

Androgen Receptor Antagonists (Antiandrogens): Structure-Activity Relationships

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Abstract: Prostate cancer, acne, seborrhea, hirsutism, and androgenic alopecia are well recognized to depend upon an excess or increased sensitivity to androgens or to be at least sensitive to androgens. It thus seems logical to use antiandrogens as therapeutic agents to prevent androgens from binding to the androgen receptor. The two predominant naturally occurring androgens are testosterone (T) and dihydrotestosterone (DHT). DHT is the more potent androgen *in vivo* and *in vitro*. All androgen-responsive genes are activated by androgen receptor (AR) bound to either T or DHT and it is believed that AR is more transcriptionally active when bound to DHT than T. The two classes of antiandrogens, presently available, are the steroidal derivatives, all of which possess mixed agonistic and antagonistic activities, and the pure non-steroidal antiandrogens of the class of flutamide and its derivatives. The intrinsic androgenic, estrogenic and glucocorticoid activities of steroidal derivatives have limited their use in the treatment of prostate cancer. The non-steroidal flutamide and its derivatives display pure antiandrogenic activity, without exerting agonistic or any other hormonal activity. Flutamide (**89**) and its derivatives, Casodex (**108**) and Anandron (**114**), are highly effective in the treatment of prostate cancer. The combination of flutamide and Anandron with castration has shown prolongation of life in prostate cancer. Furthermore, combined androgen blockade in association with radical prostatectomy or radiotherapy are very effective in the treatment of localized prostate cancer. Such an approach certainly raises the hope of a further improvement in prostate cancer therapy. However, all antiandrogens, developed so-far display moderate affinity for the androgen receptor, and thus moderate efficacy *in vitro* and *in vivo*. There is thus a need for next-generation antiandrogens, which could display an equal or even higher affinity for AR compared to the natural androgens, and at the same time maintain its pure antiandrogenic activity, and thus providing improved androgen blockade using possibly antiandrogens alone.

Introduction

Prostate cancer (PC), benign prostatic hyperplasia (BPH), acne, seborrhea, hirsutism and androgenic alopecia are well known to be sensitive to androgens [1,2] and to respond to androgen receptor antagonist (antiandrogen) therapy [3-7]. The two predominant naturally occurring androgens are testosterone (T) and dihydrotestosterone (DHT). DHT is the more potent androgen and *in vitro* expression studies have also shown that DHT is more potent in inducing transcription activation than testosterone [8]. Testosterone and DHT can, however, have some different biological functions. T-mediated functions are anabolic (muscle mass

increase, penis enlargement, scrotum enlargement and vocal cord enlargement) and spermatogenesis (male sex drive and performance), and DHT-mediated effects are increased facial and body hair, acne, scalp hair recession and prostate enlargement. All androgen-responsive genes are activated *in vitro* by AR bound to either T or DHT. AR is more transcriptionally active when bound to DHT than to testosterone.

Although castration (orchiectomy or treatment with an LHRH-agonist) causes a 90-95% reduction in serum testosterone (T) concentration [9-12], a much smaller effect is seen on the only meaningful parameter of androgenic action, namely the intraprostatic concentration of dihydrotestosterone (DHT), the most active androgen. In fact, after elimination of testicular androgens by medical or surgical castration, the intraprostatic concentration of DHT remains at

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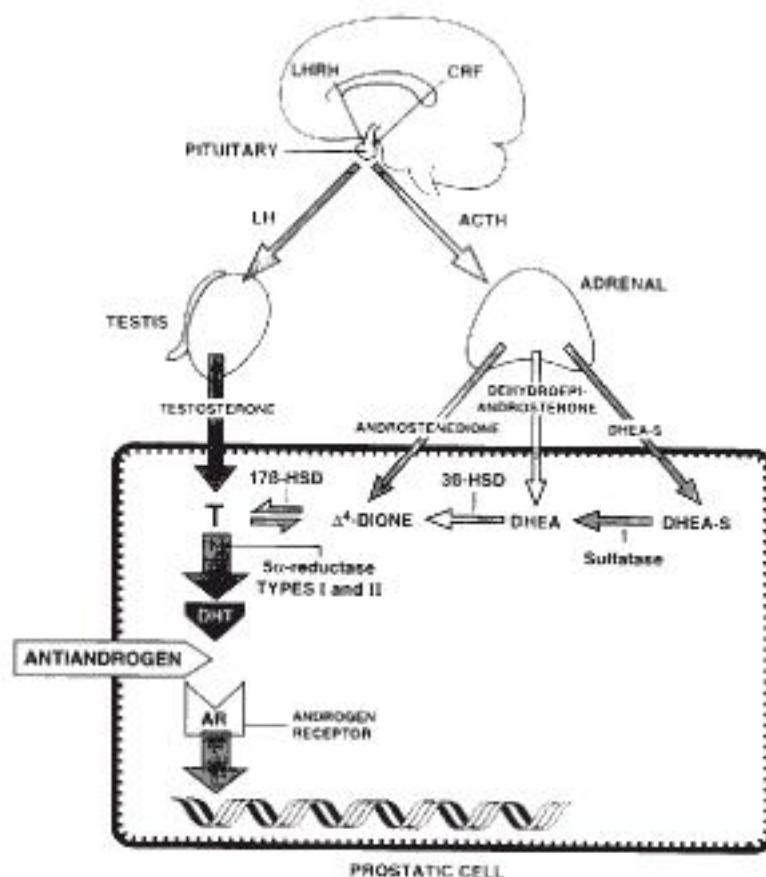


Fig. (1). Intracrine activity of the human prostate or biosynthetic steps involved in the formation of the active androgen DHT from testicular testosterone as well as from the adrenal precursors DHEA, DHEA-S and androstenedione (Δ^4 -dione) in human prostatic tissue.

approximately 40% of that measured in intact men. The importance of extratesticular androgens is also well illustrated by the finding that 40-50% of androgen metabolites remain in the circulation after castration in men [13-15]. Since recent studies show (Fig. 1) that an important proportion of androgens are produced in the peripheral tissues, including the skin and prostate, from the adrenal precursors dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S), we therefore elucidated the structure of cDNAs encoding the enzymes required for such a transformation, namely 3-hydroxysteroid dehydrogenase/5- Δ^4 isomerase [16-20], 17-hydroxysteroid dehydrogenase [21,22], and their corresponding genes. The structure of the cDNAs and genes encoding the two types of 5-reductase were also elucidated [23-26]. Since all these enzymes are expressed in the skin, prostate, and other peripheral tissues, and serum DHEA and DHEA-S are at similar levels in women and men; it is therefore not surprising that the serum concentration of the metabolites of androgens are present in women at levels 60 to 75% of those found in men of the same age.

Androgen Receptor

The androgen receptor (AR) is a member of the steroid/nuclear receptor superfamily, in which all members share basic structural and functional homology. Members of the superfamily are ligand-dependent nuclear transcription factors, and consist of three basic functional domains: the DNA binding domain, the ligand binding domain and the amino-terminal domain. However, despite the similarity in structure and function of the receptor superfamily, activation of different receptors elicits highly specific cellular responses. By studying the functional domains of receptors, and how the receptors control transcription regulation responses to different activation signals, we are beginning to understand the mechanisms controlling the specificity of receptor action. Many different naturally occurring mutations have been identified in the AR, and the study of these has allowed the localization of amino acids required for different receptor functions. These investigations, combined with *in vitro* mutagenesis studies and structural comparisons with other members of the

receptor superfamily [27,28], have allowed a greater elucidation of regions of the AR involved in ligand and DNA binding, dimerization, nuclear localization and transactivation.

AR binds ligands with high affinity, thus resulting in transformation of the receptor, associated with an increase in affinity for DNA [29,30], as a result of dissociation of heat shock proteins and a change in receptor conformation. The exact mechanisms of receptor transformation is not known: following ligand binding, the receptor changes to a more compact conformation, other conformation changes occur concomitantly with the dissociation of heat shock proteins and, then, dimerization, phosphorylation and DNA-binding occur [31].

The crystal structure of the retinoid X receptor (RXR)- ligand binding domain has been determined by Bourguet et al. [32]. A large hydrophobic cavity is predicted to form the ligand binding pocket and evidence suggests that the same structure is present in other members of the nuclear receptor superfamily, including the AR [33,34]. This would be comprised of hydrophobic amino acids between approximately 735 and 787, and approximately 855 and 865, while the full-length receptor comprises 919 amino acids [35] (Fig. 2). Penetration of the RXR- hydrophobic cavity by ligand requires some conformational changes of the

amino acid side chains. The ligand binding domain structures of the ligand-bound retinoic acid receptor (RAR) and thyroid hormone receptor (TR) suggest that the C-terminal -amphipathic helix flips over to seal the ligand binding domain and stabilize ligand binding, exposing a novel transactivation surface [36,37].

Mechanisms of Antiandrogen Action

Since an essential step in the action of androgens in target cells is binding to the androgen receptor (Fig. 3), a logical approach for neutralizing the androgens is the use of antiandrogens or compounds which prevent the interaction of T and DHT with the androgen receptor. Since prostate cancer is so highly sensitive to androgens, the antiandrogen used should be a compound having high specificity and affinity for the androgen receptor while not possessing any androgenic, estrogenic, progestational, glucocorticoid or any other hormonal and antihormonal activity. The mechanism by which antiandrogens act may be either directly by interaction with the androgen receptor or indirectly through some nonreceptor-mediated action or metabolism or nonspecific antimetabolite activity.

The two classes of antiandrogens presently available are the steroidal derivatives, all of which possess mixed agonistic and antagonistic androgenic

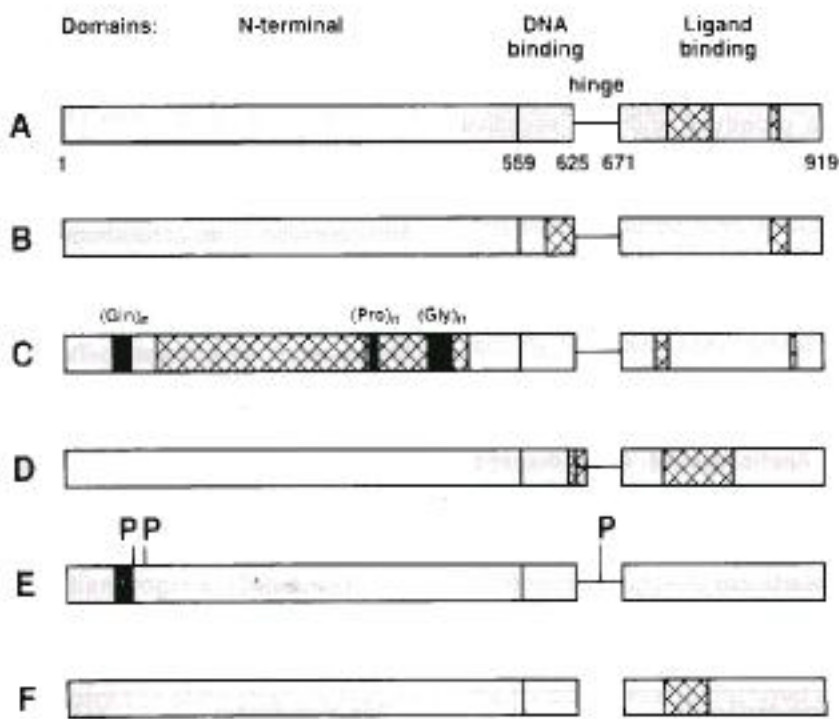


Fig. (2). Localization of AR functional regions. (A) Regions involved in the formation of the hydrophobic binding pocket. (B) Dimerization interfaces. (C) Transactivation domains. (D) Regions involved in nuclear localization. (E) Phosphorylated Ser residues. (F) Hsp90 binding site.

