN) [199, 226].

While most biochemical studies have been focused on the rat ventral prostate, some groups began to investigate the presence of cellular receptor proteins in other androgen-sensitive tissues. ARs have been reported in seminal vesicles [227, 228], sebaceous glands [229–231], testis [230, 232], epididymis [227, 233, 234], kidney [235], submandibular gland [236, 237], pituitary, and hypothalamus [238–244], bone marrow [245, 246], liver [247], and androgen-sensitive tumors [248, 249]. Although DHT is the active androgen in rat ventral prostate, it is not the only functioning form in other androgen-sensitive cells. In ventral prostate and seminal vesicles, DHT is readily formed but is metabolized only slowly and therefore can accumulate and bind to receptors. A comparison of the binding kinetics for testosterone and DHT also showed that testosterone dissociates faster, implying an extended retention of DHT by the AR [250]. In other tissues, such as brain, kidney or skeletal muscle, DHT is not readily formed and is metabolized quickly compared to testosterone. Species variations have also been demonstrated, the most striking example being the finding that 5α-androstane-3α,17α-diol interacts specifically with cytosolic receptor protein from dog prostate [251] and may be the active androgen in this species [252]. Apparently, the need for a 17β-hydroxyl is not essential in all species.

Thus, current findings indicate that AR proteins vary in steroid specificity among different tissues from the same species, as well as among different species. Nevertheless, the basic molecular mechanism of action of the androgens in androgen-sensitive tissues is consistent with the results of the studies on rat ventral prostate.

The manner whereby the androgens exert their anabolic effects has not been studied so extensively. Indeed, the conversion of testosterone to DHT was shown to be insignificant in skeletal and levator ani muscles, which suggests that the androgen-mediated growth of muscle is due to testosterone itself [253, 254]. Classical steroid receptors for testosterone are found in the cytoplasm of the levator ani and quadriceps muscles of the rat [255, 256]. Unlike the AR in the prostate, DHT had a lower affinity than testosterone for the AR in muscle. Notably, ARs have also been identified in other muscle tissues, including cardiac muscle [257–262].

In addition to the genomic mechanisms, nongenomic pathways for androgen action through the AR have been reported in various tissues, including spermatogenesis

[263], oocytes [264], skeletal muscles [265], and prostate cancer cells [266]. Several characteristics of possible nongenomic pathways include a rapid timeframe for effects (varying from seconds to hours), the regulation of androgen-responsive genes that do not contain androgen response elements, and alterations of intracellular signaling pathways. The rapid activation of kinase signaling pathways, such as the activation of MAP kinase and ERK kinase pathways, and the modulation of intracellular calcium levels, are two examples of the nongenomic mechanisms of action of androgens and ARs.

4 Synthetic Androgens

4.1 Current Drugs on the Market

Currently available synthetic androgens used as therapeutics are listed in the following table.

Generic name (structure)	*Trade name*	*U.S. manufacturer*	*Chemical class*	*Dose*
Testosterone enanthate (**31**)	Delatestryl	Various suppliers	Androstane	Injection: 200 mg ml^{-1}
Testosterone cypionate (**33**)	Depotestosterone, Andronate	Various suppliers	Androstane	Injection: 100 mg ml^{-1} 200 mg ml^{-1}
Testosterone pellets (**1**)	Testopel	Bartor Pharmacal	Androstane	Pellets: 75 mg
Testosterone transdermal system (**1**)	Androderm, Testoderm	Various suppliers	Androstane	Transdermal: 12.2 mg 24.3 mg
Testosterone gel (**1**)	AndroGel 1%	AbbVie	Androstane	Gel: 1% testosterone
	Testim 1%	Auxilium	Androstane	Gel: 1% testosterone
Testosterone, buccal system (**1**)	Striant	Columbia	Androstane	Mucoadhesive: 30 mg
Methyltestosterone (**5**)	Methyltestosterone	Various suppliers	Androstane	Tablets: 10 mg 25 mg Tablets (buccal): 10 mg
	Methitest	Global	Androstane	Capsules: 10 mg
	Testred	Valeant	Androstane	Capsules: 10 mg
	Android	Valeant	Androstane	Capsules: 10 mg
	Virilon	Star	Androstane	Capsules: 10 mg
Fluoxymesterone (**36**)	Halotestin	Pfizer	Androstane	Tablets: 2 mg 5 mg 10 mg
	Fluoxymesterone	Various suppliers	Androstane	Tablets: 10 mg
Danazol (**77**)	Danazol	Various suppliers	Androstane	Capsules: 50 mg 100 mg 200 mg
Testolactone (**38**)	Teslac	Bristol-Myers Squibb	Androstane	Tablets: 50 mg

4.2 Therapeutic Uses and Bioassays

The primary uses of synthetic androgens are the treatment of disorders of testicular function and of cases with decreased testosterone production. Several types of clinical condition result from testicular dysfunction. Information on the biochemistry and mechanism of action of testosterone that has accumulated over the past 30 years has greatly aided in the elucidation of the underlying pathophysiology of these diseases. Two reviews describe in greater detail the mechanisms involved in disorders of testicular function and androgen resistance [267, 268].

Hypogonadism arises from the inability of the testis to secrete androgens, and can be caused by various conditions. These hypogonadal diseases can, in many cases, result in disturbances in sexual differentiation and function and/or sterility. Primary hypogonadism is the result of a basic disorder in the testes, while secondary hypogonadism results from the failure of pituitary and/or hypothalamic release of gonadotropins and thus a diminished stimulation of the testis. Usually, primary hypogonadism is not recognized in early childhood (with the exception of cryptorchidism) until the expected time of puberty. This testosterone deficiency is corrected by androgen treatment for several months, at which time the testes are evaluated for possible development. Long-term therapy is necessary if complete testicular failure is present. Patients with Klinefelter's syndrome, a disease in which a genetic male has an extra X chromosome, have low testosterone levels and can also be treated by androgen replacement.

Male pseudohermaphroditism incorporates disorders in which genetically normal men do not undergo normal male development:

- *Testicular feminization* is observed in patients who have normal male XY chromosomes but the male genitalia and accessory sex glands do not develop; rather, the patients have female external genitalia. These patients are unresponsive to androgens and have defective ARs [269–271].
- An alternative *male pseudohermaphroditism* results from a deficiency of the enzyme 5α-reductase [272, 273]. Since DHT is necessary for early differentiation and development, the patients again develop female genitalia; later, some masculinization can occur at the time of puberty due to elevated testosterone levels in the blood.
- *Reifenstein syndrome* is an incomplete pseudohermaphroditism. In these patients, the androgen levels are normal, 5α-reductase is present, and elevated LH levels are found. Partially deficient ARs are present in these patients [269, 271].

In most cases of male pseudohermaphroditism, androgen replacement has little or no effect, and thus steroid treatment is not recommended.

Deficiencies of circulating gonadotropins lead to secondary hypogonadism. This condition can be caused by disorders of the pituitary and/or hypothalamus, resulting in diminished secretions of neurohormones. The lack of stimulation of the seminiferous tubules and the Leydig cells due to the low levels of these neurohormones decreases androgen production. Drugs such as neuroleptic phenothiazines and the stimulant marijuana can also interfere with the release of gonadotropins. The use of androgens in secondary hypogonadism is symptomatic.

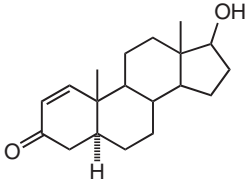
Synthetic androgens have also been used in women for the treatment of endometriosis, abnormal uterine bleeding, and menopausal symptoms, but their utility is severely limited by the virilizing side effects of these agents. Two weak androgens – calusterone and 1-dehydrotestolactone – have been used clinically in the treatment of mammary carcinoma in women. The mode of action of these drugs in the treatment of breast cancer is unknown, but it is not simply related to their androgenicity [274]. More recent evidence on the ability of these compounds to inhibit estrogen biosynthesis catalyzed by aromatase suggests that they effectively lower estrogen levels *in vivo* [150].

The various analytical methods used to establish the androgenic properties of steroidal substances have been reviewed by Dorfman [275]. Traditionally, androgens have been assayed using the capon comb growth method, and by using the seminal vesicles and prostate organs of rodents. Increases in the weight and/or growth of the capon comb have been used to denote androgenic activity following injection or topical application of a solution of the test compound in oil [276]. A number of minor modifications of this test have been described [277–279]. Increases in the weight of the seminal vesicles and ventral prostate of immature castrated male rats has provided another measure of androgenic potency [280–283]. In this case, the test compound is administered either intramuscularly or orally and the weight of the target organs is compared with those of control animals. *In-vitro* evaluations of the relative affinity of potential androgens for the AR have also become an important tool in assessing the biological activity of androgens [123, 284].

4.3 Structure–Activity Relationships for Steroidal Androgens

4.3.1 Early Modifications

Most of the early structure–activity relationship studies concerned minor modifications of testosterone and other naturally occurring androgens. Studies in animals [285] and humans [286] showed the l7β-hydroxyl function to be essential for androgenic and anabolic activity. In certain cases, esterification of the 17β-hydroxyl group not only enhanced but also prolonged the anabolic and androgenic properties [287].



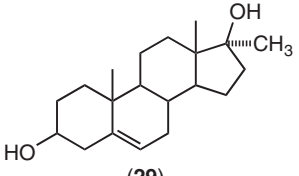
(**28**)

Reduction of the A-ring functional groups has variable effects on activity. For example, the conversion of testosterone to DHT has little effect or may even increase potency in a variety of bioassay systems [288–290]. The 1-dehydro isomer of testosterone (**28**) and related compounds are potent androgenic and anabolic steroids [285]. On the other hand, changing the A/B *trans* stereochemistry of known androgens such as androsterone (**3**) and DHT to the A/B *cis*-etiocholanolone (**19**) and 5β-dihydrotestosterone (**14**), respectively, drastically reduces both the anabolic and androgenic properties [291–293]. These observations established the importance of the A/B *trans* ring juncture for activity.

4.3.2 Methylated Derivatives

The discovery that C-17α-methylation conferred oral activity on testosterone

prompted the synthesis of additional C-17α-substituted analogs. Increasing the chain length beyond methyl invariably led to a decrease in activity [294]. However, as a result of these studies, 17α-methylandrost-5-ene-3β,17β-diol (methandriol, **29**) was widely evaluated in humans as an anabolic agent and showed no clinical advantage of methandriol over 17α-methyltestosterone (**5**) [295].



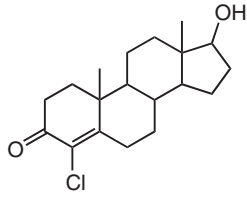
(**29**)

4.3.3 Ester Derivatives

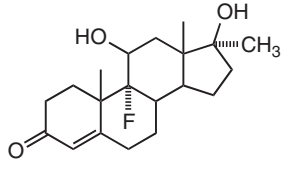
As early as 1936 it was recognized that the esterification of testosterone at the 17β-hydroxy moiety markedly prolonged the activity of this androgen when it was administered parenterally [296]. This modification enhances the lipid solubility of the steroid and, after injection, permits a local depot effect. The acyl moiety is usually derived from a long-chain aliphatic or arylaliphatic acid such as propionic, heptanoic (enanthoic), decanoic, cyclopentylpropionic (cypionic), or β-phenylpropionic acid (**30**–**34**).

4.3.4 Halo Derivatives

In general, the preparation of halogenated testosterone derivatives has been therapeutically unrewarding. 4-Chloro-17β-

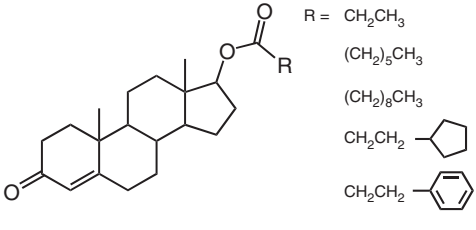


(**35**)



(**36**)

hydroxyandrost-4-en-3-one (chlorotestosterone, **35**) and its derivatives are the only chlorinated androgens that have been used clinically, albeit sparingly [297]. The introduction of a 9α-fluoro and an 11β-hydroxy substituents (analogous to synthetic glucocorticoids) yields 9α-fluoro-11β, 17β-dihydroxy-17α-methylandrost-4-en-3-one (fluoxymesterone; Halotestin, **36**), which is an orally active androgen exhibiting an approximately fourfold greater oral activity than 17α-methyltestosterone. Early clinical studies with fluoxymesterone indicated an anabolic potency that was 11-fold that of the unhalogenated derivative [298–300], but nitrogen balance studies revealed an activity that was only threefold that of 17α-methyltestosterone [301]. Because of the lack of any substantial separation of anabolic and androgenic activity, halotestin is used primarily as an

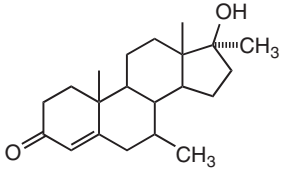


(30) propionate
(31) heptanoate (enanthate)
(32) decanoate
(33) cyclopentylpropionate (cypionate)
(34) β-phenylpropionate

orally effective androgen, particularly in the treatment of mammary carcinoma [302, 303].