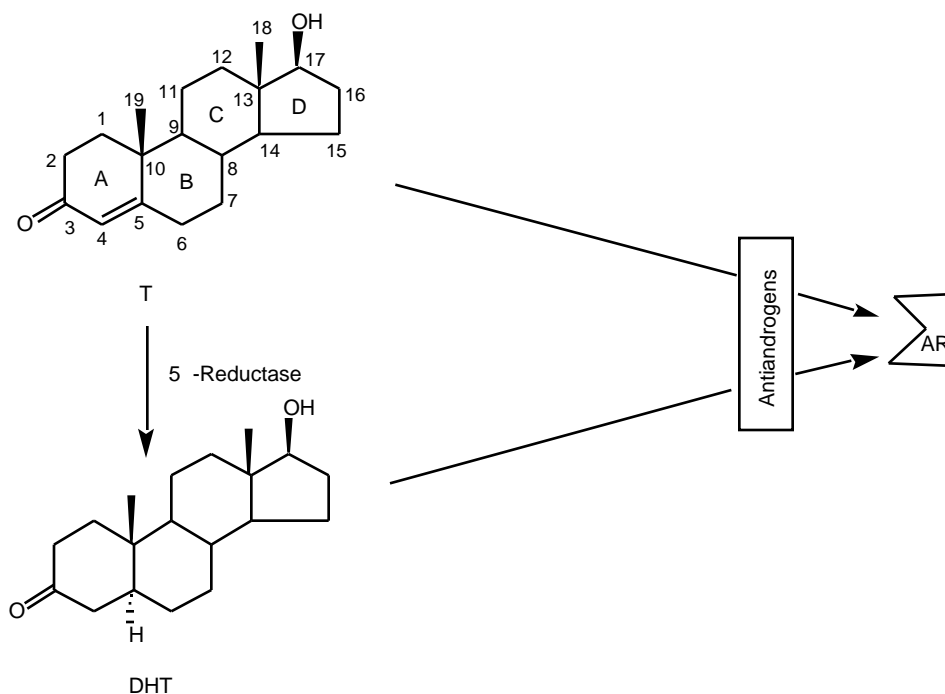


Fig. (3). Biological action of testosterone (T) and dihydrotestosterone (DHT) to androgen receptor (AR).

activities, and the non-steroidal derivatives or the pure antiandrogens of the class of flutamide, which block the androgen receptor without exerting any agonistic or any other hormonal activity. The higher efficacy of pure antiandrogens in preventing the binding of androgens to the androgen receptor has been demonstrated in a series of experimental models, including the rat ventral prostate, the level of mRNAs encoding the subunits of prostatic binding protein, ornithine decarboxylase activity as well as a growth of androgen-sensitive tumors [38-41]. As therapeutic agents, antiandrogens can be used to treat various androgen-sensitive diseases, either by topical or by systemic administration (Table 1).

Topical Treatment

Up till now, no topically active antiandrogen has been available and only some women with female

androgenization are currently treated by systemic administration of flutamide, cyproterone acetate or spironolactone [42]. For obvious reasons, such treatment cannot be applied to treat male acne and male pattern baldness [43,44]. An antiandrogen useful to treat skin disorders must be active topically and act through cutaneous androgen receptors and has to be devoid of systemic activity.

Systemic Treatment

In contrast to topical treatment, systemic administration of an antiandrogen inhibits androgenic action in all target tissues and not only at the desired target site. It thus interferes with the androgen-dependent negative feedback mechanisms regulating the secretion of androgens. The feedback action is exerted via the hypothalamo-pituitary-testicular axis. Androgens decrease the secretion of hypothalamic

Table 1. Clinical Application of Antiandrogens

| Androgen effect | Application | Route of administration |
|---|---|-------------------------|
| Sexual behavior | Hypersexuality | Systemic |
| Hypothalamo-pituitary-testicular hormone (H.P.T.) secretion | Study of H.P.T. axis activity | Systemic |
| Bone maturation | Precocious puberty | Systemic |
| Sebaceous gland function | Acne, hyperseborrhea | Topical |
| Hair growth | Hirsutism, male pattern alopecia | Topical or systemic |
| Growth of androgen-dependent tumors | Androgen-dependent tumors (prostate cancer) | Systemic |

LHRH and decrease the sensitivity of pituitary LH secretion to LHRH, thus decreasing the release of LH and, consequently, of testosterone secretion [45]. Suppressing androgen action at the hypothalamo-pituitary level will result in an increase in plasma LH and testosterone concentrations which can, at least partially, overcome the effect of the antiandrogen. This has been shown in the case of pure antiandrogens. For the treatment of prostate cancer, combination of chemical (LHRH agonist) or surgical castration with systemic antiandrogens have been shown to prevent the effect of the antiandrogen-induced increase in plasma testosterone [46-51].

Antiandrogens

This section will provide an overview of the development in antiandrogen structure-activity relationships (SAR) in the context of both *in vitro* potency, *in vivo* efficacy in animal models, and clinical results in the human, where available. Where the *in vitro* potency of antiandrogens is not available, SAR will be deduced from *in vivo* efficacy, although, it may not provide the real potency of antiandrogens. Since the data presented have been collected in a number of laboratories over the past few decades from a variety of tissues as sources of AR, comparison of the activity of compounds cannot be made rigorously unless they have been assayed in the same system. Correlation between receptor binding affinity and biological activity has been actively pursued to facilitate rapid and simple assessment of the characteristics of antiandrogens. One significant deficiency of measurements of receptor binding affinity is that such studies do not distinguish between agonists and antagonists. Compounds which show non-stimulatory effect on androgen-sensitive parameters such as Shionogi cells and other models should be considered as pure antiandrogens (antagonists). There are few reviews which cover partial SAR of antiandrogens [42,52,53]. In the present review, attempts are made to provide full SAR of all classes of antiandrogens, except for a few, where no SAR data are available.

Steroidal Antiandrogens

Testosterone and Dihydrotestosterone Derivatives as Antiandrogens (Table 2)

Large-scale correlation studies between the structure, binding affinities, and activities of agonists first of all led to the recognition of the structural features or combinations of features that are associated with high affinity and high activity [54,55]. The natural hormones T and 5 α -DHT, at low doses, bind

exclusively to the androgen receptor, although 5 α -DHT is a more potent competitor than T.

The dramatic decrease in the RBAs of androstanediol (**4**), and androstanedione (**5**) illustrate the need for a "17 α -hydroxy-3-one" structure for effective binding to AR. Nor-testosterone (**6**) displays higher affinity than its parent. Introduction of a 17 α -methyl group into T or 5 α -DHT somewhat decreases AR binding (compare **2** and **7**). Combination of 17 α -methyl substitution and unsaturation leads compound RU 1881 (**8**) which displays high affinity for AR and PR, and also binds with GR. Methyltrienolone (RU 1881) is a highly potent androgen. The flat and flexible nature of this molecule explains its lack of specificity. Removal of the 3-keto group results in the significant loss of binding affinity of compound **9**. In general, most compounds which display high affinity for the androgen receptor were androgen receptor agonists. Substituting the A- and B-ring with methyl groups decreased binding to AR and a gem-dimethyl group even further reduced the binding affinity of compound RU 2956 (**10**). This compound showed a mixed androgenic/antiandrogenic activity *in vivo* [56]. This initial study provided valuable information on the affinity of testosterone, dihydrotestosterone, and their derivatives and, moreover, on the functional groups which are responsible for high affinity, and the substituents and their positions, which decrease binding affinity.

WIN17665 (**11**) and SH434 (**12**) displayed no significant antiandrogenic activity *in vivo* [58-61]. Topical application of 17 α -propyltestosterone (WIN 17665) gave a dose-related regression of the hamster flank organ and sebaceous gland size. 17 α -Propylmesterolone (SH 434), another compound of this class, reduced both sebaceous gland size and sebogenesis significantly in a dose-dependent manner. Both compounds did not show any change in prostate weight and, thus, possess little or no systemic activity on topical administration. SH 434 has been shown to be effective in acne patients [75]. Other modifications in the testosterone skeleton, for instance, introduction of gem-dimethyl group at the 16-position, gave topically active antiandrogens **14-16**. However, compounds **13-19**, when given subcutaneously, showed very little effect on reduction of prostate weight. Only moderate *in vivo* potency of all compounds suggests that these antiandrogens have an insufficient *in vivo* half-life and/or activity.

The well-known aldosterone antagonist (spironolactone) also displayed antiandrogenic activity in the rat and human when compared to flutamide after 6 months of therapy [42]. Spironolactone (**20**) reduced hirsutism score by 30% whereas, for flutamide, this

score completely decreased to normal. Moreover, spironolactone caused only a 50% reduction in acne and seborrhea (no effect on hair loss) after 3 months of therapy. In the same randomized study, flutamide caused an 80% decrease in the above scores, including hair loss. A number of other modifications in

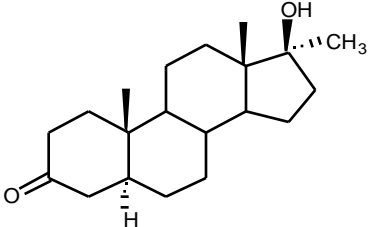
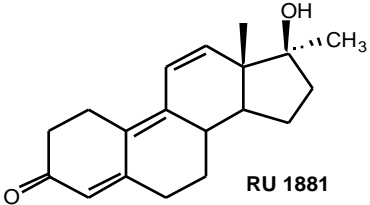
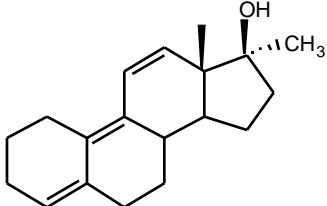
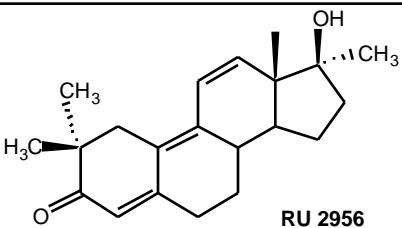
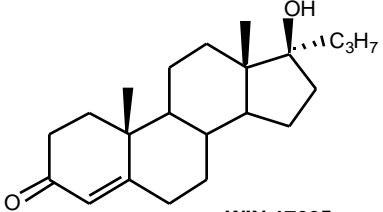
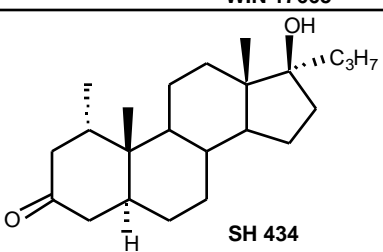
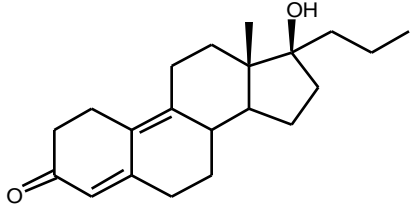
the structure of spironolactone gave other antiandrogens. For instance, lactone **24** reduced the ventral prostate weight in the rat by 39% at the oral dose of 5 mg per day while subcutaneous administration (3 mg/kg) of compound **21** reduced prostate size by 72%.

Table 2. Testosterone and Dihydrotestosterone Derivatives as Antiandrogens

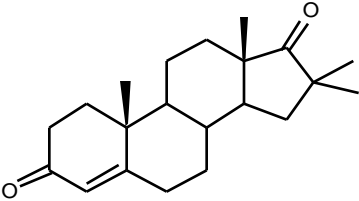
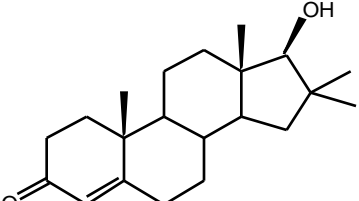
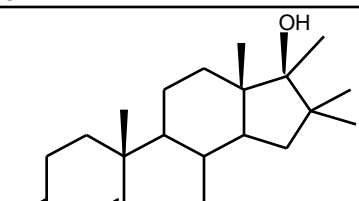
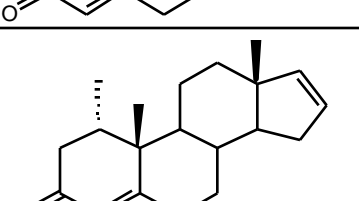
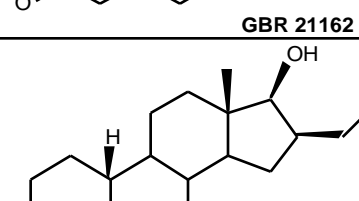
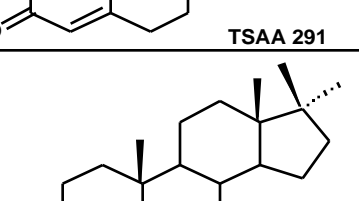
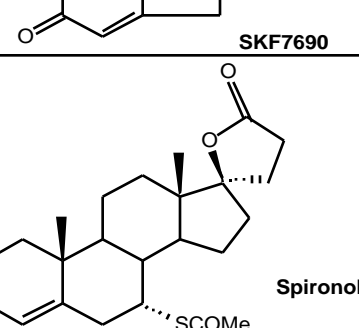
| No | Structure | Androgenic/antiandrogenic activity | | | Ref. |
|----|--|---|--|---------------------------|------------|
| | | RBA | Shio. cell proli.(IC ₅₀ , nM) | % Red. of VP wt (mg/kg/d) | |
| 1 | <p>Chemical structure of Testosterone (T): A steroid nucleus with a ketone group at C-3, a double bond at C-4, and a hydroxyl group at C-17.</p> | 100 19/97% inh. of rAR @ 0.001/ 1 μM^a | - | - | [53,54,57] |
| 2 | <p>Chemical structure of Dihydrotestosterone (DHT): A steroid nucleus with a ketone group at C-3, a hydrogen at C-4, and a hydroxyl group at C-17.</p> | 120 70/100% inh. of rAR @ 0.001/ 1 μM^a | - | - | [53,54,57] |
| 3 | <p>Chemical structure: A steroid nucleus with a ketone group at C-3, a hydrogen at C-4, and a hydroxyl group at C-17.</p> | 5-10 | - | - | [53,54,57] |
| 4 | <p>Chemical structure: A steroid nucleus with a ketone group at C-3, a hydrogen at C-4, a hydroxyl group at C-17, and a hydroxyl group at C-21.</p> | 10-15 | - | - | [53,54,57] |
| 5 | <p>Chemical structure: A steroid nucleus with a ketone group at C-3, a hydrogen at C-4, and a ketone group at C-20.</p> | 1-5 | - | - | [53,54,57] |
| 6 | <p>Chemical structure: A steroid nucleus with a ketone group at C-3, a double bond at C-4, and a hydroxyl group at C-17.</p> | 150-200 | - | - | [53,54,57] |

^aSingh SM, Labrie F et al. (1998) Unpublished results.

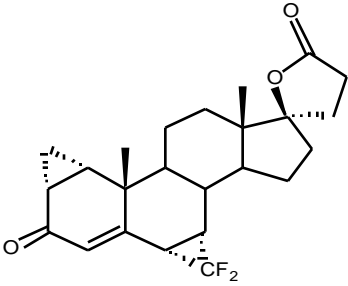
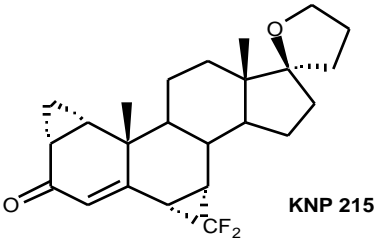
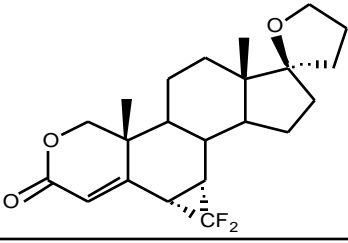
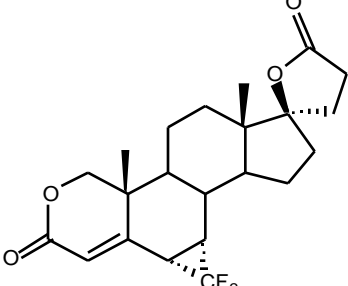
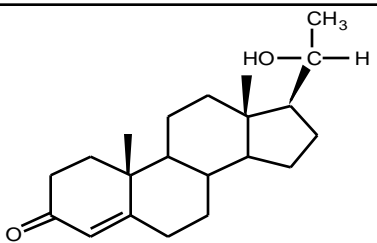
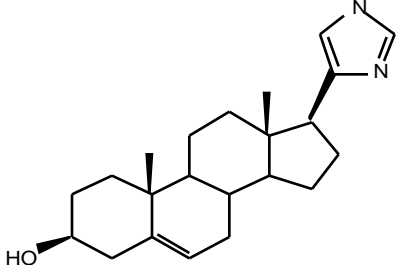
(Table 2). contd.....

| No | Structure | Androgenic/antiandrogenic activity | | | Ref. |
|----|--|---|---|--|-------------------|
| | | RBA | Shio. cell proli.(IC ₅₀ , nM) | % Red. of VP wt (mg/kg/d) | |
| 7 |  | 106 | - | - | [53,54,57] |
| 8 |  RU 1881 | 200-300 70/99% inh. of rAR @ 0.001/1 μm ² | - | - | [53,54,57] |
| 9 |  | < 1 | - | - | [53,54,57] |
| 10 |  RU 2956 | 50-75 | - | - | [53,54,56, 57] |
| 11 |  WIN 17665 | - | - | 49% dec. in seb. g. @ 200 μg/ham./3 wk | [58-60] |
| 12 |  SH 434 | - | - | 63% dec. in seb. g. @ 5 | [60,61] |
| 13 |  | 5.7 | - | 19 @ 750, po | [62] |

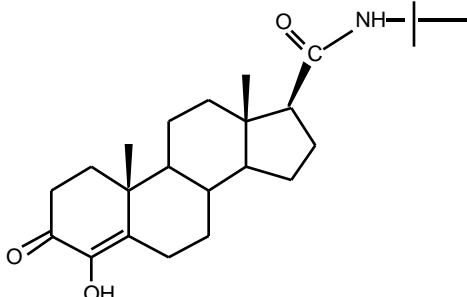
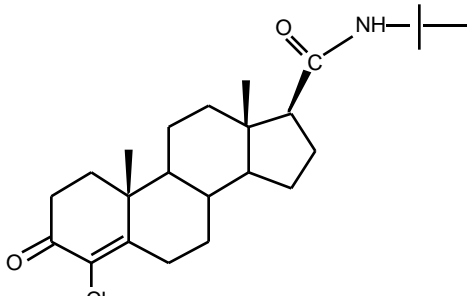
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| No | Structure | Androgenic/antiandrogenic activity | | | Ref. |
|----|---|------------------------------------|---|------------------------------|---------|
| | | RBA | Shio. cell proli.(IC ₅₀ , nM) | % Red. of VP wt (mg/kg/d) | |
| 14 |  | - | - | - | [63] |
| 15 |  | - | - | - | [63] |
| 16 |  | - | - | - | [63] |
| 17 |  GBR 21162 | - | - | - | [64] |
| 18 |  TSAA 291 | - | - | 58 @ 2.4, rat, sc | [65,66] |
| 19 |  SKF7690 | - | - | 38 @ 200, sc | [67-69] |
| 20 |  Spironolactone | - | - | 33 @ 3, rat, po | [70] |

(Table 2). contd.....

| No | Structure | Androgenic/antiandrogenic activity | | | Ref. |
|----|---|------------------------------------|---|------------------------------|------|
| | | RBA | Shio. cell proli.(IC ₅₀ , nM) | % Red. of VP wt (mg/kg/d) | |
| 21 |  | - | - | 72 @ 3, sc | [71] |
| 22 |  KNP 215 | - | - | 51 @ 10, po | [72] |
| 23 |  | - | - | 54 @ 10, po | [72] |
| 24 |  | - | - | 39 @ 5, po | [72] |
| 25 |  | - | >50% h WT AR inh. @ 0.1 μM | - | [73] |
| 26 |  | - | 46% h WT AR inh. @ 1 μM 50% LNCaP AR inh. @ 1 μM | - | [73] |

(Table 2). contd.....

| No | Structure | Androgenic/antiandrogenic activity | | | Ref. |
|----|---|------------------------------------|---|------------------------------|------|
| | | RBA | Shio. cell proli.(IC ₅₀ , nM) | % Red. of VP wt (mg/kg/d) | |
| 27 |  | - | 242 | - | [74] |
| 28 |  | - | 170 | - | [74] |

Compounds **25** and **26**, primarily developed as inhibitors of human 17(-hydroxylase/C₁₇₋₂₀ lyase, also displayed the antagonist and agonist effects on the wild-type human AR and on the mutant human AR present in LNCaP cells [76]. Compound **26** displayed a 46% reduction in transcriptional activity at 1 μ M and more than 50% reduction of activity of the wild-type AR was obtained by compound **25** at 0.1 μ M. However, only compound **26** showed antagonistic (50% at 1 μ M) effect on LNCaP AR. A variety of C4 substituted C17 *t*-butylamide steroids developed as inhibitors of 5 - reductase also inhibited DHT-stimulated Shionogi cell proliferation. Thus, compounds **27** and **28** displayed moderate antiproliferative activity.

Synthetic Progestins as Antiandrogens (Table 3)

Synthetic progestins, primarily prepared as progestins, also showed significant antiandrogenic activities together with androgenic activities [57,77]. CPA (**29**), the 6-chloro-1,2-methylene derivative of 17 -acetoxyprogesterone, exhibited high affinity for the rat androgen receptor (RU 1881 as a reference compound; RBA=158/203). Removal of the 1,2-methylene group gave chlormadinone acetate (CDA, **30**) with increased androgen binding, especially at short incubation times. Replacement of the chlorine by a methyl (MGA, **31**) slightly decreased binding whereas

further removal of the C6 double bond (MPA, **32**) modified binding kinetics.

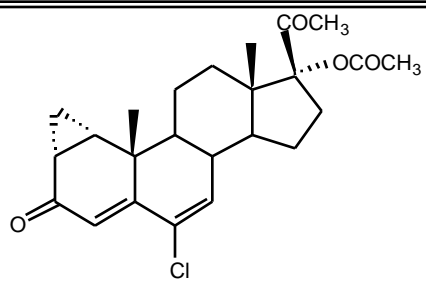
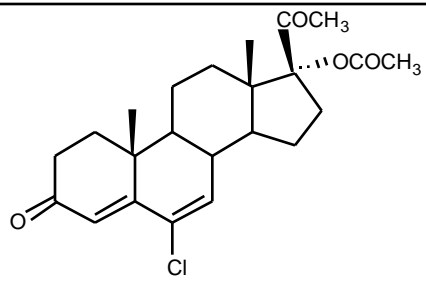
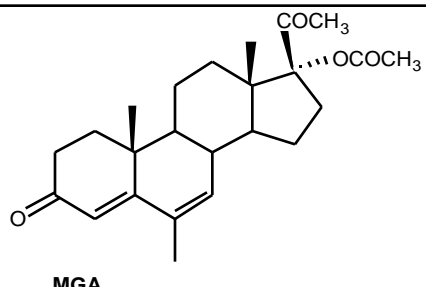
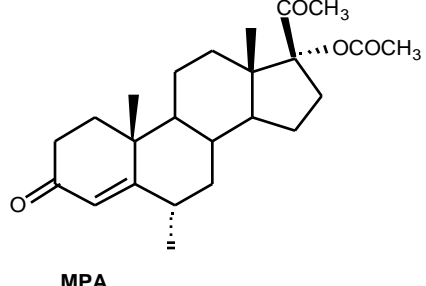
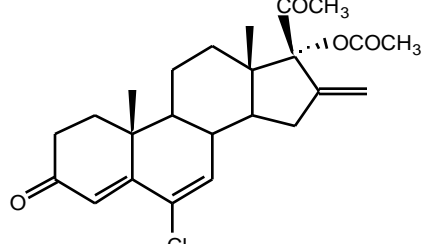
The RBA of MPA did not decrease with the incubation time, thus indicating a stronger association with AR than other synthetic progestins and explaining the relatively high androgenic activity of the compound (**32**). Other synthetic progestins **29-31** also exhibited androgenic activity on other androgen-sensitive parameters. Thus, the compounds stimulated cell growth in an androgen-sensitive clone of the mouse mammary carcinoma Shionogi SC-115 cells and their agonist activities were completely blocked by flutamide [38,39]. They also exhibited significant androgenic activity in ZR-75-1 cells co-transfected with hAR (DHT=100% at 0.1 μ M). Moreover, these progestins also exhibit glucocorticoid and antimineralocorticoid activities which seriously limit their tolerance, efficacy, and use, particularly where an optimal blockade of androgens is required, especially in prostate cancer.

Recently, other modifications in the A-, B-, C-, and D-rings of the progestin skeleton were carried out, in the hope, to obtain more potent antiandrogens. 2-Oxachlormadinone acetate (**37**) and 2-azachlormadinones (**39,40**) gave significant *in vivo* antiandrogenic activity. Thus, the potency of 2-oxachlormadinone, TPZ-4238, was the highest in the new progestin series. At the dose of 6 mg/kg/day, TPZ-4238 reduced rat ventral prostate weight by 75%. TPZ-

4238 (37), when compared with CDA, presently used in the medical management of BPH in Japan, produced a regression in canine BPH at the dosage of 0.1 mg/kg/day. This compound was 5 times more effective

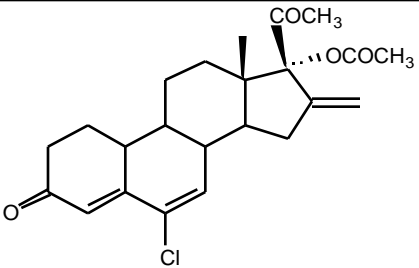
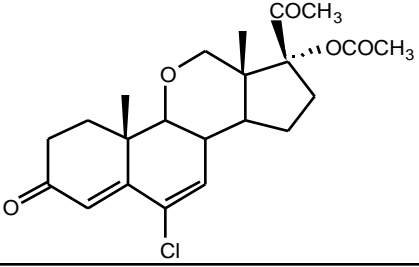
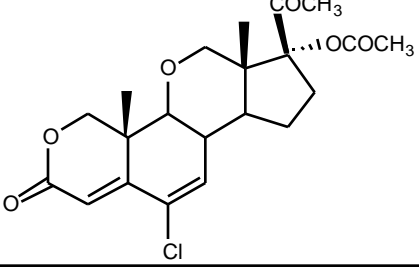
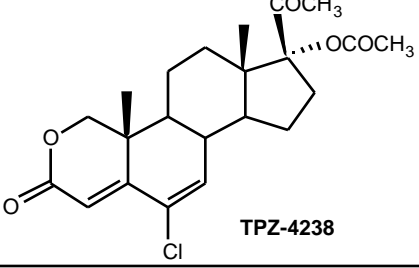
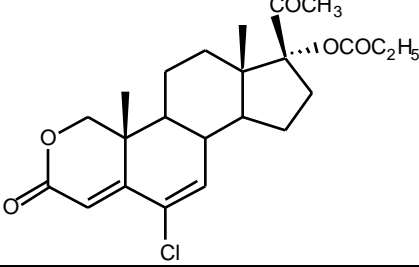
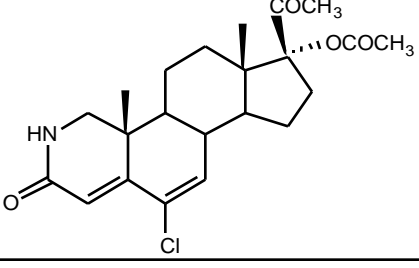
than CDA (3 mg/kg/day) [81,82]. The clinical significance of CPA and others will be discussed in the later part of the review.