4.3.5 Other Androgen Derivatives

Several synthetic steroids having weak androgenic activity have also been utilized in patients. 7β,17α-Dimethyltestosterone (calusterone, **37**) and 1-dehydrotestolactone (Testlac, **38**) are very weak androgenic agents that have been used in the treatment of advanced metastatic breast cancer [304–306].



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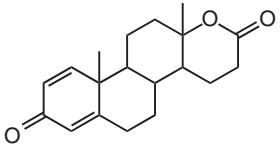


(**38**)

4.3.6 Summary of Structure–Activity Relationships of Steroidal Androgens

As with other areas of medicinal chemistry, the desire to relate chemical structure to androgenic activity has attracted the attention of numerous investigators. Although it is often difficult to interrelate biological results from different laboratories, androgenicity data from the same laboratory afford useful information. In evaluating the data, care must be taken to note not only the animal model employed but also the mode of administration. For example, marked differences in androgenic activity can be found when compounds are evaluated in the chick comb assay (local application) as opposed to the rat ventral prostate assay (subcutaneous or oral). The chick comb assay measures "local androgenicity," and is believed to minimize such factors as absorption, tissue distribution, and metabolism, which complicate the interpretation of *in-vivo* data in terms of hormone–receptor interactions.

Furthermore, although the rat assays correlate well for various C_{19} steroids with what is eventually found in humans, few studies of comparative pharmacology have been performed. Indeed, DHT may not be the principal mediator of androgenicity in all species. For example, a cytosol receptor protein has been found in normal and hyperplastic canine prostate that is specific for 5α-androstane-3α,17α-diol [250].

Since the presence of the 17β-hydroxyl group was demonstrated at a very early stage to be an important feature for androgenic activity in rodents, most investigators interested in structure–activity relationships maintained this function and modified other parts of the testosterone molecule. Three observations can be made based on these studies: (i) the 1-dehydro isomer of testosterone is at least as active as testosterone; (ii) the 1- and 4-keto isomers of testosterone and DHT have variable activities; and (iii) the 2-keto isomers of testosterone and DHT consistently lack appreciable activity.

The first attempt to ascertain the minimal structural requirements for androgenicity was made by Segaloff and Gabbard [307]. Whereas, the oxygen function at position 3 could be removed from testosterone with little reduction in androgenic activity, removal of the hydroxyl group from position 17 sharply reduced the androgenicity. As a continuation of these studies, the hydrocarbon nucleus, 5α-androstane (**39**), was synthesized [307], and it too was found to possess androgenicity when applied

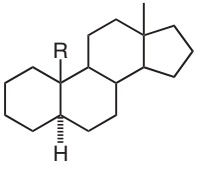
topically or given intramuscularly in the chick comb assay (albeit at high doses). On the other hand, it subsequently emerged that the 19-nor analog, 5α-estrane (**40**), had less than 1% of the androgenic activity of testosterone propionate in castrated male rats [308].

Nonetheless, the studies of Segaloff and Gabbard set the stage for a more thorough analysis of 3-deoxy testosterone analogs by Syntex scientists [309, 310]. The relative androgenicity of the isomeric A-ring olefins of 3-deoxy testosterones was the order $\Delta^1 > \Delta^2 > \Delta^3 > \Delta^4$. The Δ^2-isomer displayed the greatest anabolic activity and the best anabolic-to-androgenic ratio.

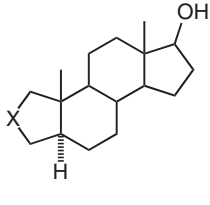
On the basis that sulfur is bioisosteric with CH=CH, Wolff and coworkers [311] synthesized the thio, seleno, and tellurio androstanes, all of which displayed androgenic activity. When the heteroatom was oxygen, however, the compound (**41**) was essentially devoid of androgenicity [312]. The oxygen analog was said to be inactive because oxygen is isosteric with CH_2 rather than CH_2=CH_2. Thus, a minimum ring size was found to be required for activity. When the oxygen atom was introduced as part of a six-membered A-ring, an active androgen resulted [312].

As with the case of the double-bond isomers, the position of the oxygen atom was found to be important. The substitution of oxygen at C-2 gives rise to the most active compound, and the order of activity was 2 > 3 ≫ 4. As pointed out by Zanati and Wolff [312], these and earlier results are consistent with the concept that "… the activity-engendering group in ring A is wholly steric and that, in principle, isosteric groups of any type could be used to construct an androgenic molecule." Further support for this idea has been obtained from X-ray crystallographic structure determinations [313]. Zanati and Wolff [314] reported that even the full steroid nucleus is not essential for activity, with 7α-methyl 1,4-seco-2,3-bisnor-5α-androstan-17β-ol (**42**) having 50% of the anabolic activity of testosterone.

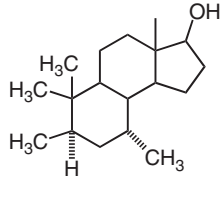
Studies by Segaloff and Gabbard [315] illustrated the marked enhancement of androgenicity achieved when a double bond was introduced at C-14. Both, 14-dehydrotestosterone and the corresponding 19-nor analog, were found to be potent androgens when applied topically. The introduction of a 7α-methyl substituent also resulted in active androgens [316]. The effects of either 7α-methyl or 14-dehydro modifications are more pronounced for 19-nortestosterone than for testosterone; the 14-dehydro modification had a greater effect on local androgenicity, whereas 7α-methylation had a more positive effect on systemic androgenicity. A marked synergism resulted when both the 14-dehydro and 7α-methyl modifications were present.



(**39**) R = CH_3
(**40**) R = H



(**41**)
X = O



(**42**)

Tab. 1 Binding affinity of various androgens for rat ventral prostate receptor protein.

Steroid	K_B (M^{-1})
5α-DHT	6.9×10^8
5β-DHT	6.4×10^7
17β-Testosterone	4.2×10^8
17α-Testosterone	2.1×10^7
Androstenedione	1.3×10^7
5α-Androstanedione	3.5×10^7
19-Nortestosterone	8.6×10^8
14-Dehydrotestosterone	4.4×10^8
14-Dehydro-19-nortestosterone	5.9×10^8
7α-CH_3-14-Dehydro-19-nortestosterone	5.0×10^8

The characterization of a specific receptor protein in androgen target tissues has made it possible to directly analyze the receptor affinity of various testosterone analogs. Liao and coworkers [284] were the first to employ this parameter for comparison with systemic androgenicity. As would be expected, the receptor affinity data did not necessarily correlate with the systemic androgenicity although, in some cases, such as with 7α-methyl-19-nortestosterone, there was a good agreement. This was not the case, however, for 19-nortestosterone. Receptor binding analyses of androgens were also performed by other groups [123, 317], and their findings are summarized in Table 1. Whereas, the importance of the A/B *trans* ring fusion and 17β-hydroxyl prevailed, the data failed to demonstrate the potency previously noted for 7α-methyl-14-dehydro-19-nortestosterone. Moreover, 19-nortestosterone displayed a receptor affinity greater than that of DHT, yet its androgenicity was much less than that of DHT.

These differences in correlations between receptor assays and *in-vivo* data should not cloud the importance of the receptor studies. The receptor assays measure affinity for the receptor protein, and this property is shared by androgens as well as antiandrogens. Moreover, such assays cannot predict the disposition and metabolic fate of an androgen following administration. A summary of the structure–activity relationships for androgens is provided in Fig. 8.

4.4 Nonsteroidal Androgens, Selective Androgen Receptor Modulators (SARMs)

Until the mid-1990s, androgen agonistic activities were limited to steroidal molecules. Synthetic modifications of

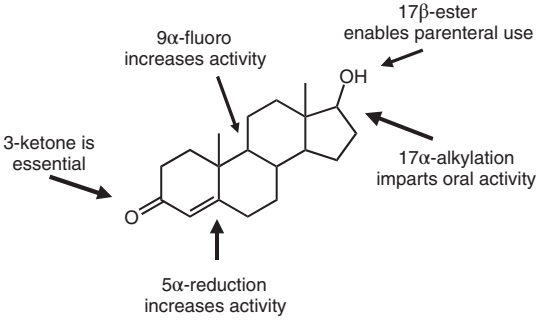
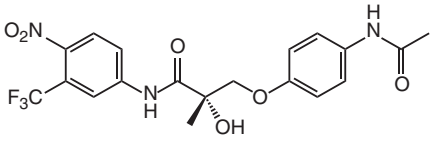


Fig. 8 Summary of structure–activity relationships for androgens.

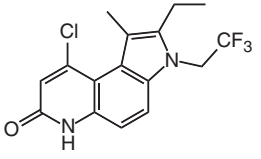
nonsteroidal androgen antagonists (see Sect. 6.2.2.2) resulted in molecules that exhibited androgen agonist activities in various tissues. These molecules are now referred to as selective androgen receptor modulators, which describes compounds that act as antagonists or weak agonists in the prostate but exhibit agonist activities in other tissues such as muscle and bone (for reviews, see Refs [319–321]).

The pharmacophores associated with SARM activity include *N*-arylpropionamides, bicyclic hydantoins, and various quinolinones. Extensive research has been performed on the *N*-arylpropionamides, derived from structural modifications of the antiandrogen bicalutamide [320]. Initial studies identified the sulfide analogs as being effective *in vitro* when binding to the AR [321], but these analogs lacked significant *in-vivo* activity due to a rapid oxidative metabolism of the sulfide [322]. Replacement of the sulfide linkage with an ether bridge resulted in potent agonist activity both *in vitro* and *in vivo* for andarine (**43**) [322–324]. Currently, these *N*-arylpropionamide SARMs are under clinical investigations for the treatment of muscle wasting, osteoporosis, and other conditions associated with aging or androgen deficiency.

Another pharmacophore that has been extensively investigated are the bicyclic and tricyclic quinolinones with several analogs, including LGD-3303 (**44**), demonstrating oral bone anabolic activities [325, 326]. Several bicyclic hydantoin derivatives have shown potent muscle anabolic activities [327, 328]. Clinical investigations of SARMs are currently underway in patients with various androgen-dependent disorders to determine beneficial pharmacological androgenic activities without unwanted side effects.



(**43**)



(**44**)

4.5 Absorption, Distribution, and Metabolism

Numerous factors are involved in the absorption, distribution, and metabolism of the synthetic androgens, and the physico-chemical properties of these steroids greatly influence the pharmacokinetic parameters. The lipid solubility of a synthetic steroid is an important factor in its intestinal absorption. The acetate ester of testosterone demonstrated an enhanced absorption from the gastrointestinal tract over both testosterone and 17α-methyltestosterone (**58**). Injected solutions of testosterone in oil result in a rapid absorption of the hormone from the injection site; however, rapid metabolism greatly decreases the biological effects of the injected testosterone. The esters of testosterone are much more nonpolar and, when injected intramuscularly, are absorbed more slowly. As a result, commercial preparations of testosterone propionate are administered every few days. Increasing the size of the ester functionality enables testosterone esters such as the ethanate or cypionate to be given in a depot injection lasting two to four weeks.

Once absorbed, the steroids are transported in the circulation, primarily in a protein-bound complex. Testosterone and other androgens are reversibly associated with certain plasma proteins, and the unbound fraction can be absorbed into target cells to exert its action. The structure–binding relationships of the natural and synthetic androgens to SHBG have been extensively investigated [63–66, 71–77]. A 17α-hydroxyl group is essential for binding, while the presence of a 17α-substituent such as the 17α-methyl moiety decreases its affinity. The 5α-reduced androgens bind with the highest affinity. A much smaller quantity of the androgen is bound to other plasma proteins, principally albumin and transcortin, or CBG.

The metabolism of synthetic androgens is similar to that of testosterone, and has been extensively studied [284, 315–327]. Introduction of the 17α-methyl group greatly retards the metabolism, thus providing oral activity. Reduction of the 4-en-3-one system in synthetic androgens to give the various α- and β-isomers occurs *in vivo* [329, 330]. Finally, aromatization of the A-ring can also occur [146–148]. One analog that demonstrates an alternate metabolic pattern is 4-chlorotestosterone, which in humans gave rise to an allylic alcohol, 4-chloro-3α-hydroxyandrost-4-en-17-one [331]. A number of other halogenated testosterone derivatives subsequently were found to take this abnormal reduction path *in vitro* [332]. It was proposed that fluorine or chlorine substituents at the 2-, 4-, or 6-position in testosterone interfere with the usual α,β-unsaturated ketone resonance so that the C-3 carbonyl electronically resembles a saturated ketone.

4.6
Toxicities

The use of androgens in women and children can often result in virilizing or masculinizing side effects. In boys, an acceleration of the sexual maturation is seen, while in girls and women the growth of facial hair and a deepening of the voice can be observed [333, 334]. These effects are reversible when medication is stopped, but prolonged treatment can produce effects that are irreversible. An inhibition of gonadotropin secretion by the pituitary can also occur in patients receiving androgens.