Table 3. Synthetic Progestins as Antiandrogens

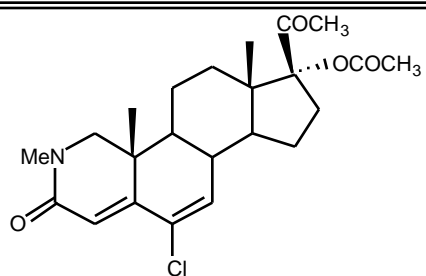
| No | Structure | Antiandrogenic activity | | Ref. |
|----|--|-------------------------|--|---------------|
| | | RBA (30 min/2 h) | % Red. of VP wt (mg/kg/d) | |
| 29 |  <p>CPA</p> | 51/16 | 66 @ 0.5/mice/bid, sc ^a | [38,39,57] |
| 30 |  <p>CDA</p> | 81/20 | 20 @ 5, po 55 @ 45, po 62 @ 0.5/mice/bid/sc ^a | [38,39,57,78] |
| 31 |  <p>MGA</p> | 67/19 | 57 @ 0.5/mice/bid/sc ^a | [38,39,57] |
| 32 |  <p>MPA</p> | 40/51 | 47 @ 0.5/mice/bid/sc ^a | [38,39,57] |
| 33 |  | - | 50 @ 10, sc | [79] |

^aSingh SM, Labrie F et al. (1998) Unpublished results.

(Table 3). contd.....

| No | Structure | Antiandrogenic activity | | Ref. |
|----|---|-------------------------|---------------------------|------------|
| | | RBA (30 min/2 h) | % Red. of VP wt (mg/kg/d) | |
| 34 |  | - | 35 @ 10, sc | [79] |
| 35 |  | - | 12 @ 2, po | [78,80] |
| 36 |  | - | 42 @ 2, po | [78,80] |
| 37 |  TPZ-4238 | - | 75 @ 6, po | [78,80-82] |
| 38 |  | - | 79 @ 6, sc | [78,80] |
| 39 |  | - | 57 @ 6, sc | [80,83] |

(Table 3). contd.....

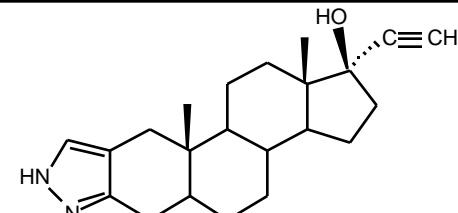
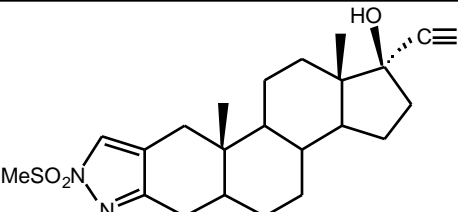
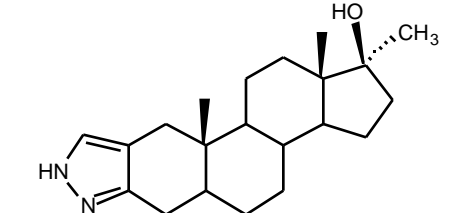
| No | Structure | Antiandrogenic activity | | Ref. |
|----|---|-------------------------|---------------------------|---------|
| | | RBA (30 min/2 h) | % Red. of VP wt (mg/kg/d) | |
| 40 |  | - | 57 @ 6, sc | [80,83] |

Antiandrogenic Steroidal Sulfonyl Heterocycles (Table 4)

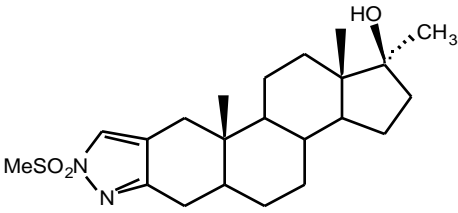
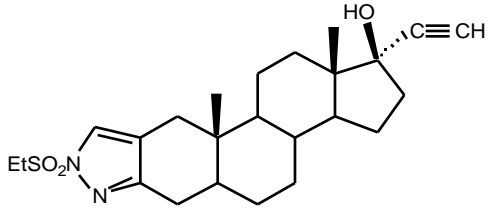
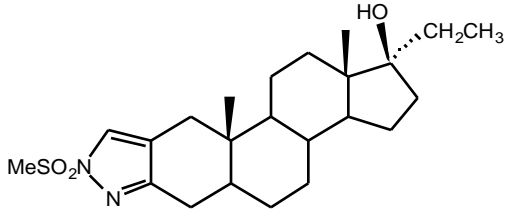
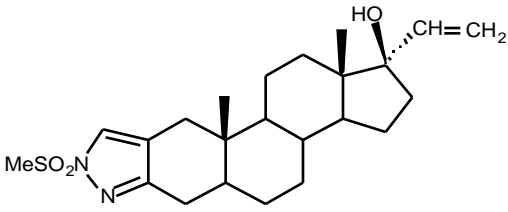
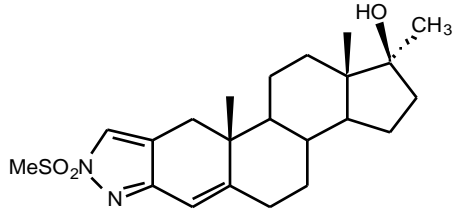
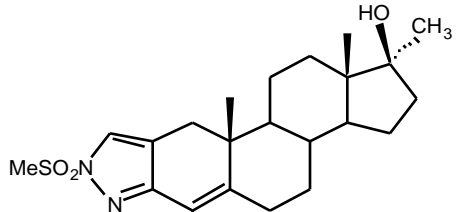
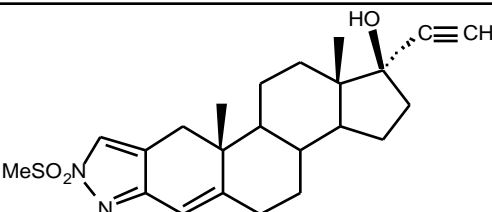
The Sterling group synthesized [84-87] and evaluated steroidal sulfonyl [3,2-c]pyrazoles and their bioisosteric sulfonyl heterocycles as androgen receptor antagonists. The parent pyrazole **41** bound strongly to the androgen receptor and displayed significant mixed androgenic/antiandrogenic activity *in vivo*. However, its 1'-methylsulfonyl derivative **42** exhibited less affinity to AR, but it was more potent than **41** *in vivo*. The 17 -

methyl analog **43** of the parent pyrazole showed the highest affinity for AR and was a potent androgen *in vivo*. However, the 1'-methylsulfonyl derivative **44** displayed moderate affinity compared to **43**, but this compound was a potent antiandrogen *in vivo*. It thus seems that this 1'-methylsulfonyl group is critical for significant binding to AR and antiandrogenic activity. The larger alkylsulfonyl groups compared to the methyl increased the receptor affinity, and showed mixed androgenic/antiandrogenic activity (compare compounds **42** and **45**).

Table 4. Antiandrogenic Steroidal Sulfonyl Heterocycles

| No | Structure | Antiandrogenic activity | | Ref. |
|----|--|-------------------------|--------------------------|---------|
| | | RBA(1 h/18 h) | ED ₅₀ (mg/kg) | |
| 41 |  | 28/0.8 (DHT: 87/88) | 50(50% VP wt inc.) | [88] |
| 42 |  WIN 49596 | 2.2/0.05 | 15 ^a | [88,89] |
| 43 |  | 164/0.8 | - | [88] |

(Table 4). contd.....

| No | Structure | Antiandrogenic activity | | Ref. |
|----|---|-------------------------|--------------------------|------|
| | | RBA(1 h/18 h) | ED ₅₀ (mg/kg) | |
| 44 |  | 16/1 | 10 ^a | [88] |
| 45 |  | 2.7/0.1 | 100 (50% VP wt inc.) | [88] |
| 46 |  | 3.0/0.1 | 41 ^a | [88] |
| 47 |  | 4.0/0.1 | 33 ^a | [88] |
| 48 |  | 12.0/0.9 | 16 ^a | [88] |
| 49 |  | 18.0/1.0 | 3 ^a | [88] |
| 50 |  | 7.0/0.05 | 14 ^a | [88] |

(Table 4). contd.....

| No | Structure | Antiandrogenic activity | | |
|----|-----------|-------------------------|--------------------------|---------|
| | | RBA(1 h/18 h) | ED ₅₀ (mg/kg) | Ref. |
| 51 | | 1.9/0.2 | 8 | [90,91] |
| 52 | | 1.4/0.16 | 17 | [90] |
| 53 | | 1.2/0.05 | 22 | [90] |
| 54 | | 5.8 | 32% @ 750 mg/kg/d | [62] |

^aAndrogenic activity was not significant.

Partial and complete saturation of the 17 -triple bond increased the affinity for AR and decreased the *in vivo* efficacy (compare compounds **42**, **46**, and **47**). In the 4-series, the binding affinity of **48** was similar to **44**. Introduction of the methyl group at the C-4 position, compound **49**, increased the affinity as well as the *in vivo* potency. A number of bioisosteric sulfonyl heterocycles were also prepared, 2'-methylsulfonyl furan **51** showing low AR affinity, but improvement of *in vivo* potency compared to **52** and **53**. Out of all, WIN 49596 (**42**) was evaluated further in a preclinical study [89]. Daily administration (20-500 mg/kg) of WIN 49596 to mature male rats for 72 days gave a significant inhibition of ventral prostate and seminal vesicle weights. At the highest dose level (500 mg/kg), the weight of the ventral prostate and seminal vesicles was reduced by 64% and 48%, respectively, without compromising reproductive function. Zanterone (WIN 49596) is in a phase II clinical trial in human for the treatment of BPH and prostate cancer in the USA.

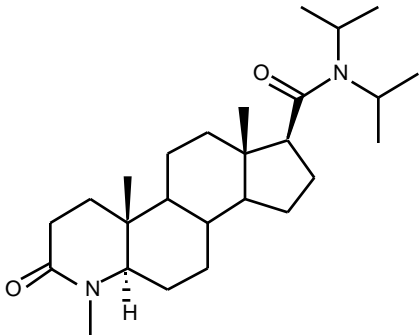
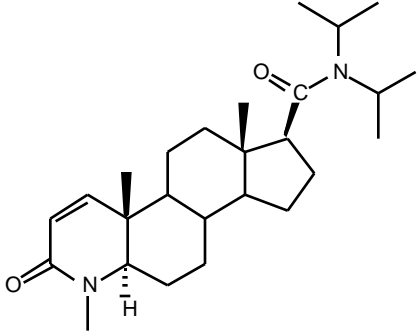
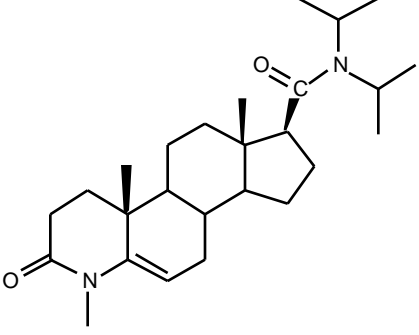
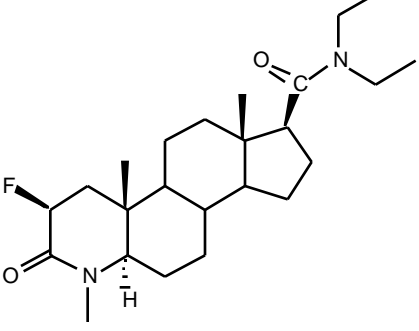
4-Azasteroids as Antiandrogens (Table 5)

4-Azasteroids primarily prepared as inhibitors of 5 α -reductase also displayed moderate to good antiandrogenic activity *in vitro* and *in vivo* [92-95]. Antiandrogenic activity varied dramatically depending upon the nature of substitution. For instance, replacement of N-CH₃ (compound **63**) by N-H (compound **62**) greatly diminished the affinity of compounds. However, introduction of the 1,2- (compare compounds **63** and **64**) or 5,6- (compare compounds **55** and **57**) double bond increased the activity relative to the parent compound. Other A-ring modifications of 4-azasteroids such as addition of 2 - fluoro (**58**) and 1,2 -epoxy (**59**) gave enhancement in antiandrogenic activity. A range of 17-substituted azasteroids was also evaluated, N-dialkyl amides and carbonyls were less effective. On the other hand, NH-aryl amides (**63-65**) displayed very strong antiandrogenic activity. Significant loss of activity was observed when NH-aryl amide (**64**) was replaced by N-