Both, males and females experience salt and water retention resulting in edema. This edema can be treated by either maintaining a low-salt diet or by using diuretic agents. Liver problems are also encountered with some of the synthetic androgens, with clinical jaundice and cholestasis often developing after the use of 17α-alkylated products [335–338]. Various clinical laboratory tests for hepatic function, such as bilirubin concentrations, bromosulfophthalein (BSP) retention and glutamate transaminase and alkaline phosphatase activities, are affected by these androgen analogs.

5
Anabolic Agents

5.1
Current Drugs on the Market

Currently available anabolic agents used as therapeutics are listed in the following table.

Generic name (structure)	*Trade name*	*U.S. manufacturer*	*Chemical class*	*Dose*
Nandrolone decanoate (**45**)	Deca-durabolin	Organon	Estrane	Injection (In Oil): 200 mg ml^{-1}
Oxandrolone (**67**)	Oxandrin	Savient	Androstane	Tablets: 10 mg 2.5 mg
Oxymetholone (**65**)	Anadrol-50	Alaven	Androstane	Tablets: 50 mg

5.2 Therapeutic Uses and Bioassays

Many synthetic analogs of testosterone were prepared in order to separate the anabolic activity of the C_{19} steroids from their androgenic activity. Although the goal of a pure synthetic anabolic that retains no androgenic activity has not been accomplished, several preparations are now available commercially that have high anabolic:androgenic ratios. Extensive reviews on anabolic agents are available [4, 10].

The primary criterion for assessing the anabolic activity of a compound is the demonstration of a marked retention of nitrogen. This nitrogen-retaining effect is the result of an increased protein synthesis and a decreased protein catabolism in the body [339]. Thus, the urinary nitrogen excretion – particularly of urea – is greatly diminished. The castrated male rat serves as the most sensitive animal model for nitrogen retention, although other animals have been used [340–342]. Another bioassay for anabolic activity involves monitoring the increase in levator ani muscle mass in rats following the administration of an anabolic agent [281, 282]. This measure of the myotrophic effect correlates well with the nitrogen retention bioassay, and the two assays are usually performed together when determining anabolic activity.

Anabolic steroids also exert other effects on the body, with skeletal mineralization and bone maturation notably being enhanced by androgens and anabolic agents [35]. Such agents decrease calcium excretion by the kidney, and this results in an increased deposition of both calcium and phosphorus in the bones. Androgenic and anabolic agents also can influence erythropoiesis (red blood cell formation) via two mechanisms of action, namely an increased production of erythropoietin and an enhanced responsiveness of the tissue [36].

These various biological activities of anabolic agents have prompted their use in a range of treatment protocols, albeit with varying success. Previously, clinical trials have demonstrated the effectiveness of anabolic steroids in inducing muscle growth and development in some diseases [343]. Anabolic steroids are effective in the symptomatic treatment of various malnourished states due to their ability to increase protein synthesis and decrease protein catabolism. The treatment of diseases such as malabsorption, anorexia nervosa, emaciation and malnutrition as a result of psychoses includes dietary supplements, appetite stimulants and anabolics [344–349]. An improved postoperative recovery with the adjunctive use of anabolic agents has been demonstrated in numerous clinical studies [344, 350–354]. However,

the usefulness of these agents in other diseases such as muscular dystrophies and atrophies, and in geriatric patients, has not been observed.

Anabolic steroids also have the ability to lower serum lipid levels *in vivo* [354–357]. The most widely studied agent in this respect is oxandrolone, which dramatically lowers serum triglycerides and, to a lesser extent, cholesterol levels at pharmacological doses [358–360]. The proposed mechanism of this hypolipidemic effect includes both an inhibition of triglyceride synthesis [361] and an increased clearance of the triglycerides [362]. The androgenic side effects of the anabolics and their lack of superiority over more efficacious hypolipidemic agents have curtailed their use in the treatment of these conditions, however.

Although now supplanted by the availability of recombinant erythropoietin, the stimulation of erythropoiesis by anabolics has resulted in the use of these agents for the treatment of various anemias [363–366]. Anemias arising from deficiencies of the bone marrow are particularly responsive to pharmacological doses of anabolic agents. The treatment of aplastic anemia with anabolics and corticosteroids has been proven effective [363–366], while secondary anemias resulting from inflammation, renal disease or neoplasia have also been shown responsive to anabolic steroid administration [36, 366–370]. Finally, synthetic anabolic agents have been prescribed for women with osteoporosis [35] and for children with delayed growth [361]. Although these applications have produced limited success, the virilizing side effects that occur have severely limited their usefulness, particularly in children.

The methods employed to determine the anabolic or myotrophic properties of steroids have been reviewed [371]. Generally, these are based on an increase in nitrogen retention and/or muscle mass in various laboratory animals. The castrated male rat is the most widely used [340], but dogs and ovariectomized monkeys have also been employed [341, 342]. Although it is generally agreed that variations in urinary nitrogen excretion relate to an increase or decrease in protein synthesis, nitrogen balance assays are not without their limitations [372]. This is partly because such studies fail to describe the shifts in organ protein and measure only the overall status of nitrogen retention in the animal [373].

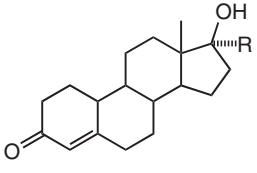
The easily accessible levator ani muscle of the rat has provided a valuable index for measuring the myotrophic activity of steroidal hormones [280]. By comparing the weight of levator ani muscle, seminal vesicles and ventral prostate with those of controls, it is possible to obtain a ratio of anabolic to androgenic activity [280, 282]. There also appears to be some correlation between the levator ani response and urinary nitrogen retention [280]. A modification of this muscle assay utilizes the parabiotic rat [374, 375] and allows for the simultaneous measurement of pituitary gonadotrophic inhibition and myotrophic activity. The suitability of the levator ani assay has been questioned on the possibility that its growth is more a result of androgenic sensitivity than of any steroid-induced myotrophic effect [375–378]. Thus, this assay is usually performed in conjunction with nitrogen balance studies or an acceleration of body growth [379].

5.3 Structure–Activity Relationships for Anabolic Agents

5.3.1 19-Nor Derivatives

An important step towards developing an anabolic agent with minimal androgenicity was taken when Hershberger and associates

[282], and later others [380, 381], found 19-nortestosterone (17β-hydroxyestr-4-en-3-one, nandrolone, **45**) to be equally myotrophic as testosterone, but only about one-tenth as androgenic. This observation prompted the synthesis and evaluation of a variety of 19-norsteroids, including the 17α-methyl (normethandrone, **46**) [382] and the 17α-ethyl (norethandrolone, **47**) [383] homologs of 19-nortestosterone.

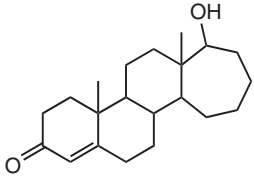


R = H (**45**) nandrolone
R = CH_3 (**46**) normethandrolone
R = CH_2CH_3 (**47**) norethandrolone

Nandrolone, in the form of a variety of esters (such as decanoate and β-phenylpropionate), and norethandrolone have been widely used clinically. The latter agent (trade name Nilevar®) was the first to be marketed in the United States as an anabolic steroid, but its androgenic [384] and progestational [385] side effects eventually led to it being replaced by other agents.

These studies stimulated the synthesis of other norsteroids, but both 18-nortestosterone [386] and 18,19-bisnortestosterone [387] were found essentially to be devoid of androgenic and anabolic properties. A contraction of the B ring led to B-norsteroids which were also lacking in androgenicity but, unlike the foregoing, this modification at least resulted in compounds with antiandrogenic activity.

Among the number of homoandrostane derivatives (those having one or more additional methylene groups included in normal tetracyclic ring system) that have been synthesized, only B-homodihydrotestosterone [388, 389] and D-homodihydrotestosterone [390, 391] have shown appreciable androgenic activity. A D-bishomo analog (**48**) was reported to be weakly androgenic [392].



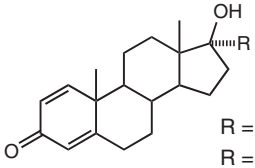
(**48**)

5.3.2 Dehydro Derivatives

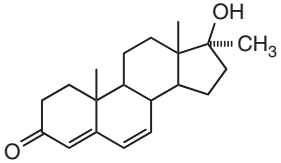
The marked enhancement in biological activity afforded by the introduction of a double bond at C_1 of cortisone and hydrocortisone prompted similar transformations in the androgens. The acetate of 17β-hydroxyandrosta-1,4-dien-3-one (**49**) [393] was as myotrophic as testosterone propionate but much less androgenic. Furthermore, 17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one (methandrostenolone, **50**) had equal to twofold the oral potency of 17α-methyltestosterone in rat nitrogen retention [394, 395] and levator ani muscle assays [396, 397]. In clinical studies, methandrostenolone produced a marked anabolic effect when given orally at doses of 1.25–10 mg per day, and was several-fold more potent than 17α-methyltestosterone [398].

In contrast to the 1-dehydro analogs, the introduction of an additional double bond at the 6-position (**51**) markedly decreased both androgenic and myotrophic activities in the rat [393, 399]. Moreover, removal of the C_{19}-methyl [400], inversion of the configuration at C_9 and C_{10} [401] and at C_8 and C_{10} [402], and reduction of the C_3-ketone

failed to improve the biological properties [403].



R = H (**49**)
R = CH_3 (**50**)



(**51**)

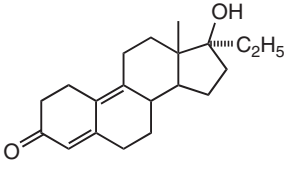
On the other hand, the introduction of unsaturation into the B, C, and D rings has given rise to compounds with significant androgenic or anabolic activities. Ethyldienolone (**52**), for example, displayed an anabolic:androgenic ratio of 5 and was slightly more active than methyltestosterone when both were given orally [404]. Segaloff and Gabbard [315] showed that the introduction of a 14–15 double bond (**53**) increased androgenicity when compared to testosterone applied locally in the chick comb assay. In contrast, a 25% decrease in androgenicity was identified in the rat ventral prostate following subcutaneous administration. Although conversion to the 19-nor analog (**54**) increased androgenicity, the anabolic activity was significantly enhanced [316].

Among a variety of triene analogs of testosterone that have been tested, only 17α-methyl-17β-hydroxyestra-4,9,11-trien-3-one (methyltrienolone, **55**) showed significant activity in rats. Surprisingly, this compound had 300-fold the anabolic potency and 60-fold the androgenic potency of 17α-methyltestosterone when administered orally to castrated male rats [405]. In this instance, however, the potent hormonal properties in rats did not correlate with later studies in humans [406–408]. In fact, a study in patients with advanced breast cancer showed methyltrienolone to have weak androgenicity and to produce severe hepatic dysfunction at very low doses [408].

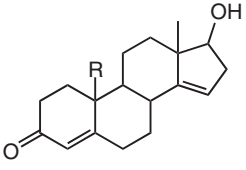
5.3.3 Alkylated Analogs

An extensive effort has been directed towards assessing the physiological effect of replacing hydrogen with alkyl groups at most positions of the steroid molecule. Although methyl substitution at C_3, C_4, C_5, C_6, C_{1l}, and C_{16} has generally led to compounds with low anabolic and androgenic activities, similar substitutions at C_1, C_2, C_7, and C_{18} have afforded derivatives of clinical significance.

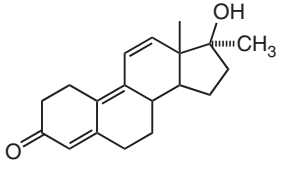
1-Methyl-17β-hydroxy-5α-androst-l-en-3-one (methenolone, **56**) as the acetate (methenolone acetate) was about fivefold more myotrophic, but only one-tenth androgenic, as testosterone propionate in animals [409]. In addition, this compound or the free alcohol represented one of the few instances of a C_{17} nonalkylated steroid



(**52**)

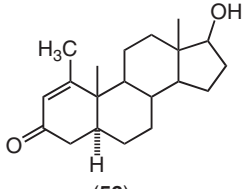


(**53**) R = CH_3
(**54**) R = H

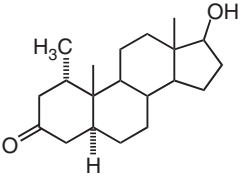


(**55**)

that possessed significant oral anabolic activity in animals [410] and in humans [411]. This effect may be related to the slow *in-vivo* oxidation of the 17β-hydroxyl group when compared with testosterone [412]. At a daily dose of 300 mg, methenolone acetate caused little virilization [413] or BSP retention [414]; by contrast, the dihydro analog, 1α-methyl-17β-hydroxy-5α-androstan-3-one (mesterolone, **57**), was found to possess significant oral androgenic activity in the cockscomb test [415] and also in clinical assays [416]. A comparison of the anabolic and androgenic activity of **56** with its A-ring congeners revealed that the double bond was necessary at C_1 for anabolic activity. For example, 1α-methyl-17β-hydroxyandrost-4-en-3-one had a much lower activity [417]. Furthermore, either reduction of the C_3 carbonyl group of **57** [418] or removal of the C_{19} methyl group [419, 420] greatly reduced both anabolic and androgenic activities in this series.

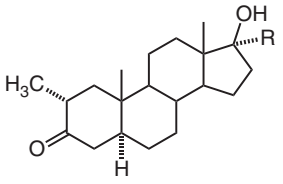


(**56**)



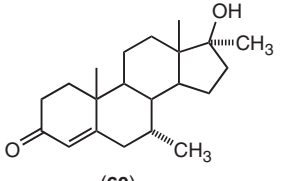
(**57**)

Among the C_2-alkylated testosterone analogs, 2α-methyl-5α-androstan-17β-ol-3-one (drostanolone, **58**) and its 17α-methylated homolog (**59**) have displayed anabolic activity both in animals [421] and in man [422]. In contrast, 2,2-dimethyl and 2-methylenetestosterone or their derivatives showed only low anabolic or androgenic activities in animals [421, 423, 424].

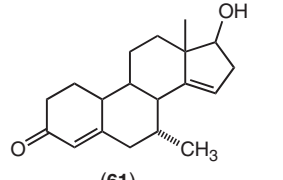


(**58**) R = H
(**59**) R = CH_3

7α,17α-Dimethyltestosterone (bolasterone, **60**) had 6.6-fold the oral anabolic potency of 17α-methyltestosterone in rats [425]. A similar activity was observed in humans at 1–2 mg per day, without many of the usual side effects [426]. Moreover, the corresponding 19-nor derivative was 41-fold as active as 17α-methyltestosterone as an oral myotrophic agent in the rat [427]. Segaloff and Gabbard [316] found 7α-methyl-14-dehydro-19-nortestosterone (**61**) to be approximately 1000-fold as active as testosterone in the chick comb assay, and about 100-fold as active as testosterone in the ventral prostate assay.



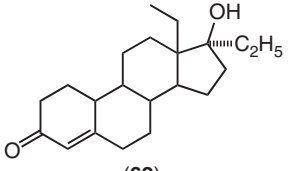
(**60**)



(**61**)

Certain totally synthetic 18-ethylgonane derivatives possessed pronounced anabolic activity. Similar to other 19-norsteroids,

13β,17α-diethyl-17β-hydroxygon-4-en-3-one (norbolethone, **62**) was found to be a potent anabolic agent in animals and in humans [428, 429]. Since it is prepared by total synthesis, the product was isolated and marketed as the racemic DL-mixture; notably, the hormonal activity resides in the D-enantiomer.



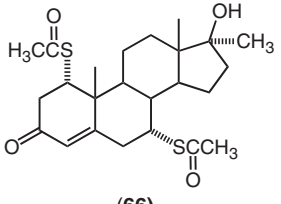
(**62**)

5.3.4 Hydroxy and Mercapto Derivatives

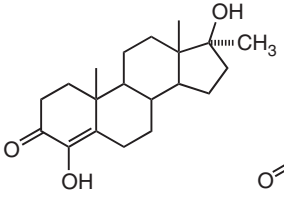
Testosterone has been hydroxylated at virtually every position on the steroid nucleus. For the most part, nearly all these substances possess no more than weak myotrophic and androgenic properties, though two striking exceptions to this are 4-hydroxytestosterone and 11β-hydroxytestosterone. 4-Hydroxy-17α-methyltestosterone (oxymesterone, **63**), for instance, had three- to fivefold the myotrophic activity but only half of the androgenic activity of 17α-methyltestosterone in rats [430]. In clinical studies, oxymesterone produced nitrogen retention in adults at a daily dose of 20–40 mg, and no adverse effect on liver function were observed [431, 432]. The introduction of an 11β-hydroxyl group in many instances resulted in a favorable effect on biological activity; for example, 11β-hydroxy-17α-methyltestosterone (**64**) was more anabolic in rats than was 17α-methyltestosterone [298], and 1.5-fold as myotrophic in humans [398].

To date, one of the most widely studied anabolic steroids has been 2-hydroxymethylene-17α-methyl-5α-androstan-17β-ol-3-one (oxymetholone, **65**). In animals, this compound was found to be threefold as anabolic but only half as androgenic as 17α-methyltestosterone [433, 434]. These results were confirmed in clinical studies [432–435].

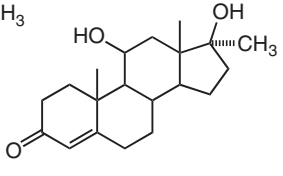
The substitution of a mercapto for a hydroxyl group has generally resulted in decreased activity. However, the introduction of a thioacetyl group at C_1 and C_7 of 17α-methyltestosterone afforded lα,7α-bis(acetylthio)-17α-methyl-17β-hydroxyandrost-4-en-3-one (thiomesterone, **66**), a compound with significant activity. Thiomesterone was 4.5-fold as myotrophic and 0.6-fold as androgenic as 17α-methyltestosterone in rats [436], and has been used clinically as an anabolic agent [437].



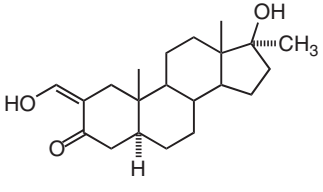
(**66**)



(**63**)



(**64**)

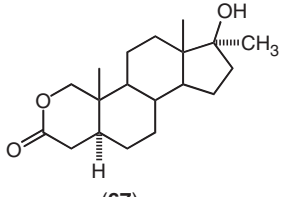


(**65**)

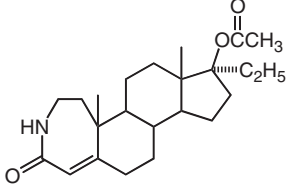
Moreover, numerous 7α-alkylthio androgens have exhibited anabolic–androgenic activity similar to that of testosterone propionate when administered subcutaneously [438, 439]. Even though no clinically useful androgen resulted, similar 7α-substitutions proved to be advantageous in the development of radioimmunoassays now employed in clinical laboratories [440]. In addition, certain 7α-arylthioandrost-4-ene-3,17-diones are effective inhibitors of estrogen biosynthesis (see Sect. 6.2.3).

5.3.5 Oxa, Thia, and Aza Derivatives

A number of androgen analogs in which an oxygen atom replaces one of the methylene groups in the steroid nucleus have been synthesized and evaluated biologically. Of these derivatives, 17β-hydroxy-17α-methyl-2-oxa-5α-androstan-3-one (oxandrolone, **67**) [441] was threefold as anabolic but only 0.24-fold as androgenic as 17α-methyltestosterone in the oral levator ani assay [442]. By contrast, only minimal responses were obtained following intramuscular administration. The 2-thia [443] and 2-aza [444] analogs were essentially devoid of activity by both routes. The 3-aza-A-homoandrostene derivative **68** displayed only 5% of the anabolic-to-androgenic activity of methyltestosterone [445].



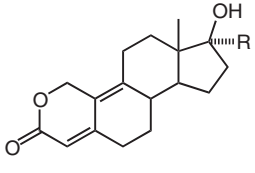
(**67**)



(**68**)

The clinical anabolic potency of oxandrolone was considerably greater than that of 17α-methyltestosterone, and provided perceptible nitrogen-sparing at a dose as low as 0.6 mg per day [446]. Moreover, at dosages of 0.25–0.5 mg kg^{-1}, oxandrolone was effective as a growth-promoting agent, without producing androgenically induced bone maturation [447]. Because of this favorable separation of anabolic from androgenic effects, oxandrolone has been one of the most widely studied anabolic steroids. Its potential utility in various clinical hyperlipidemias was discussed in Sect. 5.2.

The significant hormonal activity noted for estra-4,9-dien-3-ones such as **52** (see Sect. 5.3.2) prompted the synthesis of the 2-oxa bioisosteres in this series. Despite the lack of a 17α-methyl group, **69** had 93-fold the oral anabolic activity of l7α-methyltestosterone, and was also 2.7-fold as androgenic. As might be expected, the corresponding 17α-methyl derivative, **70**, was the most active substance in this series, with myotrophic and androgenic effects that were 550-fold and 47-fold, respectively, that of 17α-methyltestosterone [448]. These two compounds differed dramatically in their progestational activity, however, with the activity of **69** being only one-tenth that of progesterone but the activity of **70** being 100-fold in the Clauberg assay [448]. The pronounced oral activity of **69** suggests that it is not a substrate for the 17β-alcohol dehydrogenase, but does represent an interesting finding.



(**69**) R = H
(**70**) R = CH_3

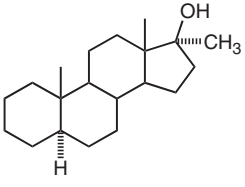
5.3.6 Deoxy and Heterocyclic-Fused Analogs

Early studies conducted by Kochakian [449] indicated that the 17β-hydroxyl group and the 3-keto group were essential for maximum androgenic activity. Based on these observations, the C_3 oxygen function was removed in the hope of reducing the androgenic potency while maintaining the anabolic activity [450]. Unfortunately, the results obtained failed to substantiate the rationale, and 17α-methyl-5α-androstan-17β-ol (**71**) was found to be a potent androgen in animals [451] and humans [452]. However, Wolff and Kasuya showed that this substance is extensively metabolized to the 3-keto derivative by rabbit liver homogenate [453]. Other deoxy analogs of testosterone have been synthesized and tested. For example, a 19-nor derivative, 17α-ethylestr-4-en-17β-ol (estrenol, **72**) had at least fourfold the anabolic activity but only one-fourth of the androgenic activity of 17α-methyltestosterone in animals [454], and was effective in humans at a daily dose of 3–5 mg [455–457]. 17α-Methyl-5α-androst-2-en-17β-ol (desoxymethyltestosterone, **73**) also offered a good separation of anabolic from androgenic activity [451, 458].

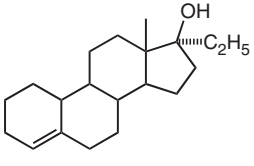
Since sulfur is considered to be isosteric with -CH=CH-, Wolff and Zanati [326] reasoned that 2-thia-A-nor-5α-androstane derivatives such as **74** should have androgenic activity. Indeed, this compound possessed high androgenic and anabolic activities, which served to verify that steric rather than electronic factors are important in connection with the structural requirements at C-2 and/or C-3 in androgens [459]. Interestingly, the selenium and tellurium isosteres in the same series were found to have good androgenic activity [460, 461]. Moreover, experiments with a ^{75}Se-labeled analog have shown **75** to selectively compete with DHT for binding to the AR in rat prostate [462].

The high biological activity noted for the 3-deoxy androstanes prompted numerous investigators to fuse various systems to the A-ring. The simplest such changes were 2,3-epoxy, 2,3-cyclopropano, and 2,3-epithioandrostanes. The 2,3α-cyclopropano-5α-androstan-17β-ol was as active as testosterone propionate as an anabolic agent [463]. While the epoxides had little or no biological activity, certain of the episulfides possessed pronounced anabolic and androgenic activities [464]. For example, 2,3α-epithio-17α-methyl-5α-androstan-17β-ol (**76**) was found to have approximately equal androgenic activity but 11-fold the anabolic activity of methyltestosterone after oral administration to rats. The 2,3β-episulfide, on the other hand, was much less active. 2,3α-Epithio-5α-androstan-17β-ol has been shown to have long-acting antiestrogenic activity, as well as some beneficial effects in the treatment of mammary carcinoma [465].

Other heterocyclic androstane derivatives have included the pyrazoles. Thus, 17β-hydroxy-17α-methylandrostano-(3,2-c)-pyrazole (stanozolol, **77**) was 10-fold



(**71**)



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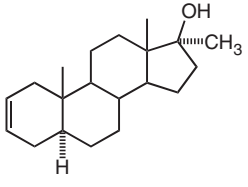
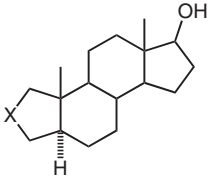
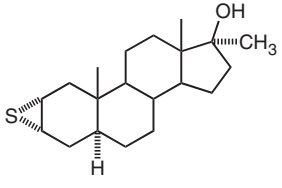


(**73**)

as active as 17α-methyltestosterone in improving nitrogen retention in rats [466], but the myotrophic activity was only twice that of 17α-methyltestosterone [467]. Stanozolol, at a dose of 6 mg per day, produced an adequate anabolic response with no lasting adverse side effects [468, 469].

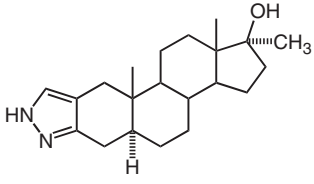


(**74**) X = S
(**75**) X = Se



(**76**)

The high activity of the pyrazoles instigated the synthesis of other heterocyclic fused androstane derivatives including isoxazoles, thiazoles, pyridines, pyrimidines, pteridines, oxadiazoles, pyrroles, indoles, and triazoles. One of the most potent was 17α-methylandrostan-17β-ol-(2,3-d)-isoxazole (androisoxazol, **78**), which exhibited an oral anabolic-to-androgenic ratio of 40 [470]. The corresponding 17α-ethynyl analog (danazol, **79**) has been of most interest clinically, as this compound is known to impede androgenic activity and inhibit pituitary gonadotropin secretion [471]. Since danazol depresses blood levels of androgens and gonadotropins, it has been studied as an antifertility agent in males [472]. Indeed, daily doses of 200 or 600 mg caused a dose-related lowering of plasma levels of testosterone and androstenedione. Moreover, in addition to inhibiting gonadotropin release, a direct inhibition of Leydig cell androgen synthesis was also observed. Other studies have shown danazol to be effective in the treatment of endometriosis, benign fibrocystic mastitis and precocious puberty [473]. Several reports have appeared relating to its disposition and metabolic fate [473, 474].