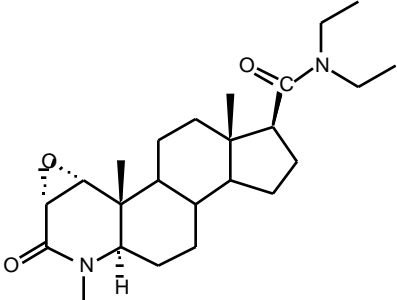
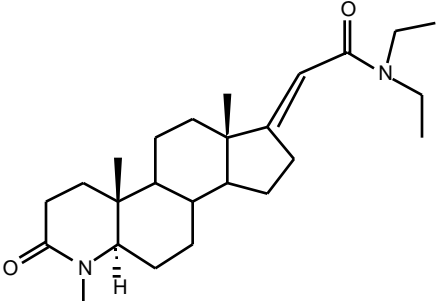
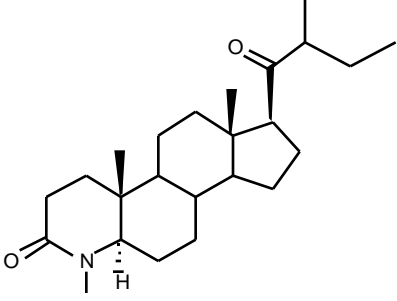
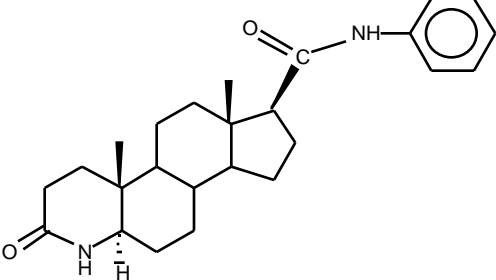
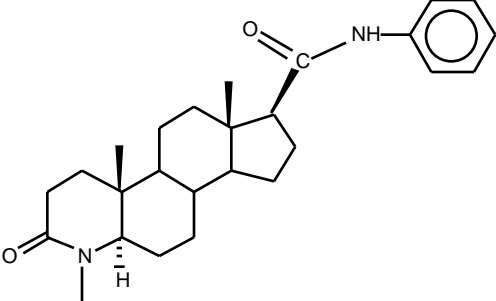
alkylaryl amide (**66**). *In vivo* assays utilizing castrated male rats, oral administration of compounds **55**, **57**, **58**, **60** and **61** in testosterone propionate treated rats caused a severe reduction of ventral prostate weight

compared to dihydrotestosterone propionate treated rats. This difference in activity versus the two androgens is due to that compounds are much more potent inhibitors of 5 α -reductase than antiandrogens.

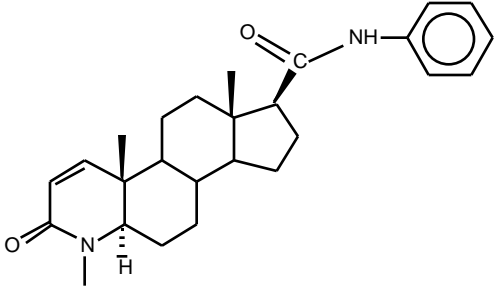
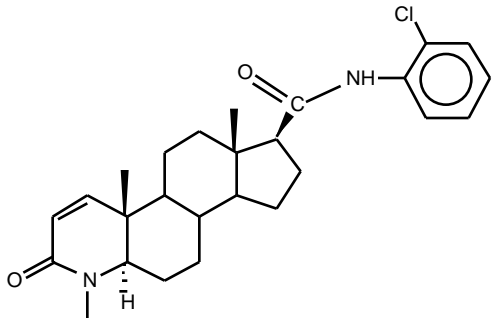
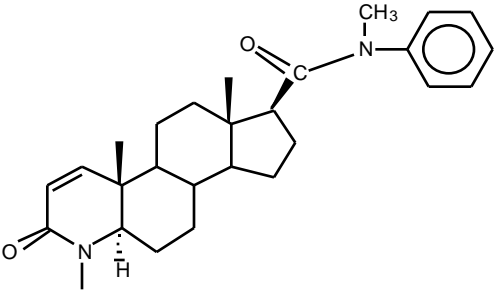
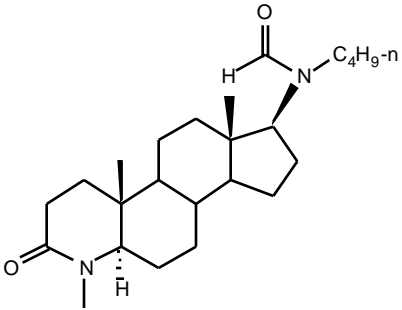
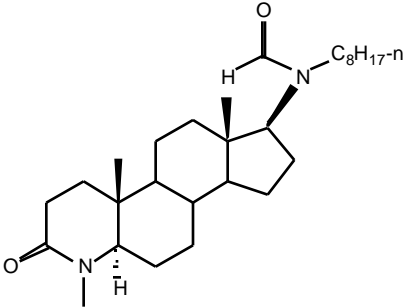
Table 5. 4-Azasteroids as Antiandrogens

| No | Structure | Antiandrogenic activity | | | Ref. |
|----|---|------------------------------------|---|--|---------|
| | | rAR or hAR (IC ₅₀ , nM) | Inh. of Shio.cell proli.(IC ₅₀ , nM) | % Red. of VP wt ^D (mg/kg/d) | |
| 55 |  | 14,000(rAR) | - | >51 @ 108 | [92,93] |
| 56 |  | 10,000(rAR) | - | - | [92] |
| 57 |  | 10,000(rAR) | - | > 25 @ 90 | [92,93] |
| 58 |  | 1800 (rAR) | - | > 23 @ 90 | [92,93] |

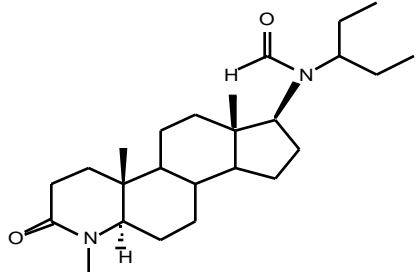
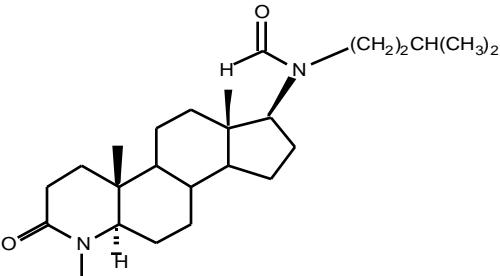
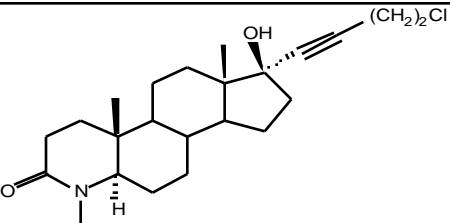
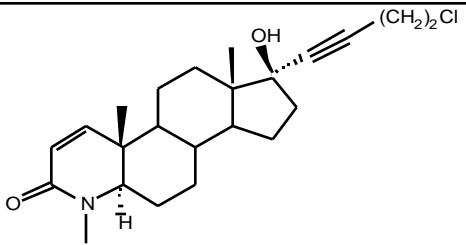
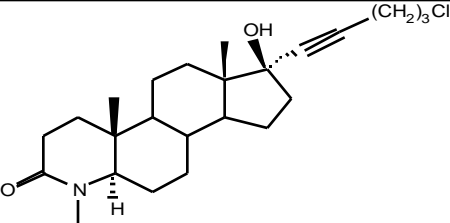
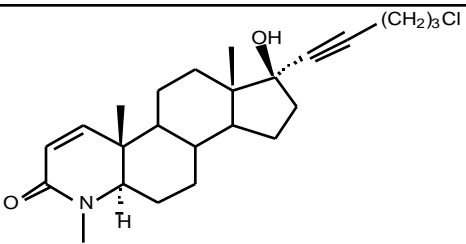
(Table 5). contd.....

| No | Structure | Antiandrogenic activity | | | Ref. |
|----|---|------------------------------------|--|---------------------------------------|---------|
| | | rAR or hAR (IC ₅₀ , nM) | Inh. of Shio.cell proli.(IC ₅₀ , nM) ^a | % Red. of VP wt(mg/kg/d) ^b | |
| 59 |  | 710(rAR) | - | - | [92] |
| 60 |  | 930(rAR) | - | >23 @ 108 | [92,93] |
| 61 |  | 420(rAR) | - | >25 @ 90 | [92,93] |
| 62 |  | 23,000(hAR) | - | - | [96,97] |
| 63 |  | 90(hAR) | - | - | [96,97] |

(Table 5). contd.....

| No | Structure | Antiandrogenic activity | | | Ref. |
|----|---|---------------------------------------|---|--|---------|
| | | rAR or hAR (IC ₅₀ , nM) | Inh. of Shio.cell proli.(IC ₅₀ , nM) ^a | % Red. of VP wt(mg/kg/d) ^b | |
| 64 |  | 5(hAR) | - | - | [96,97] |
| 65 |  | 8(hAR) | - | - | [96,97] |
| 66 |  | 6,000(hAR) | - | - | [96,97] |
| 67 |  | - | 166 | - | [98] |
| 68 |  | - | 50 | - | [98] |

(Table 5). contd....

| No | Structure | Antiandrogenic activity | | | Ref. |
|----|---|---------------------------------------|---|--|------|
| | | rAR or hAR (IC ₅₀ , nM) | Inh. of Shio.cell proli.(IC ₅₀ , nM) ^a | % Red. of VP wt(mg/kg/d) ^b | |
| 69 |  | - | 90 | - | [98] |
| 70 |  | - | 46 | - | [98] |
| 71 |  | - | 250 | - | [99] |
| 72 |  | - | 95 | - | [99] |
| 73 |  | - | 129 | - | [99] |
| 74 |  | - | 67 | - | [99] |

^aAntagonism was performed on Shionogi mouse mammary carcinoma cells in the presence of DHT (0.3 nM). Reference compound: hydroxyflutamide; IC₅₀=54 nM.

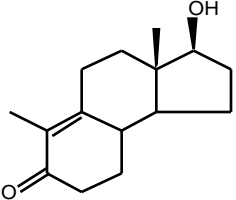
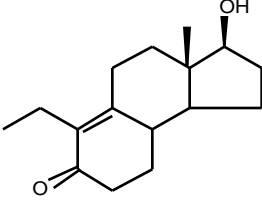
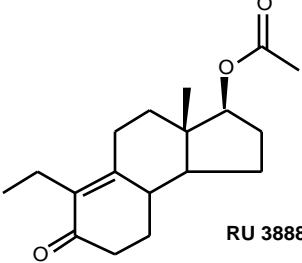
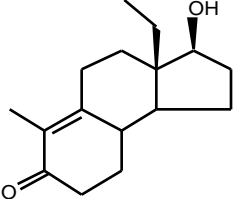
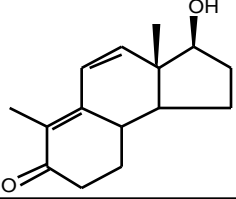
^bDihydrotestosterone propionate (0.4 mg/kg/day). Oral administration of antiandrogens.

In the C17 reversed amide class, in general, antiandrogenic activity increased as the N-alkyl chain length increased. For instance, compound **68** showed better antiproliferic activity than **67** on DHT-stimulated Shionogi cell proliferation. 17-Hydroxy-17-(1-chloroalkyn-1'-yl)-4-azasteroids, another class of 4-azasteroids, also showed good antiandrogenic activity. In this class, introduction of the 1,2-double bond also increased the potency (compare compounds **71**, **72**, **73**, and **74**). The C4 and C5-chain lengths showed similar activity. Replacement of the chloro group by the bromo or iodo group also gave similar activity, but the corresponding hydroxy group gave inactive compounds.

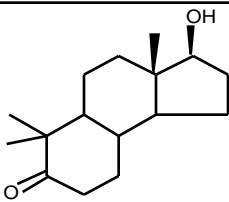
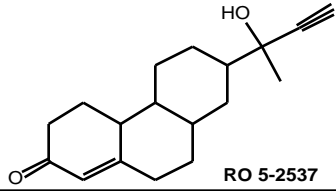
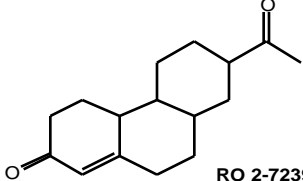
Des-A-steroidal Antiandrogens (Table 6)

In the steroid series, the presence of the 3-keto and 17-hydroxy groups is essential for interaction with the androgen receptor, and is important for biological activity. In the des-A-steroidal series, where the distance (8.9 Å) between the two oxygen functions (3-keto and 17-OH groups) is much shorter than in the steroid series (10.9 Å), the tricyclic derivatives showed noticeable affinity for the androgen receptor. A methyl group at the C10 position and conjugated double bond in the B- and C-ring enhanced the receptor affinity. When administered subcutaneously to immature castrated rats, compounds **75-79** reduced prostate weight and the most active compound was **76**.