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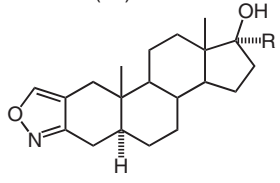


(**78**) R = H
(**79**) R = C≡CH

5.3.7 Esters and Ethers

Since the esterification of testosterone markedly prolongs its activity, it was unsurprising that this approach to increasing drug latency would be extended to the anabolic steroids. The acyl moiety is usually derived from a long-chain aliphatic or arylaliphatic acid such as heptanoic (enanthoic), decanoic, cyclopentylpropionic, and β-phenylpropionic. For example, no less than 12 esters of 19-nortestosterone (nandrolone) have been used clinically as long-acting anabolic agents [475, 476].

In the case of nandrolone, the duration of action and the anabolic-to-androgenic ratio were each increased with the chain length of the ester group [477, 478]. The decanoate and laurate esters, for instance, were active at six weeks after injection. Clinically, nandrolone decanoate appeared to be the most practical, as a weekly dose

of 25–100 mg produced a marked nitrogen retention [479, 480].

Since the 17α-alkyl group has been implicated as the cause of hepatotoxic side effects of oral preparations, the effect of esterification on oral efficacy has attracted much attention. For example, the esterification of dihydrotestosterone with short-chain fatty acids resulted in oral anabolic and androgenic activities in rats [481]. Moreover, esters of methenolone possessed appreciable oral anabolic activity [482]. Unfortunately, follow-up studies with steroid esters in humans have not been reported.

The manner by which steroid esters evoke their enhanced activity and increased duration of action has puzzled investigators for many years. The classical concept has been that esterification delays the rate at which the steroid is absorbed from the site of injection, thus preventing its rapid destruction. However, other factors must also be involved as the potency and prolongation of action are known to vary markedly with the nature of the esterifying acid.

James and coworkers shed much light on this problem by studying the effect of various aliphatic esters of testosterone on rat prostate and seminal vesicles, and correlating androgenicity with lipophilicity and the rate of ester hydrolysis by liver esterases [483, 484]. Peak androgenic response was observed with the butyrate ester, which was also the most readily hydrolyzed, while the more lipophilic valerate ester was slightly less androgenic (in a quantitative sense) but its action was longer-lasting. James and colleagues concluded that the ease of hydrolysis controls the weight of the target organs, whereas lipophilicity was responsible for the duration of the androgenic effect. These results also explained the low androgenic activity that had been previously noted for hindered trimethylacetate (pivalate) esters, which would be expected to be resistant to *in-vivo* hydrolysis.

The effect of etherification on anabolic or androgenic activities has been studied less rigorously. Replacement of the 17β-OH with l7β-OCH_3 markedly reduced androgenic activity but had no great effect on the ability to counteract cortisone-induced adrenal atrophy in male rats [485]. A series of 17β-acetals [486, 487], alkyl ethers [488], and 3-enol ethers [489, 490], however, showed significant activity when given orally [491–493]. For example, the cyclohexyl enol ether of 17α-methyltestosterone, when given orally, was fivefold as myotrophic as 17α-methyltestosterone [493].

Other ethers such as the tetrahydropyranol [494, 495] and trimethylsilyl have oral anabolic and androgenic activities in animals. The trimethylsilyl ether of testosterone (silandrone) showed a protracted activity following injection [496], and after oral dosing had twice the anabolic and androgenic activities of 17α-methyltestosterone. Solo *et al.* [497] evaluated a variety of ethers that would not be expected to be readily cleaved *in vivo*, and found them to be almost devoid of anabolic and androgenic activities. These findings provided additional support for the need of a free 17β-hydroxyl for androgenic activity.

5.3.8 Summary of Structure–Activity Relationships

Synthetic modifications of C_{19} steroids have resulted in the enhancement of anabolic activity, even though a pure synthetic anabolic agent which retains no androgenic activity has not yet been accomplished. Structural changes in two regions of the testosterone molecule have resulted in the greatest enhancement of the anabolic/androgenic ratio. The first region is the C-17 position of the testosterone

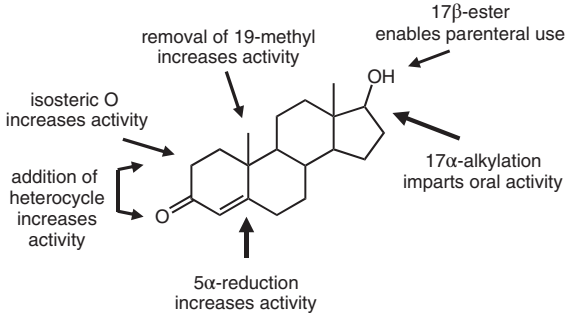


Fig. 9 Summary of structure–activity relationships for anabolic agents.

molecule. The introduction of a 17α-alkyl functionality, such as a 17α-methyl or a 17α-ethyl group, greatly increases the metabolic stability of the anabolic and decreases the *in-vivo* conversion of the 17β-alcohol to the 17-ketone by 17β-hydoxysteroid dehydrogenases. In addition, esterification of the 17β-alcohol enhances the lipid solubility of the steroids, thus providing injectable preparations for depot therapy.

The A ring of testosterone is the second region in which structural modifications can be made to increase anabolic activity. Removal of the C-19 methyl group results in the 19-nortestosterone analogs, which have slightly higher anabolic activities. A major impact on the structure–activity relationships of anabolic agents can be observed with modifications at the C-2 position. For example, a bioisosteric replacement of the carbon atom at position 2 with an oxygen provides a threefold increase in anabolic activity, as is seen with oxandrolone. Finally, the greatest effects were observed with the addition of heterocyclic rings fused at positions 2 and 3 of the A ring. The two heterocycles that have led to the greatest changes are the pyrazole and the isoxazole rings, as seen in stanozolol and androisoxazole, respectively. In these anabolics, the 3-ketone of testosterone is replaced by the bioisosteric 3-imine. Stanozolol, which contains the pyrazole ring at C-2 and C-3, shows the greatest increases when compared to testosterone. The anabolic activities of nitrogen retention and myotrophic activity for several common anabolic agents are listed in Table 2, while a summary of their structure–activity relationships is shown in Fig. 9.

5.4 Absorption, Distribution, and Metabolism

The absorption, distribution, and metabolism of the various anabolic steroids is quite similar to the pharmacokinetic properties of the endogenous and synthetic androgens discussed earlier in the chapter [498]. Again, lipid solubility is critical for the absorption of these agents following oral or parenteral administration. The 17α-methyl group retards the metabolism of the compounds and provides orally active agents. Other anabolics such as methenolone are orally active without a 17α-substituent, indicating that these steroids are poor substrates for 17α-hydroxysteroid dehydrogenase [411, 499]. Reduction of the 4-en-3-one system in synthetic anabolics to give the various α- and β-isomers occurs *in vivo* [329]. The 3-deoxy agent 17α-methyl-5α-androstan-17α-ol was shown to be

Tab. 2 Comparison of anabolic activities.

Compound	*Number*	*Trade names*	*Anabolic activity*	
			Nitrogen retention	*Myotrophic activity*
Testosterone	**1**	Android-T Malestrone Oreton Primotest Virosterone	1.0	1.0
19-Nortestosterone Nandrolone	**45**	Nerobolil Nortestonate	0.8	1.0
Normethandrone	**46**	Methalutin Orgasteron	4.0	4.5
Norethandrolone	**47**	Nilevar Solevar	3.9	4.0
Methandrostenolone Methandienone	**50**	Danabol Dianabol Nabolin Nerobil	0.6	1.4
Drostanolone	**58**	Drolban Masterone	—	1.3
Oxymetholone	**65**	Adroyd Anadrol Anadroyd Anapolon Anasterone Nastenon Protanabol Synasteron	2.75	2.8
Oxandrolone	**67**	Anavar Provita	3.0	3.0
Estrenol	**72**	Duraboral-O Maxibolin Orabolin Orgaboral Orgabolin	1.7	2.0
Stanozolol	**77**	Stanozol Winstrol Tevabolin	10.0	7.5
Androisoxazole	**78**	Androxan Neo-ponden	1.5	1.7

extensively converted to the 3-keto derivative by liver homogenate preparations [453]. The metabolic fates of stanozolol and danazol have been reported [473, 474], with the major metabolites being heterocyclic ring-opened derivatives and their deaminated products. Finally, both the unchanged anabolics and their metabolites are primarily excreted in the urine as glucuronide or sulfate conjugates.

5.5 Toxicities

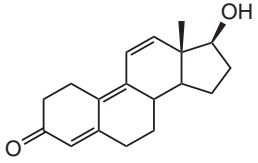
The major side effect of anabolic steroids is the residual androgenic activity of the molecules, with virilizing actions being undesirable in adult males as well as in females and children. Many anabolic steroids can also suppress the release of gonadotropins from the anterior pituitary, leading to lower levels of circulating hormones and potential problems of reproductive function. Headaches, acne and elevated blood pressure are common symptoms in individuals taking anabolics, while the salt and water retention induced by these agents can produce edema.

The most serious toxicities resulting from the use of anabolic steroids relate to subsequent liver damage, including jaundice and cholestasis, that can occur after the administration of the 17α-alkylated C_{19} steroids [335–338]. Individuals who have received anabolic agents over an extended period have also developed hepatic adenocarcinomas [500–502]. Such clinical reports serve to underscore the inherent risks associated with anabolic steroid use in amateur athletes, for no demonstrable benefits.

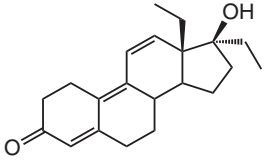
5.6 Abuse of Anabolic Agents

The myotrophic effects of testosterone and other anabolic steroids have led to the use and abuse of these agents by athletes [503–506]. Conflicting reports on the effectiveness of anabolics to increase strength and power in healthy males have resulted from early clinical trials. Several groups have found no significance differences between groups of male, college-age students receiving anabolics and weight-training, and other (control) students receiving placebo plus weight-training in double-blind studies [507–510]. Whilst other studies have reported some improvement in strength and power, they involved only small numbers of subjects or were single-blind in design [511–514]. More recently, a small randomized study on "supraphysiologic" doses of an anabolic (testosterone enanthate, 600 mg per week) in normal men demonstrated an enhancement of muscle size when such doses were combined with strength training [515]. Studies using higher doses of an anabolic or using multiple forms of anabolic agents simultaneously – a practice referred to as "anabolic stacking" – are lacking. Anabolic steroids also exhibit an anticatabolic effect; that is, a reversal of the catabolic effects of glucocorticoids released in response to stress. Such effects would enable individuals to recover more quickly following strenuous workouts.

Currently, an alarming percentage of professional and amateur athletes utilize anabolic steroids [516, 517], which are readily available "on the street." The abuse of these steroids for increasing strength and power is banned in intercollegiate and international sports, and very sensitive assays such as radio-immunoassay and gas chromatography/mass spectrometry (GC/MS) have been developed for measuring levels of anabolic agents in urine and blood [356]. More recently, "designer" anabolic steroids were introduced in attempts to evade anti-doping detection methods. Between 2002 and 2005, laboratories accredited by the World Anti-Doping Agency (WADA) developed sensitive GC/MS and liquid chromatography (LC)/MS/MS assay methods for designer steroids such as norbolethone **62**, desoxymethyltestosterone **73**, trenbolone **80**, and tetrahydrogestrinone **81** [517–520].



(**80**)



(**81**)

6
Androgen Antagonists

A majority of recent research efforts in the area of androgens has concentrated on the preparation and biological activities of androgen antagonists. An androgen antagonist is defined as … a substance which antagonizes the actions of testosterone in various androgen-sensitive target organs and, when administered with an androgen, blocks, or diminishes the effectiveness of the androgen at various androgen-sensitive tissues. Androgen antagonists may act to block the action of testosterone at several possible sites. First, androgen antagonists may block the conversion of testosterone to its more active metabolite, DHT. Second, competition for the high-affinity binding sites on the AR molecule may account for antiandrogenic effects. Finally, certain agents such as LHRH agonists can act in the pituitary to lower gonadotropin secretion via gonadotropic receptor downregulation, and thus diminish the production of testosterone by the testis. The substances described in the following section act through at least one of these mechanisms. Several reviews on androgen antagonists are available [521 – 527].

6.1
Current Drugs on the Market

Currently available androgen antagonists used as therapeutics are listed in the following table.

Generic name (structure)	*Trade name*	*U.S. manufacturer*	*Chemical class*	*Dose*
Antiandrogens				
Cyproterone acetate (**82**)	Androcur	Schering AG	Pregnane	
Flutamide (**95**)	Eulexin	Various suppliers	Nonsteroidal	Tablet: 125 mg
Nilutamide (**97**)	Nilandron	Aventis	Nonsteroidal	Tablets: 50, 150 mg
Bicalutamide (**101**)	Casodex	AstraZeneca	Nonsteroidal	Tablet: 50 mg
Enzalutamide (**106**)	Xtandi	Astellas	Nonsteroidal	Capsule: 40 mg
5α-Reductase inhibitors				
Finasteride (**108**)	Proscar	Merck	Androstane	Tablet: 5 mg
	Propecia	Merck	Androstane	Tablet: 1 mg
Dutasteride (**112**)	Avodart	GlaxoSmithKline	Androstane	Capsule: 0.5 mg
17,20-Lyase inhibitors				
Abiraterone (**132**)	Zytiga	Cadia	Androstane	Tablet: 250 mg
Aromatase inhibitors				
Exemestane (**140**)	Aromasin	Pfizer	Androstane	Tablet: 25 mg
Anastrozole (**141**)	Arimidex	AstraZeneca	Nonsteroidal	Tablet: 1 mg
Letrozole (**142**)	Femara	Novartis	Nonsteroidal	Tablet: 5 mg

6.2 Antiandrogens

6.2.1 Therapeutic Uses

Antiandrogens are agents that compete with endogenous androgens for the hormone-binding site on the AR. These agents have therapeutic potential in the treatment of acne, virilization in women, hyperplasia and neoplasia of the prostate, baldness and male contraception, and clinical studies have demonstrated their potential therapeutic benefits. The application of antiandrogens to the treatment of prostatic carcinoma and for the treatment of BPH has also been investigated. Antiandrogens are effective for the treatment of prostate cancer when combined with androgen ablation, such as surgical (orchiectomy) or medical (LHRH agonist) castration.

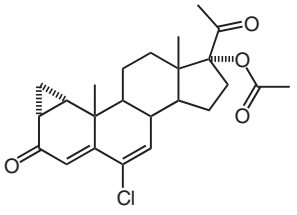
6.2.2 Structure–Activity Relationships for Antiandrogens

Steroidal Agents Several steroidal and nonsteroidal compounds with demonstrated antiandrogenic activity have been utilized clinically [523]. The first compounds to be used as antiandrogens were the estrogens and progestins [528], and steroidal estrogens and diethylstilbesterol are still used for the treatment of prostatic carcinoma [529–532], exerting their action via a suppression of the release of pituitary gonadotropins. Progestational compounds have also been utilized for antiandrogenic actions, but with limited success [533]. The inherent hormonal activities of these compounds, and the development of more selective antiandrogens, have limited the clinical applications of estrogens and progestins as antiandrogens.

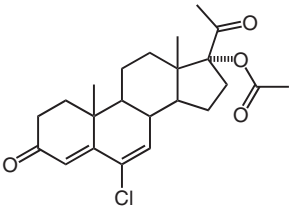
One modified progestin that is a potent antiandrogen and has minimal progestational activity is the agent cyproterone acetate (**82**). This compound was originally prepared in the quest for orally active progestins, but was quickly recognized for its ability to suppress gonadotropin release [25, 534–541]. It was shown later that cyproterone acetate also bound with high affinity to the AR, and thus competed with DHT for the binding site [25, 166, 542, 543]. Cyproterone acetate has received the most clinical attention of steroidal agents in antiandrogen therapy [544–555], having provided satisfactory results in the treatment of acne, seborrhea, and hirsutism [544–550]. The therapeutic effectiveness of cyproterone acetate in treating prostatic carcinoma has been reported [551–555], and it was also shown to be a good alternative to estrogens for treating prostate cancer, when combined with androgen ablation [556, 557]. Unfortunately, this combination did not improve disease-free survival or overall survival when compared to castration alone.

Other pregnane compounds that exhibit antiandrogenic actions by binding to the AR include chlormadinone acetate (**83**), medroxyprogesterone acetate (**84**), medrogestone (**85**), A-norprogesterone (**86**), and gestonorone capronate (**87**) [558–562]. Medrogestone also exerts antiandrogenic effects by inhibiting 5α-reductase and thus preventing the formation of DHT [563, 564]. Gestonorone capronate interferes with the uptake process in target cells [562].

Several androstane derivatives have demonstrated antiandrogenic properties. In 1964, 17α-methyl-β-nortestosterone (**88**) was the first to be prepared and tested for antihormonal activity [565], but within the next decade several other androstane analogs were synthesized and found to possess antiandrogenic activity [566–572]; these included BOMT (**89**), R2956 (**90**), and oxendolone (TSAA-291; **91**). As expected, the mechanism of antiandrogenic action



(**82**)



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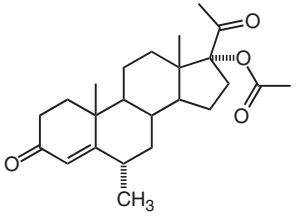

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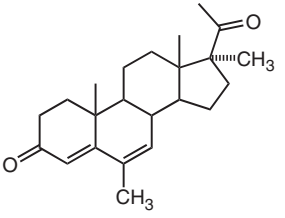

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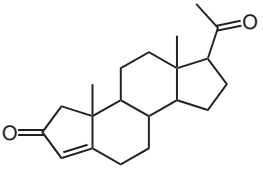
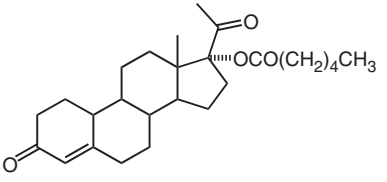


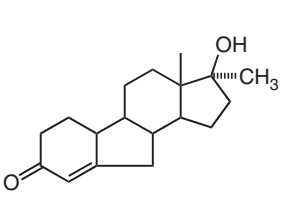
(**86**)



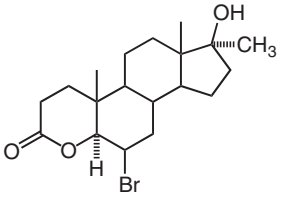
(**87**)

of these synthetic steroids is competition with androgens for the binding sites on the receptor molecule [543, 573–576]. Numerous A and B ring-modified steroids were examined for their antiandrogenic activities and the ability to bind to the AR [577, 578], thus confirming that the structural requirements of the AR binding site could accommodate some degree of flexibility in the A and/or B rings of antiandrogenic molecules. Heterocyclic-substituted A-ring antiandrogens such as zanoterone (WIN 49,596; **92**) further supported these conclusions on the structure–activity relationships of steroidal antiandrogens. Additional A-ring heterocycles identified as novel antiandrogens are the thiazole (**93**) and oxazole (**94**) [579, 580]. The optimal substitutions on the A-ring heterocyclic androstanes for *in-vivo* antiandrogenic activity are the methylsulfonyl group at the N-1′ position and a 17α-substituent (e.g., 17α-methyl or 17α-ethinyl).

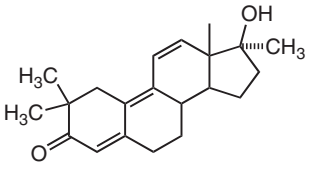
Nonsteroidal Agents The absolute requirement of a steroidal compound for interaction with the AR was invalidated when the potent nonsteroidal antiandrogen flutamide (Eulexin, **95**) was introduced [581, 582]. Subsequent receptor studies [576, 583, 584] showed that this compound competed with DHT for the binding sites. The side chain of flutamide allows sufficient flexibility for the molecule to assume a structure similar to an androgen. In addition, a hydroxylated metabolite (**96**)



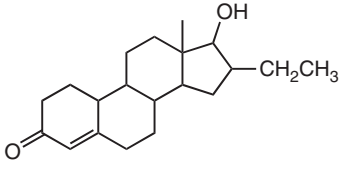
(**88**)



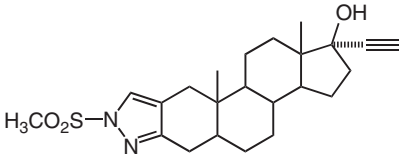
(**89**)



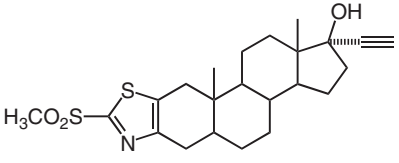
(**90**)



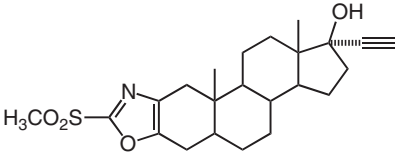
(**91**)



(**92**)



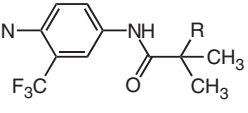
(**93**)



(**94**)

has been identified, is a more powerful antiandrogen *in vivo*, and has a higher affinity for the receptor than the parent compound [576, 585]. Important factors in the structure–activity relationships of flutamide and analogs are the presence of an electron-deficient aromatic ring and a powerful hydrogen bond donor group.

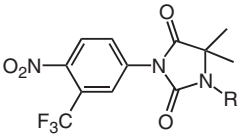
(**95**) X = H
(**96**) X = OH



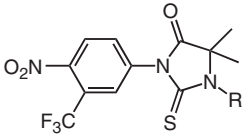
Flutamide has been evaluated extensively for the treatment of prostate cancer. Notably, large double-blind studies in prostate cancer patients, using a combination of flutamide with an LHRH agonist (as a medical castration), resulted in an increased number of favorable responses and increased overall survival when compared to an LHRH agonist or surgical castration [586, 587].

Nilutamide (Anandron, **97**) and related nilutamide analogs (**98–100**), and bicalutamide (Casodex, **101**) are other nonsteroidal antiandrogens with a similar electron-deficient aromatic ring, and have been shown to interact with the AR to varying degrees [528, 588]. Nilutamide and bicalutamide are pure antiandrogens, and are effective in suppressing testosterone-stimulated cell proliferation [589]. Both compounds have demonstrate an effective therapy against prostate cancer [590–594].

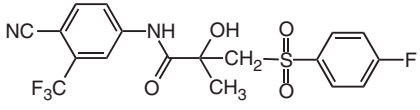
Other aryl-substituted nonsteroidal compounds have also been identified as antiandrogens. DIMP (**102**) is a phthalimide derivative that showed a weak affinity for the AR and poor *in-vivo* activity [576, 595]. A series of tetrafluorophthalimides (e.g., **103**) demonstrated moderate activity



(**97**) R = H
(**98**) R = $(CH_2)_4OH$

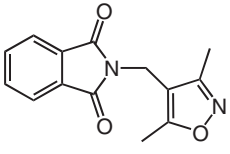


(**99**) R = CH_3
(**100**) R = $(CH_2)_4OH$

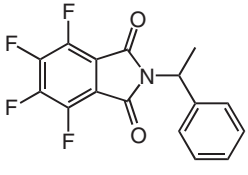


(**101**)

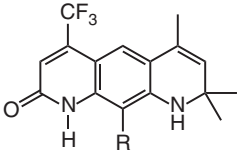
as antiandrogens in cell proliferation assays [596].



(**102**)

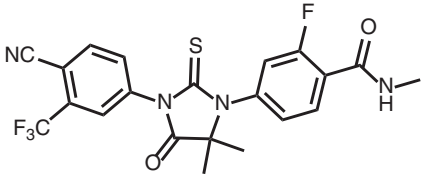


(**103**)

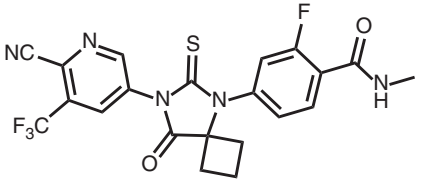


(**104**) R = H
(**105**) R = CH_3

A series of 1,2-dihydropyridono[5,6-*g*]quinolines was identified as novel nonsteroidal antiandrogens, employing a cell-based screening approach [597]. Several analogs (e.g., **104** and **105**) demonstrated excellent *in-vivo* activity, reducing rat ventral prostate weight without affecting serum levels of gonadotropins and testosterone.



(**106**)



(**107**)

Several biarylthiohydantoins have demonstrated potent androgen antagonist activities in preclinical and clinical studies. Enzalutamide (formerly referred to as MDV3100; **106**) exhibited a high affinity for the AR, antagonized androgen-induced gene expression, and did not display any agonist activities in LNCaP prostate cancer cells *in vitro* and in xenograft mouse models [598, 599]. Among related analogs, enzalutamide exhibited excellent pharmacokinetic properties and was selected for clinical trials. In both Phase I and II trials, enzalutamide demonstrated antitumor activity in patients with castration-resistant prostate cancer [600], and was approved for

use by the FDA in 2012. A related analog, ARN-509 (**107**), has shown greater *in-vivo* activity in xenograft models [601].