Table 6. Des-A-steroidal Antiandrogens

| No | Structure | Antiandrogenic activity | | Ref |
|----|---|-------------------------|--|-----------|
| | | RBA ^a | % Red. of VP wt (mg/kg/d/sc) | |
| 75 |  | 4 | 27 @71 ^b | [100,101] |
| 76 |  | 1 | 59 @71 | [100,101] |
| 77 |  RU 38882 | - | 50 @71 80 @ 1/ham.(flank organ wt.) | [100-102] |
| 78 |  | 5 | 10 @ 71 | [100,101] |
| 79 |  | 8 | 25 @ 71 | [100,101] |

(Table 6). contd....

| No | Structure | Antiandrogenic activity | | Ref |
|----|--|-------------------------|------------------------------|-----------|
| | | RBA ^a | % Red. of VP wt (mg/kg/d/sc) | |
| 80 |  | 2 | 49 @ 71 | [100,101] |
| 81 |  RO 5-2537 | - | 54 @ 240 ^c | [103,104] |
| 82 |  RO 2-7239 | - | 78 @ 17 ^d | [105] |

^aRBA condition: rat prostate, 0°C, [3H]-testosterone, 0.7% ethanol, 24h.^bReference compound for *in vivo* experiment: cyproterone acetate (90% at 14 mg/kg/day). 0.7 mg/kg/day of testosterone propionate.^cTestosterone propionate (1.5 mg/kg/day).^dTestosterone. (2 mg/kg/day).

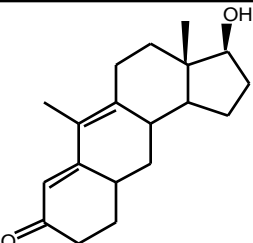
However, these compounds were weak antiandrogens compared to cyproterone acetate (**29**). Similar results were obtained when these compounds were tested locally on one hamster flank organ. Compound **77** reduced flank organ weight by 80% while under these conditions, compound **77** was more potent when compared to cyproterone acetate [102]. In another class of des-A-steroids, Ro 5-2537 showed weak antiandrogenic activity and no androgenic activity under the assay conditions used. Moreover, compound **81** also displayed progestational and uterotrophic activities. Another tricyclic derivative, compound **82**, showed potent antiandrogenic activity

along with antimyotrophic activity. Finally, this study provided valuable information that the tetracyclic structure, i.e. steroid backbone, is not essential for the androgen receptor affinity and antiandrogenic activity.

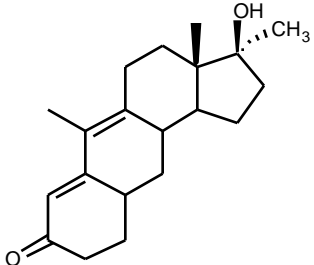
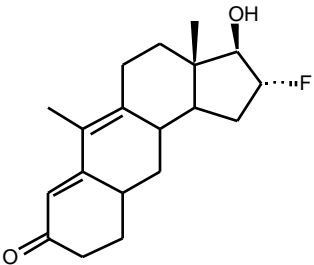
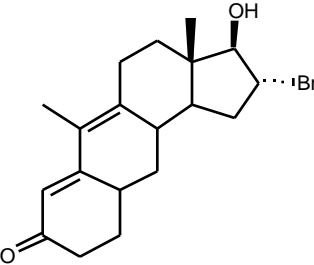
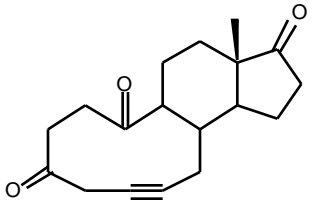
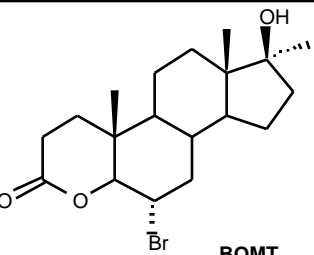
Other Steroidal Antiandrogens (Table 7)

Anthrasteroids **83-86** showed significant antiandrogenic activity *in vivo*, when administered subcutaneously. Compounds **83** and **86** also inhibited the androgen-dependent tumor growth (Shionogi-carcinoma 115) *in vivo*.

Table 7. Other Steroidal Antiandrogens

| No | Structure | Antiandrogenic activity | | | Ref. |
|----|---|-------------------------|--------------------------------|---------------------------|-------|
| | | RBA | % Inh. of tumor wt (mg/mice/d) | % Red. of VP wt (mg/kg/d) | |
| 83 |  | - | 92 @ 2 | 43 @ 25, sc | [106] |

(Table 7). contd.....

| No | Structure | Antiandrogenic activity | | | Ref. |
|----|---|-------------------------|--------------------------------|---------------------------|-----------|
| | | RBA | % Inh. of tumor wt (mg/mice/d) | % Red. of VP wt (mg/kg/d) | |
| 84 |  | - | - | 41 @ 25, sc | [106] |
| 85 |  | - | - | 52 @ 25, sc | [106] |
| 86 |  | - | 69 @ 2 | - | [106] |
| 87 |  | 50 | - | no inh. @ 12 | [107,108] |
| 88 |  BOMT | 2.7 | - | - | [109] |

Non-steroidal Antiandrogens

Flutamide Derivatives as Antiandrogens (Table 8)

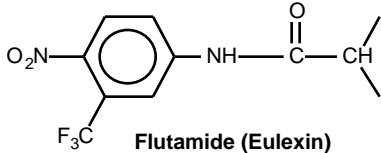
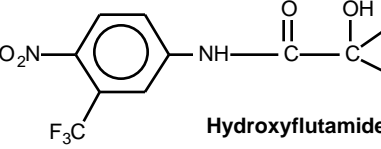
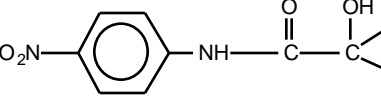
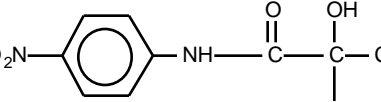
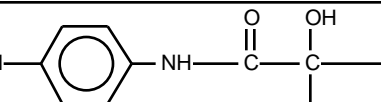
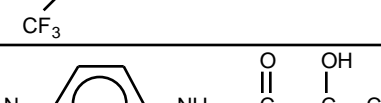
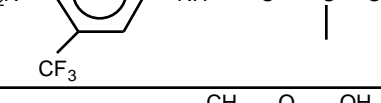
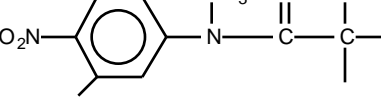
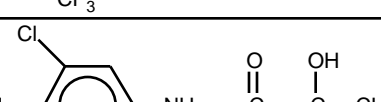
Flutamide and its derivatives are the most used and studied antiandrogens. Early development of flutamide (**89**) and its clinically proven efficacy led to the development of a series of its derivatives. Three non-

steroidal antiandrogens, i.e. flutamide (**89**), Anandron (**114**), and Casodex (**108**), have shown clinical benefits in the treatment of prostate cancer. The compound having the longest and largest clinical experience is flutamide, the first compound in prospective and randomized studies to be shown to prolong life in prostate cancer when associated with medical or surgical castration [46,110,111]. The clinical studies of the three non-steroidal antiandrogens will be

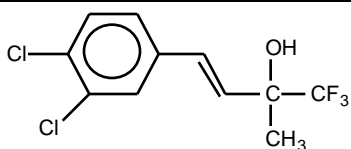
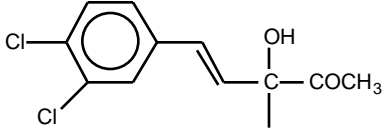
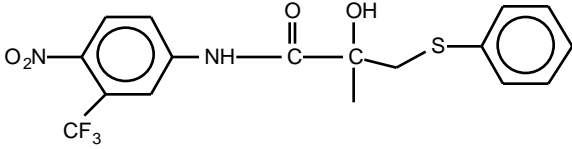
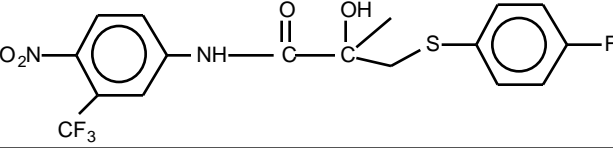
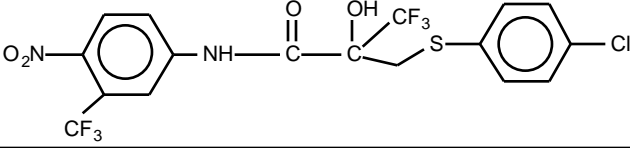
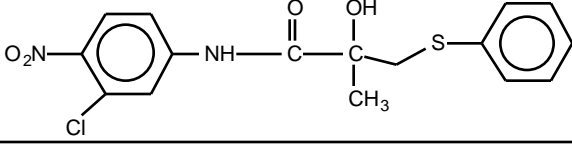
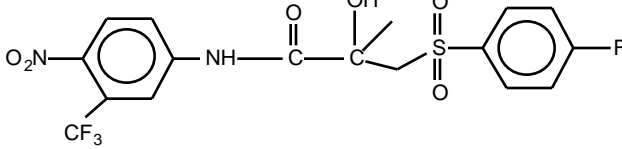
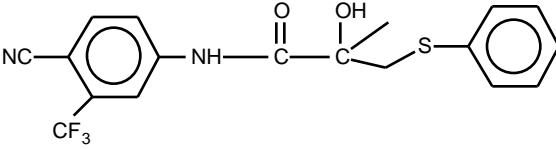
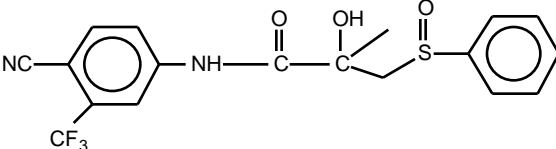
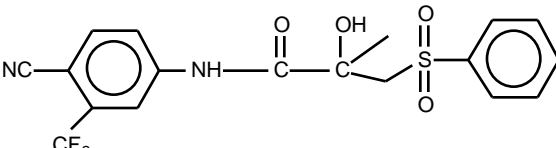
discussed in the later part of the review. Clinically, the most widely studied non-steroidal antiandrogen is flutamide, i.e. 2-methyl-4'-nitro-3'-(trifluoromethyl)propionanilide (**89**). 2-Hydroxylated derivative **90** of flutamide is the active metabolite. Structure-activity relationships infer that the most active compounds contain electron-withdrawing substituents in the aromatic ring and a branched alkyl chain to the amide

carbonyl. Compounds with two electron-withdrawing substituents on the aromatic gave a better potency than the one substituent (compare **90:91**; RBA=2.1:0.2). When the CF₃ group was introduced at the C2 position of anilide having only one electron-withdrawing substituent, antiandrogenic activity of compound increased by five-fold (compare **91:92**; RBA=0.2:0.9).

Table 8. Flutamide Derivatives as Antiandrogens

| No | Structure | Antiandrogenic activity | | Ref. |
|----|---|---|----------------------------|-------|
| | | RBA | ED ₅₀ (mg/kg/d) | |
| 89 |  <p style="text-align: center;">Flutamide (Eulexin)</p> | < 0.2 | 0.5, sc 80% @ 12, po | [112] |
| 90 |  <p style="text-align: center;">Hydroxyflutamide</p> | 2.1 IC ₅₀ =72 nM (Shio. cell) | 0.5, sc | [112] |
| 91 |  | < 0.2 | - | [112] |
| 92 |  | 0.9 | 5.0, sc | [112] |
| 93 |  | 6.6 | 0.15, sc | [112] |
| 94 |  | 15.1 | 0.12, sc | [112] |
| 95 |  | < 0.2 | - | [112] |
| 96 |  <p style="text-align: center;">AA560</p> | - | 93% @ 4, po | [113] |
| 97 |  <p style="text-align: center;">RU 22273</p> | <0.2 | >10, sc | [112] |

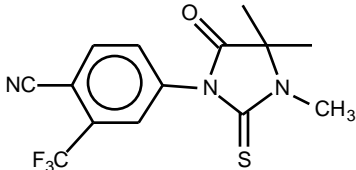
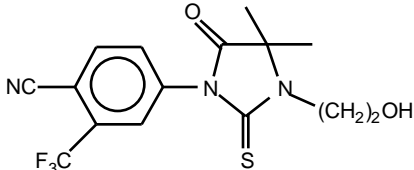
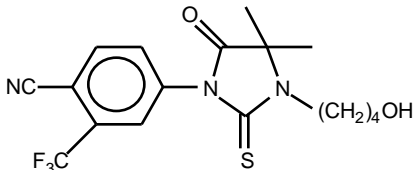
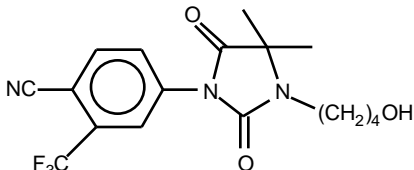
(Table 8). contd.....

| No | Structure | Antiandrogenic activity | | Ref. |
|-----|---|-------------------------|----------------------------|-------|
| | | RBA | ED ₅₀ (mg/kg/d) | |
| 98 |  | <0.2 | 1.5, sc | [112] |
| 99 |  | 0.4 | 0.2, po | [112] |
| 100 |  | - | 1.0, po | [114] |
| 101 |  | - | 1.1, po | [114] |
| 102 |  | - | 23% @ 25(agonist) | [114] |
| 103 |  | - | 0.5, po | [114] |
| 104 |  | - | 0.4, po | [114] |
| 105 |  | - | 1.7, po | [114] |
| 106 |  | - | 1.4, po | [114] |
| 107 |  | - | 1.8, po | [114] |

(Table 8). contd.....

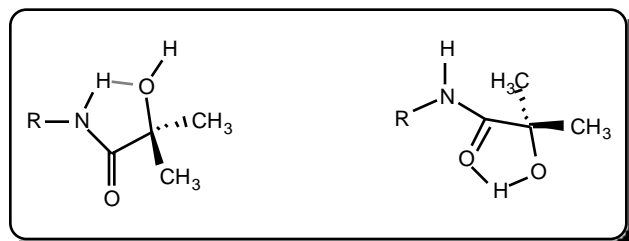
| No | Structure | Antiandrogenic activity | | Ref. |
|-----|--------------------------------------|--|------------------------------------|--------------|
| | | RBA | ED ₅₀ (mg/kg/d) | |
| 108 | <p>Casodex (Bicalutamide)</p> | IC ₅₀ =243 nM(Shio. cell) | 0.5, po 64% @ 12, po | [114-116] |
| 109 | | - | 30.0, po | [116] |
| 110 | | - | 0.5, po | [116] |
| 111 | | - | 1.1, po | [114] |
| 112 | | - | 10, po | [114] |
| 113 | | - | 2.0, po | [114] |
| 114 | <p>RU 23908 (Anandron)</p> | 4.5(T: 100) IC ₅₀ =412 nM (Shio. cell) | 58% @ 10, ip 94% @ 125, sc | [57,117-121] |
| 115 | <p>RU 22860</p> | - | 46% @ 10, ip | [57] |
| 116 | <p>RU 22930</p> | - | 60% red. of seb. g. @ 5 mg/ham. | [56] |

(Table 8). contd.....

| No | Structure | Antiandrogenic activity | | Ref. |
|-----|--|---|--------------------------------|-------|
| | | RBA | ED ₅₀ (mg/kg/d) | |
| 117 |  RU 56187 | ⁹² (R1881: 290) (DHT: 180) | 60 @ 1.0, po | [122] |
| 118 |  RU 57073 | 163 | 45 @ 1.0, po | [122] |
| 119 |  RU 59063 | 300 | 23 @ 1.0, po | [122] |
| 120 |  RU 58841 | Ka=1.4 nM (T; Ka=0.7 nM) ham. (F.O.) | 52% red. of F.O. @ 100 µg/ham. | [123] |

In a compound **94**, where all three factors were present, the highest potency of the series was obtained. SAR revealed several factors which were responsible for high antiandrogenic activity.

1. An electron-deficient aromatic ring.
2. A powerful hydrogen bond donor group.
3. Fixed conformers involved in intramolecular hydrogen binding.



Replacement of the anilide by the alkene gave weakly active compounds such as **97**. The weak

activity of this compound can be attributed to the lack of possible intramolecular hydrogen bonding or the poor hydrogen-bond donor capability. Thus, the only way to increase electron-donor ability is to introduce electron-withdrawing groups to hydroxy. Thus, compounds **98** and **99** having powerful donor showed stronger *in vivo* activities.

In the Casodex series, compounds with the cyano or nitro group at the 4'-position and the chloro **103** or trifluoromethyl group **100** at the 3'-position of the anilide ring gave improved antiandrogenic activity. In general, 2-trifluoromethyl compounds, such as **102**, showed a mixed agonist/antagonist activity. Antiandrogenic activities of the sulfide **105**, sulfoxide **106** and sulfone **107** were comparable *in vivo* and sulfones were the major metabolites of the sulfides *in vivo*. In the case of the arylthio analogs, para-substituted groups decreased activity, except for chloro, which had little effect on potency. However, the fluoro group increased potency (compare compounds **107** and **108**). R-Casodex (**110**) was 60 times more

potent than S-Casodex (**109**) *in vivo*. In the alkylthio series, potency was optimum for the ethylthio analog **111** and decreased with increasing size of the alkyl group **112**.

Anandron [(5,5-dimethyl-3-{4'-nitro-3'-(trifluoromethyl)phenyl}-2,4-imidazolidine dione)] exhibited very low affinity for AR. While, it competes for either labelled T or RU 1881 binding to cytosol from castrated rat prostate. Anandron (**114**) has shown efficacy in the treatment of prostate cancer when added to castration [124]. Other modifications, such as nitrogen to oxygen **115**, has little effect on the potency of the compounds. Dichloro analog also showed similar efficacy. RBAs decreased on changing the alkyl group to an alcohol moiety. However, N-substituted arylthiohydantoins exhibited relatively high binding affinity to the rat androgen receptor. The RBA of RU 59063 (**119**) was 3 times that of testosterone, and 100 times that of non-steroidal antiandrogens. RU 59063 could be useful as a marker for AR. Furthermore, unlike other markers of AR, it was devoid of any binding to other steroid receptors. *In vivo*, another analog, RU 56187 (**117**) showed high antiandrogenic activity. In rats, compound **117** was 3 and 10 times more active than Casodex and Anandron, respectively. RU 58841 (**120**) displayed 2 times less affinity than T for the hamster flank organ (F.O.) androgen receptor. However, activity was similar for the human androgen receptor. *In vivo*, when applied topically, it provided 52% regression of flank organ area at 100 µg/hamster, while being devoid of antiandrogenic activity on other accessory sex organs.

Flutamide (**89**), Casodex (**108**), and Anandron (**114**) are the most studied pure antiandrogens *in vitro* and *in vivo* and are presently used in the treatment of prostate cancer. Recently, considerable efforts are being made for the development of pure antiandrogens more potent than flutamide, the first pure antiandrogen. Considering the rapidly rising interest in antiandrogens, especially following the demonstration that the addition of flutamide to an LHRH agonist prolongs life [51], comparison of the three was made, using the most appropriate *in vitro* and *in vivo* assays, to assess the biological characteristics of flutamide, Casodex, and Anandron. Thus, the two-fold stimulation of Shionogi cell proliferation caused by a 10-day exposure to 1 nM testosterone was competitively reversed by incubation with OH-FLU (**90**) (IC₅₀=72 nM), Casodex (243 nM) and Anandron (412 nM). Moreover, marked increase in GCDP-15 release induced by 1 nM testosterone was blocked by OH-FLU (35 nM), Casodex (142 nM) or Anandron (75 nM) in ZR-75-1 cells. These data demonstrate that the antiandrogenic activity of OH-FLU is 3.4-fold more potent than Casodex and 5.7-fold more potent than

Anandron. Furthermore, OH-FLU is 4.1 times more potent than that of Casodex in inhibiting testosterone-induced GCDP-15 secretion in ZR-75-1 cells. These data show a greater difference in potency in favor of OH-FLU [120]. The *in vivo* study also is in close agreement with the study *in vitro*. Flutamide is about three times more potent than Casodex in inhibiting ventral prostate and seminal vesicle weight in orchietomized rats supplemented with 4-dione [125].

Quinoline Derivatives as Antiandrogens (Table 9)

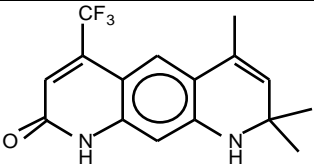
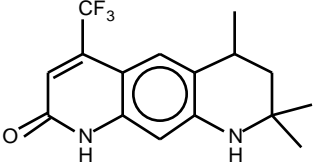
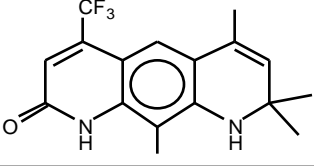
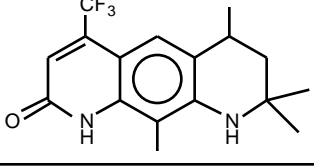
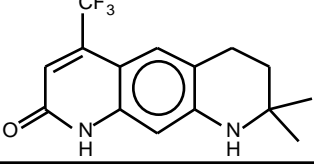
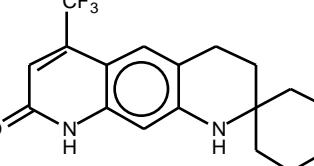
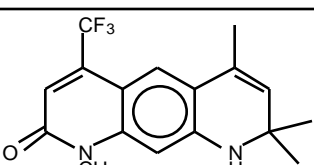
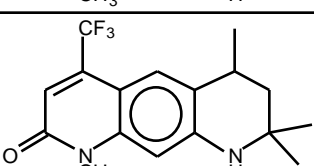
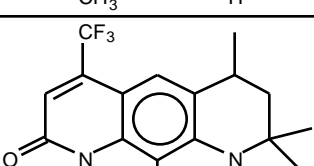
In 1998, workers from Ligand described a series of 1,2-dihydropyridono[5,6-g] quinoline derivatives as androgen receptor antagonists [126-132]. Quinoline **121** showed potent antiandrogenic activity along with antiprogesterin activity (IC₅₀=49 nM). Introduction of the C9 methyl group increased the selectivity between AR (IC₅₀=23 nM) and PR (IC₅₀=3346 nM) for compound **123**. Reduction of the 3,4-double bond of quinoline derivatives did not affect the potency. However, it greatly improved *in vivo* efficacy. In general, alkylation at the bottom part of molecules increased the activity. Methylation of the pyridone nitrogen **127-129** did not affect the antiandrogenic activity, but it enhanced the selectivity for AR over PR. A clear trend in activity did not arise from the alkylation of the quinoline nitrogen. However, methylation of both nitrogen **130** and **131** did not change the activity. Chemical substitution at C2 was essential for antiandrogenic activity. Substitution at the 2, 3, and 4 positions was tolerable.

Compounds **121**, **124**, **125**, and **127** were evaluated for their *in vivo* efficacy. Reduction of testosterone propionate (1 mg/kg, sc) induced ventral prostate in castrated rats at oral administration (30 mg/kg, po) of once a day for 3 days was 57, 55, 71, and 49%, respectively. Whereas flutamide gave a 100% reduction of VP weight, unsubstituted at the C4 position **125** gave the highest *in vivo* efficacy of the series.

Cyclocymopol Analogs as Antiandrogens (Table 10)

Cyclocymopol monomethyl ether was isolated from a crude organic extract of the marine alga *C. barbata* and exhibited activity against the human androgen receptor and human progesterone receptor [133-136]. The acetate **139** of cyclocymopol monomethyl ether was a weak antiandrogen. However, the selectivity was significant for AR over PR (IC₅₀ > 10,000 nM). SAR study revealed that bromo, hydroxy, and methoxy on

Table 9. Quinoline Derivatives as Antiandrogens

| No | Structure | Antiandrogenic activity ^a | | Ref. |
|-----|---|---|-----------------------------------|-------|
| | | hAR in CV-1 cells (IC ₅₀ , nM) | hAR Binding (K _i , nM) | |
| 121 |  | 28 | 115 | [126] |
| 122 |  | 26 | 76 | [126] |
| 123 |  | 23 | 82 | [126] |
| 124 |  | 22 | 85 | [126] |
| 125 |  | 27 | 26 | [126] |
| 126 |  | 35 | 9 | [126] |
| 127 |  | 34 | 81 | [126] |
| 128 |  | 73 | 46 | [126] |
| 129 |  | 31 | 40 | [126] |

(Table 9). contd.....

| No | Structure | Antiandrogenic activity ^a | | Ref. |
|-----|-----------|---|-----------------------------------|-------|
| | | hAR in CV-1 cells (IC ₅₀ , nM) | hAR Binding (K _i , nM) | |
| 130 | | 46 | 39 | [126] |
| 131 | | 19 | 17 | [126] |
| 132 | | 30 | 73 | [126] |
| 133 | | 27 | 54 | [126] |
| 134 | | 159 | 650 | [126] |

^aOH-FLU: hAR (IC₅₀=15 nM) and hAR binding (IC₅₀=27 nM).

the aromatic ring were not necessary for the optimal activity (compare compounds **135** and **140**). In fact, replacement by the nitro group gave the most active compound **135** of the series. Moreover, the bromo group in the cyclohexyl ring was also not essential for activity (compare compounds **139** and **140**). Replacement of the exo-cyclic double bond **140** by cyclopropyl **142** or hydroxy **143** did not affect the activity. The gem-dimethyl group was essential for activity. Majority of the compounds also showed PR antagonist activity.