6.2.3 Absorption, Distribution, and Metabolism

The steroidal antiandrogens exhibit similar pharmacokinetic properties to the androgens and anabolic agents, with the lipophilicity of the compounds influencing absorption both orally and from injection sites [602–606]. Reduction of the 3-ketone and 4,5 double bond are common routes of metabolism [602]. An unusual metabolite of cyproterone acetate, 15α-hydroxycyproterone acetate, was isolated and identified in both animals and humans [607]. The nonsteroidal antiandrogen flutamide is rapidly absorbed and extensively metabolized *in vivo* [608, 609] and, as noted above, the hydroxy metabolite (**96**) of flutamide is a more potent antiandrogen [576, 585]. The major metabolite of bicalutamide is the glucuronide, which has comparable *in-vivo* activity [610]. Finally, the antiandrogens are primarily excreted as glucuronide and sulfate conjugates in the urine.

6.2.4 Toxicities

Adverse side effects of these agents have been identified from various clinical trials. In particular, testicular atrophy and decreased spermatogenesis have been observed during treatment with cyproterone acetate [611, 612]. Antiandrogens can also impair libido and result in impotence [613], while certain antiandrogens (e.g., cyproterone acetate and medrogesterone) also exhibit inherent progestational activity, suppress corticotropin release, and have some androgenic effects [614–616]. No hormonal activities were observed for the nonsteroidal antiandrogens, such as flutamide [609]. On the other hand, many nonsteroidal antiandrogens exhibit other endocrine side effects, such as elevated serum levels of gonadotropins and testosterone. Gynecomastia, nausea, diarrhea and liver toxicities have been observed in patients receiving nonsteroidal antiandrogens [617]. Resistance to antiandrogen therapy has also been observed in prostate cancer patients [618].

6.3 Enzyme Inhibitors

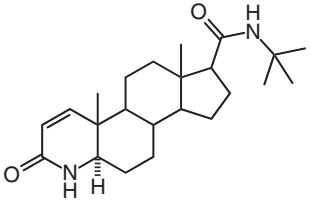
Enzymes involved in the biosynthesis and metabolism of testosterone represent attractive targets for drug design and drug development. To suppress of the synthesis of androgenic hormones and androgen precursors offers a viable therapeutic approach for the treatment of various androgen-mediated disease processes, and is an important endocrine treatment for prostate cancer. A potent inhibition of Type 2 5α-reductase in androgen target tissues, and the resultant decrease in DHT levels, will provide a selective interference with androgen action within those target tissues and no alterations of other effects produced by testosterone, other structurally related steroids, and other hormones such as corticoids and progesterone. The cytochrome $P450_{17\alpha}$ enzyme complex displays two enzymatic activities: 17α-hydroxylation to produce 17α-hydroxysteroids; and C_{17}-C_{20} bond cleavage (17,20-lyase activity) to produce androgens. In the male, this enzyme is found in both testicular and adrenal tissues, with these organs providing circulating androgens in the blood. An effective inhibition of this microsomal enzyme complex would eliminate both testicular and adrenal androgens and remove the growth stimulus to androgen-dependent prostate carcinoma. Synthetic androgen analogs that inhibit the oxidative

metabolism of androgens to estrogens can serve as potential therapeutic agents for controlling estrogen-dependent diseases such as hormone-dependent breast cancer.

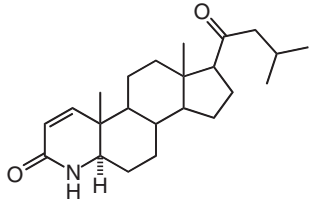
6.3.1 5α-Reductase Inhibitors

The most extensively studied class of 5α-reductase inhibitors is the 4-azasteroids [619], which includes the drug finasteride (Proscar, **108**). Finasteride, which is the first 5α-reductase inhibitor to be approved in the United States for the treatment of BPH, has an approximately 100-fold greater affinity for Type 2 5α-reductase than for the Type 1 enzyme, demonstrating an IC_{50} value of 4.2 nM for Type 2 5α-reductase [620]. In humans, finasteride decreases prostatic DHT levels by 70–90% and reduces prostate size [621], while testosterone tissue levels are increased. Clinical trials have demonstrated a sustained improvement in BPH disease and a reduction in PSA levels [622, 623]. Related analogs (**109–111**) have also demonstrated effectiveness both *in vitro* and *in vivo* [624–628]. These agents were originally designed to mimic the putative 3-enolate intermediate of testosterone and serve as transition-state inhibitors [625, 626]. Subsequently, finasteride was shown to produce time-dependent enzyme inactivation [627] and to function as a mechanism-based inactivator. The structure–activity relationships for the 4-azasteroids illustrate the stringent requirements for the inhibition of human Type 2 5α-reductase [628]. The 5α-reduced azasteroids are preferred, a 1,2-double bond can be tolerated, and the nitrogen can be substituted with only hydrogen or small lipophilic groups. Lipophilic amides or ketones are preferred as substituents at the C-17β position.

Several 6-azasteroids, such as dutasteride (**112**) and **113**, were prepared as extended mimics of the enolate transition state and have also demonstrated a potent inhibition of 5α-reductase [629]. Although the 6-azasteroids are more effective inhibitors of Type 2 5α-reductase, some analogs also exhibit a good inhibition of Type 2 5α-reductase. Alkylation of the nitrogen can be tolerated, but a 1,2-double bond



(**108**)



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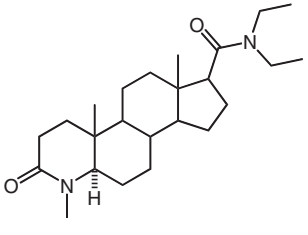

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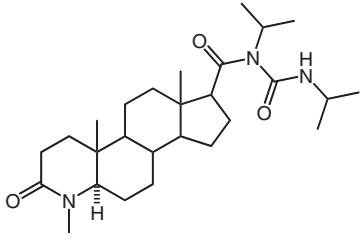

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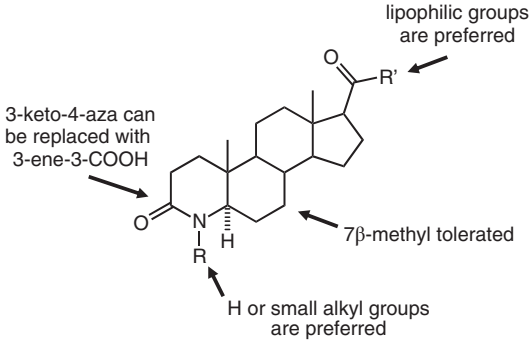
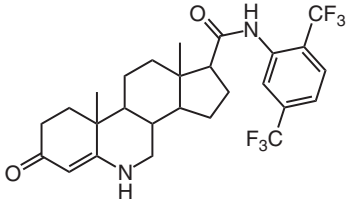
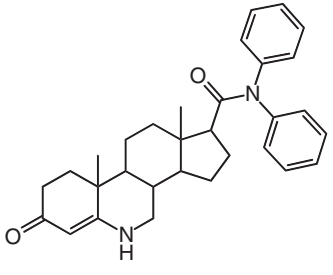


Fig. 10 Summary of structure–activity relationships for 5α-reductase inhibitors.

decreases the inhibitory activity in this series. The best inhibitors contain large lipophilic substituents at the C-17β position. A summary of the structure–activity relationships for steroidal 5α-reductase inhibitors is provided in Fig. 10.



(**112**)



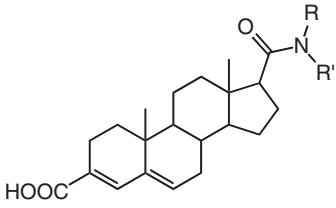
(**113**)

Androstadiene 3-carboxylic acids **114** and **115** were designed as transition-state inhibitors, and have demonstrated a potent uncompetitive inhibition of Type 2 5α-reductase [630, 631]. Epristeride (SK&F 105,657; **114**) has demonstrated the ability to lower serum DHT levels by 50% in clinical trials [632, 633]. Other analogs with acidic functionalities at the C-3 position include other androstene carboxylic acids (**116**, **117**) and estratriene carboxylic acids (**118**) [634]. The allenic secosteroid (**119**) has been demonstrated as a potent irreversible inhibitor of 5α-reductase, even though it was originally developed as an irreversible inhibitor of 3β-hydroxysteroid dehydrogenase/Δ4,5-isomerase [635–637]. Finally, selective and potent inhibitors of Type 1 5α-reductase were developed based on the 4-azacholestane MK-386 (**120**) [638].

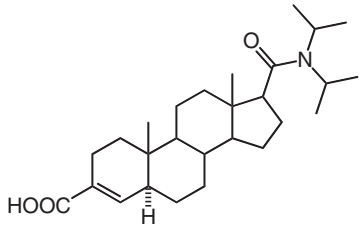
Several nonsteroidal 5α-reductase inhibitors have been developed based on the azasteroid molecule, or from high-throughput screening methods. Examples of these nonsteroidal inhibitors include the benzoquinolinone (**121**), an aryl carboxylic acid (**122**), and FK143 (**123**) [639–641].

6.3.2 17,20-Lyase Inhibitors

Both, nonsteroidal and steroidal agents have been examined as inhibitors of 17α-hydroxylase/17,20-lyase. The nonsteroidal agents studied most extensively are aminoglutethimide (**124**) and ketoconazole (**125**), both *in vitro* and in clinical trials. Objective response rates for treatment of prostate cancer in relapsed patients were observed with high doses of aminoglutethimide [642]



(**114**) R = H; R' = $C(CH_3)_3$
(**115**) R = R' = $CH(CH_3)_2$



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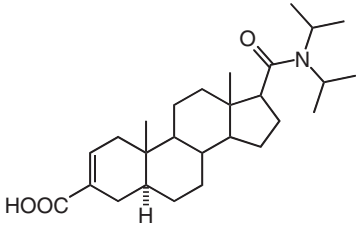

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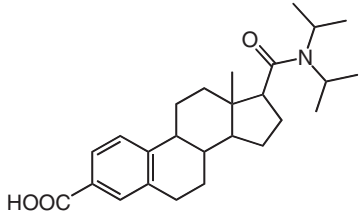

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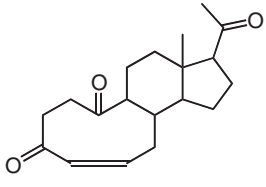
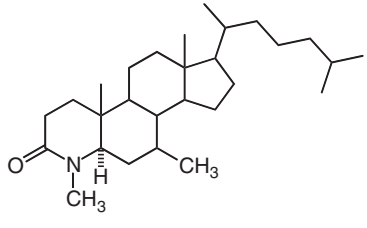
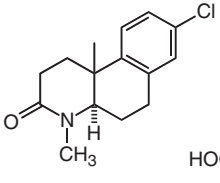


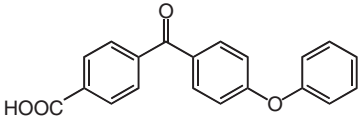
(**119**)



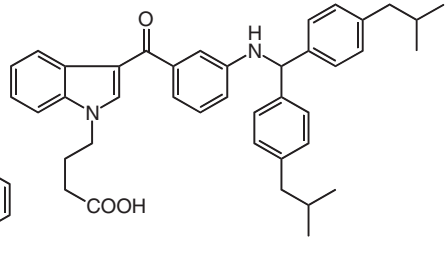
(**120**)



(**121**)

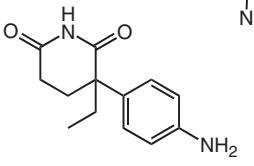


(**122**)

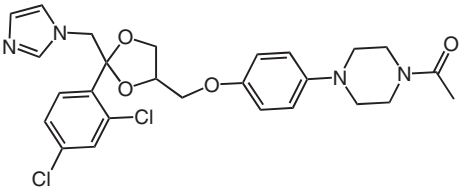


(**123**)

and high doses of ketoconazole [643], but both agents produced frequent adverse side effects. A third nonsteroidal agent that has received extensive preclinical evaluation is the benzimidazole analog, liarozole (**126**), which produced a reduction in plasma levels of testosterone and androstenedione *in vivo* [644]. Other nonsteroidal agents



(**124**)



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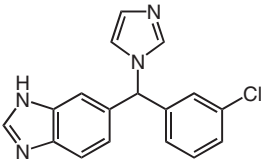

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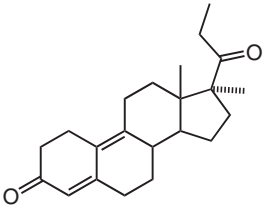
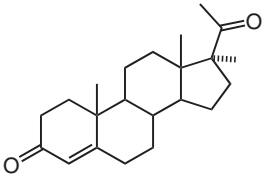
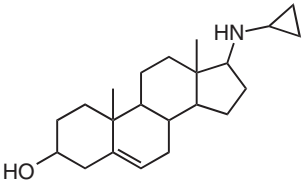


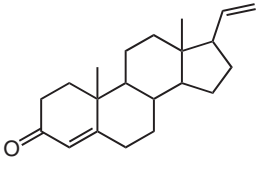
(**127**)



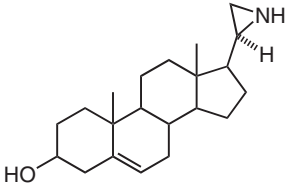
(**128**)



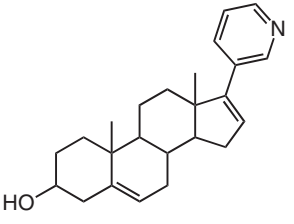
(**129**)



(**130**)



(**131**)



(**132**)

reported to exhibit 17α-hydroxylase/17,20-lyase inhibitory activity *in vitro* include other imidazole analogs [645], nicotine [646], bifluranol analogs [647], and pyridylacetic acid esters [648]. In general, high doses of nonsteroidal agents are needed to produce significant *in-vitro* or *in-vivo* activity. Another potential problem with these agents is the nonspecific inhibition of other cytochrome P450 enzymes involved in either steroidogenesis or liver metabolism.

A few studies of steroidal inhibitors of 17α-hydroxylase/17,20-lyase have been reported. An extensive analysis of the specificity of steroid binding to testicular microsomal cytochrome P450 identified several steroids exhibiting binding

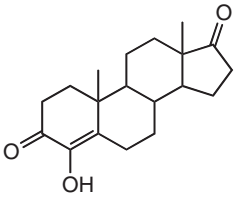
affinity [649]. One of these, promegestrone (**127**), has been utilized in the kinetic analysis of purified cytochrome $P450_{17\alpha}$ [650]. An affinity label inhibitor, 17-bromoacetoxyprogesterone (**128**), alkylates a unique cysteine residue on purified cytochrome $P450_{17\alpha}$ [651]. Potential mechanism-based inhibitors include 17β-(cyclopropylamino)-5-androsten-3β-ol (**129**; 652) and 17β-vinylprogesterone (**130**; 653). To date, all of these inhibitors exhibit apparent K_i-values in the micromolar (μM) range, while the apparent K_m for progesterone is 140 nM. The 17β-aziridinyl analog (**131**) and 17β-pyridyl derivative (abiraterone, **132**) also exhibited similar inhibitory activity [654, 655].

In a small clinical study, abiraterone (**132**) demonstrated clinical efficacy in suppressing testosterone levels in males with prostate cancer [656]. In patients with metastatic castration-resistant prostate cancer who had already received docetaxel, abiraterone plus low-dose prednisone improved overall survival [657], leading to FDA approval in 2011. Subsequently, a large clinical trial in men with metastatic castration-resistant prostate cancer without previous chemotherapy demonstrated that abiratone supplemented with prednisone resulted in progression-free survival and improved clinical outcomes [658].

6.3.3 C_{19} Steroids as Aromatase Inhibitors

Aromatase is the enzyme complex that catalyzes the conversion of androgens into the estrogens. This enzymatic process is the rate-limiting step in estrogen biosynthesis, and converts C_{19} steroids, such as testosterone and androstenedione, into the C_{18} estrogens, estradiol and estrone, respectively. The inhibition of aromatase has been an attractive approach for examining the roles of estrogen biosynthesis in various physiological or pathological processes. Furthermore, effective aromatase inhibitors can serve as potential therapeutic agents for controlling estrogen-dependent diseases such as hormone-dependent breast cancer. Investigations on the development of aromatase inhibitors began during the 1970s and have expanded greatly over the past four decades, with summaries of research into steroidal and nonsteroidal aromatase inhibitors having been presented at several international conferences on aromatase [659–667] and several reviews also being published [668–675].

Steroidal inhibitors that have been developed to date build upon the basic androstenedione nucleus, and incorporate chemical substituents at varying positions on the steroid. These inhibitors bind to the aromatase cytochrome P450 enzyme in the same manner as the substrate androstenedione. Even though the steroidal aromatase inhibitors are C_{19} steroids, these agents exhibit no significant androgenic activity. A limited number of effective inhibitors with substituents on the A ring have been reported. Several steroidal aromatase inhibitors contain modifications at the C-4 position, with 4-hydroxyandrostenedione (4-OHA; formestane; **133**) being the prototype agent. Initially, 4-OHA was thought to be a competitive inhibitor, but was later shown to produce enzyme-mediated inactivation [149, 676, 677]. *In vivo*, 4-OHA inhibits the reproductive process [678] and causes a regression of hormone-dependent mammary rat tumors [679, 680]. 4-OHA is also effective in the treatment of advanced breast cancer in postmenopausal women [681–683], and has been approved in the United Kingdom for the treatment of breast cancer. Thus, the spatial requirements of the A-ring for binding of the steroidal inhibitor to aromatase are rather restrictive, permitting only small structural modifications to be made.



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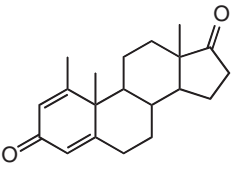

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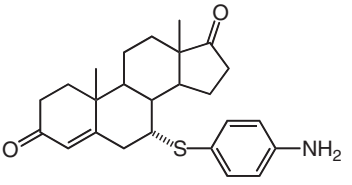
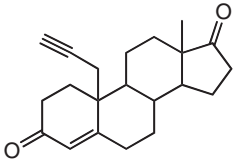


(**135**)

An incorporation of the polar hydroxyl group at C-4 enhances inhibitory activity. 1-Methyl-1,4-androstadiene-3,17-dione (**134**) is a potent inhibitor of aromatase *in vitro* and *in vivo* [684], whereas bulky substituents at the 1α-position led to poor inhibitors [685]. At the C-3 position, replacement of the ketone with a methylene provided an effective inhibition [686].

More extensive structural modifications may be made on the B-ring of the steroid nucleus. Bulky substitutions at the C-7 position of the B-ring have provided several very potent aromatase inhibitors [685]. For example, 7α-(4′-amino)phenylthio-4-androstene-3,17-dione (7α-APTA; **135**) is a very effective competitive inhibitor, with an apparent K_i of 18 nM. This inhibitor has also been shown effective in inhibiting aromatase in cell cultures [687, 688] and in treating hormone-dependent rat mammary tumors [688, 689]. The evaluation of various substituted aromatic analogs of 7α-APTA provided no correlation between the electronic character of the substituents and the inhibitory activity [690]. Investigations of various 7-substituted 4,6-androstadiene-3,17-dione derivatives [691, 692] have suggested that only those derivatives which can project the 7-aryl substituent into the 7α pocket are effective inhibitors. Overall, the most effective B-ring-modified aromatase inhibitors are those with 7α-aryl derivatives, with several analogs having two- to tenfold greater affinity for the enzyme than the substrate. These results suggest that additional interactions occur between the phenyl ring at the 7α-position and amino acids at or near the enzymatic site of aromatase, resulting in an enhanced affinity.

Numerous modified androstenedione analogs have been developed as effective mechanism-based aromatase inhibitors. The first of these compounds to be designed, 10-propargyl-4-estrene-3,17-dione (PED; MDL 18,962; **136**), was synthesized and studied independently by three research groups [693–695]. MDL 18,962 has an electron-rich alkynyl function on the C-19 carbon atom, the site of aromatase-mediated oxidation of the substrate. Although the identity of the reactive intermediate formed is not known, an oxirene and a Michael acceptor have been suggested. This agent is an effective inhibitor *in vitro* and *in vivo* [695–700]. Other approaches to C-19-substituted, mechanism-based inhibitors containing latent chemical groups have provided a limited number of inhibitors, including the difluoromethyl analog (**137**) [701] and a thiol (**138**) [702, 703]. Another series of mechanism-based inhibitors has been developed from more detailed biochemical investigations of several inhibitors which originally were thought to be competitive inhibitors. These inhibitors can be grouped into general categories of 4-substituted



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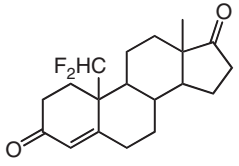

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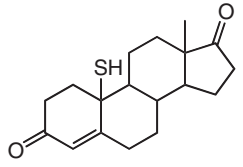

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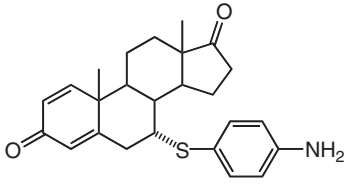

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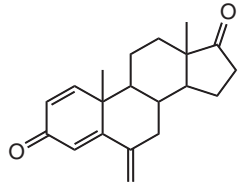

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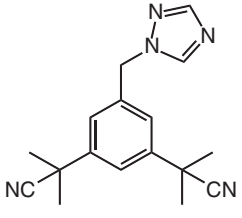
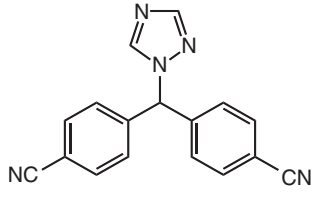


(**141**)



(**142**)

androst-4-ene-3,17-diones such as 4-hydroxyandrostenedione (**133**) [677], substituted androsta-1,4-diene-3,17-diones such as 7α-(4′-amino)-phenylthioandrosta-4,6-diene-3,17-dione (7α-APTADD; **139**) [704], and 6-methyleneandrost-4-ene-3,17-dione (exemestane; **140**) [705]. Exemestane (Aromasin®) was originally marketed as second-line therapy for the treatment of breast cancer patients in whom tamoxifen treatment had failed. Exemestane has been approved as a first-line therapy in women with advanced breast cancer, and as an adjuvant therapy in hormone-dependent breast cancer patients [706]. Two nonsteroidal aromatase inhibitors, anastrozole (Arimidex; **141**) and letrozole (Femara; **142**), were developed simultaneously and approved as first-line therapy in women with advanced breast cancer and as an adjuvant therapy in hormone-dependent breast cancer patients [706].

7 Summary

The steroid testosterone is the major circulating sex hormone in males, and serves as the prototype for the androgens, the anabolic agents, and androgen antagonists. Endogenous androgens are biosynthesized from cholesterol in various tissues of the body, with the majority of the circulating androgens produced in the testes under stimulation of the gonadotropin, LH. One critical aspect of testosterone and its biochemistry is that this steroid is converted in

various cells to other active steroidal agents. For example, the reduction of testosterone to DHT is necessary for the androgenic actions of testosterone to be effected in some androgen target tissues such as the prostate, whereas testosterone itself appears to be the active androgen in muscle. On the other hand, the oxidation of testosterone by the enzyme aromatase to yield estradiol is crucial for certain actions in the central nervous system. Investigations of these enzymatic conversions of circulating testosterone continue to be a fruitful area of biochemical research on the roles of steroid hormones in the body. Additionally, the elucidation of the mechanism of action of androgens in various target tissues is receiving on-going attention. The actions of androgens are considered due to their binding to the androgen nuclear receptor, followed by dimerization of the receptor complex and binding to a specific DNA sequence. This binding of the homodimer to the androgen response element leads to gene expression, stimulation of the synthesis of new mRNA, and subsequent protein biosynthesis. Other actions of testosterone, particularly the anabolic actions, appear to be mediated through a similar nuclear receptor-mediated mechanism. Nongenomic pathways for androgen action through the androgen receptor have also been reported, and include a rapid activation of kinase signaling pathways, such as the activation of MAP kinase and ERK kinase pathways, and the modulation of intracellular calcium levels. Many of the intricate biochemical events that occur during the action of androgens in their target cells remain to be further clarified. Nevertheless, receptor studies of new agents represent an important biological tool in the evaluation of the compounds for later, in-depth, pharmacological testing.

Synthetic androgens and anabolic agents were first created to impart oral activity to the androgen molecule, to separate the androgenic effects of testosterone from its anabolic effects, and to improve upon its biological activities. Over the years, these research efforts have provided several effective drug preparations for the treatment of various androgen-deficient diseases, for the therapy of diseases characterized by muscle wasting and protein catabolism, for postoperative adjuvant therapy, and for the treatment of certain hormone-dependent cancers. Unfortunately, however, some of these synthetic anabolics have also been abused by athletes. During the past decade, nonsteroidal androgen agonists have been identified that are referred to as selective androgen receptor modulators. These compounds exhibit agonist activities in tissues such as muscle and bone, yet act as antagonists or weak agonists in the prostate gland. Finally, steroidal and nonsteroidal agents have both been developed as androgen antagonists, the two major categories of which are the antiandrogens (which block the interactions of androgens with the androgen receptor) and the inhibitors of androgen biosynthesis and metabolism. These compounds have therapeutic potential in the treatment of acne, virilization in women, hyperplasia and neoplasia of the prostate, baldness, and also for male contraception. A number of androstane derivatives are also currently being developed as inhibitors of aromatase for the treatment of hormone-dependent breast cancer. Thus, the numerous biological effects of the male sex hormones testosterone and dihydrotestosterone, and the varied chemical modifications of the androstane molecule, have resulted in the development of effective medicinal agents for the treatment of androgen-related diseases.

Acknowledgments

The present text is a modified and updated version of the chapter written by the present author, entitled "Male Sex Hormones, Analogs, and Antagonists," in *Burger's Medicinal Chemistry and Drug Discovery, Seventh Edition, Volume 5* (eds D. Abraham and D. Rotella), John Wiley & Sons, New York, pp. 153–217, 2010 [6].

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