Phthalimide Derivatives as Antiandrogens (Table 11)

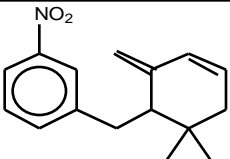
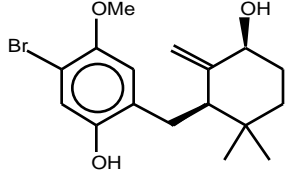
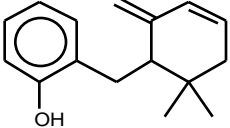
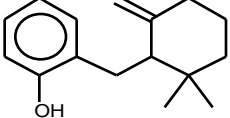
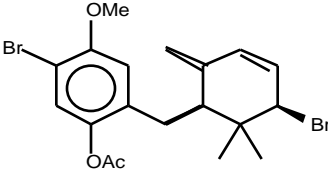
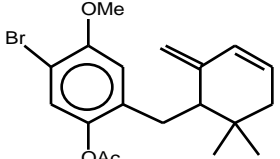
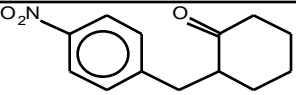
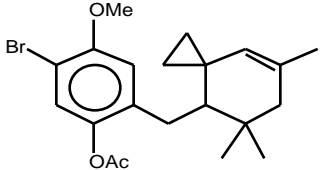
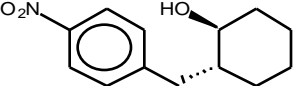
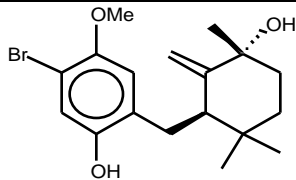
Antiandrogenic activity of various phthalimide analogs was evaluated against testosterone (10 nM)-stimulated Shionogi cells proliferation. Tetrafluorophthalimides **149-152** showed potent antagonistic activity. The activity of both enantiomers

was comparable. A chiral center next to the nitrogen atom was essential for the high potency of the fluoro compounds. Other phthalimide derivatives showed moderate activities and were comparable to flutamide (34% inhibition at 1 μM). The inhibitory activity on androgen-induced activation of the nuclear androgen receptor was also evaluated in a CAT assay, and it well correlated to the antiandrogenic activity evaluated by the growth inhibition assay in Shionogi cells.

Other Non-steroidal Antiandrogens (Table 12)

1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene (**154**), the major and persistent metabolite of DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) inhibits androgen binding to the androgen receptor. The *in vitro* potency of *p,p'*-DDE (**154**) was comparable to hydroxyflutamide (OH-Flu; 50% inhib. at 0.2 μM). In adult rats, treatment with *p,p'*-DDE (200 mg/kg, po) for 4 days reduced seminal vesicle (16%) and ventral

Table 10. Cyclocymopol Analogs as Antiandrogens

| No | Structure | Antiandrogenic activity | Ref |
|-----|---|--|-----------|
| | | hAR (IC ₅₀ , nM) ^a | |
| 135 |  | 66 | [135,136] |
| 136 |  | 180 | [135,136] |
| 137 |  | 210 | [135,136] |
| 138 |  | 220 | [135,136] |
| 139 |  | 230 | [135,136] |
| 140 |  | 240 | [135,136] |
| 141 |  | 250 | [135,136] |
| 142 |  | 250 | [135,136] |
| 143 |  | 290 | [135,136] |
| 144 |  | 300 | [135,136] |

^aAntiandrogenic activity was performed on the human androgen receptor utilizing CV-1 cells.

Table 11. Phthalimide Derivatives as Antiandrogens

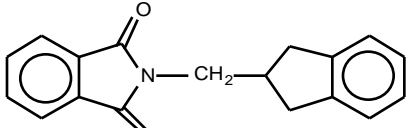
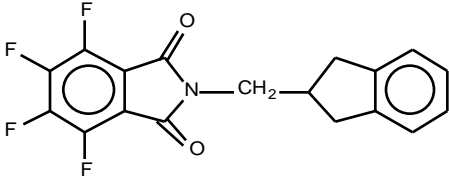
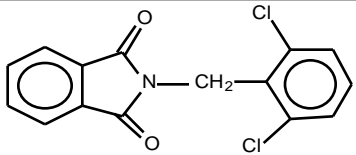
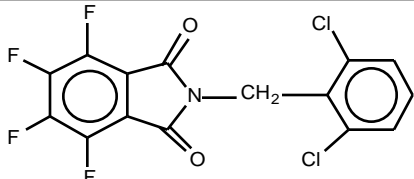
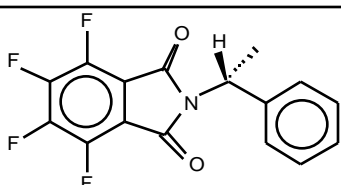
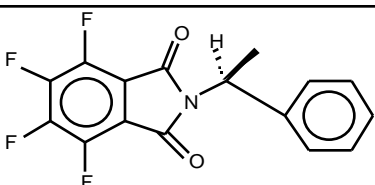
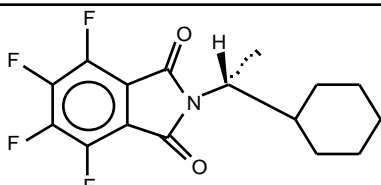
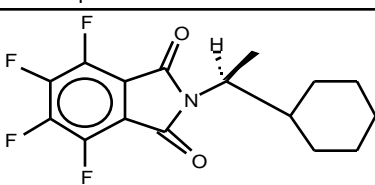
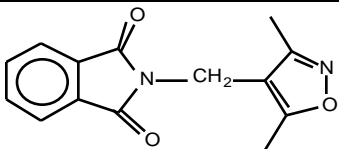
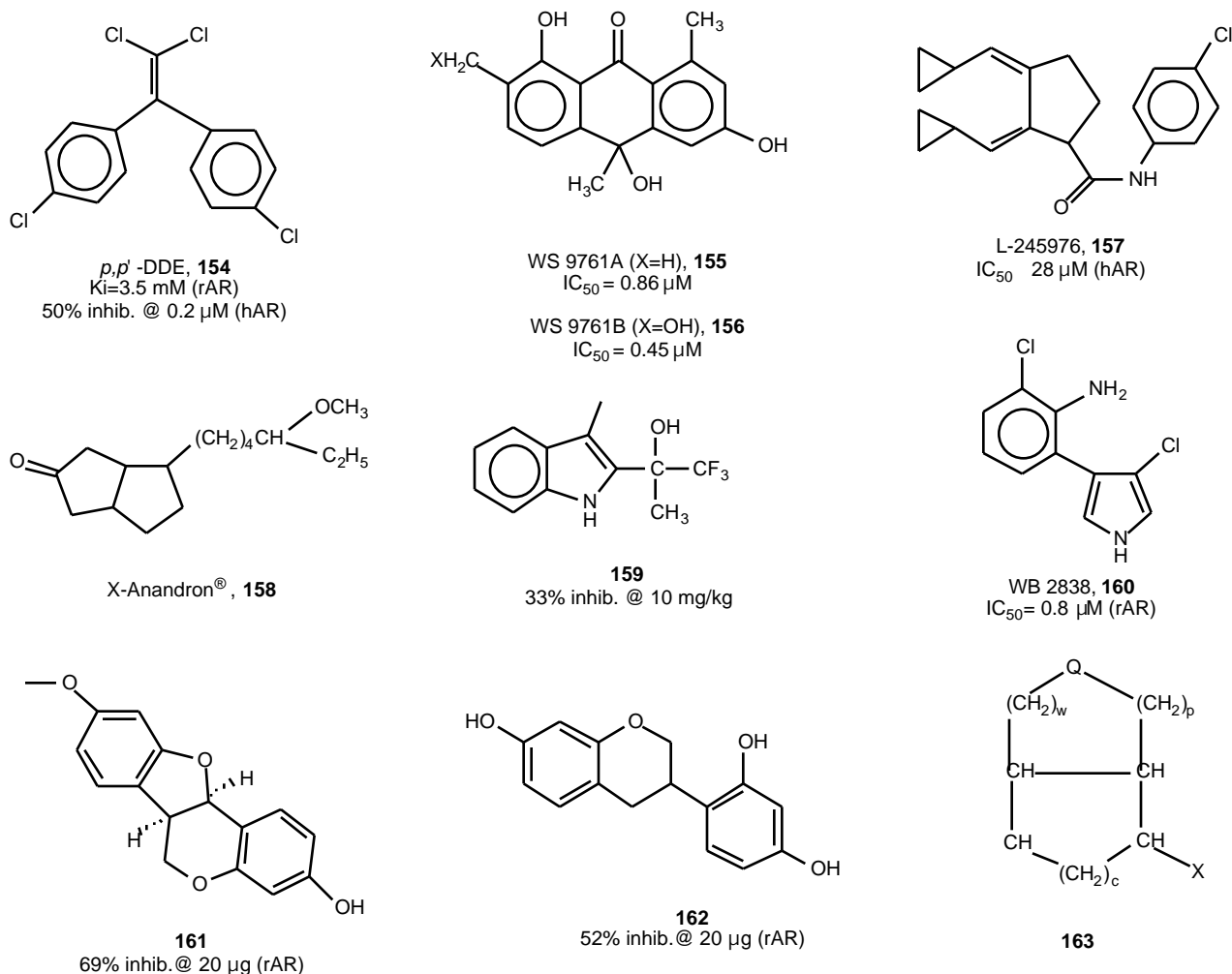
| No | Structure | Antiandrogenic activity | Ref. |
|-----|---|------------------------------------|-------|
| | | Shio cells (% inhib. at 1 μ M) | |
| 145 |  | 44 | [137] |
| 146 |  | 39 | [137] |
| 147 |  | 31 | [137] |
| 148 |  | 55 | [137] |
| 149 |  | 84 | [137] |
| 150 |  | 97 | [137] |
| 151 |  | 98 | [137] |
| 152 |  | 95 | [137] |
| 153 |  DIMP | (RBA: 2.55) (R1881: RBA:100) | [109] |

Table 12. Other Non-steroidal Antiandrogens

prostate weight (30%). These results support the hypothesis that the antiandrogenic effects of DDT on the male reproductive system are mediated by p,p' -DDE [138]. Compounds **155** and **156**, isolated from the fermentation broth of a *Streptomyces* strain microorganism, were moderately active as antiandrogens [139,140]. In DDT1 cells, L-245976 (**157**) completely blocked the action of testosterone (10 nM) at 10 μ M, whereas hydroxyflutamide at 1 μ M had a similar effect. Moreover, compound **157** also exhibited low affinity for AR (IC_{50} 28 μ M) compared to hydroxyflutamide (IC_{50} 100 nM) [141]. Antiandrogen **158** blocked DHT binding to androgen receptors from fibroblasts of frontal skin from alopecia patients by 80%, and of fibroblasts from facial skin by 78-93% [142]. Other non-steroidal antiandrogens **159-163** have also been reported to display good antiandrogenic activity [112,143-145].

Clinical Results

Over the past thirty years, little progress has been made in the development of potent antiandrogens.

Few of them have shown a promising response in pre-clinical studies. Moreover, clinical studies remain to be done to further define their efficacy in the treatment of prostate cancer and skin related diseases. So far, flutamide and its derivatives have displayed good clinical benefits in human, and are extensively used. The clinical results of these antiandrogens are summarized in this last section of the review.

Cyproterone Acetate (CPA; **29**; Table 3)

An EORTC study compared the clinical results of CPA, DES and MPA in 210 patients. The efficacy of CPA and DES was similar when compared for progression and survival rates while MPA was less effective [146,147]. When compared to flutamide, this progestin has significant intrinsic androgenic and estrogenic activities. CPA causes estrogen-like complications such as thrombosis, cardiovascular side effects, gynecomastia, and adverse effects on serum lipoproteins [148-150]. Virilization effects were seen in all the female fetuses examined when pregnant guinea pigs were given cyproterone acetate, thus providing

early evidence of androgenic activity [151]. The effects of flutamide and the steroidal derivatives, cyproterone acetate, chlormadinone acetate, megestrol acetate and medroxyprogesterone acetate were compared *in vivo* in female nude mice bearing androgen-sensitive Shionogi tumors. All steroidal compounds stimulated tumor growth while flutamide had no stimulatory effect [51]. Thus, CPA due to its intrinsic properties stimulates androgen-sensitive parameters and cancer growth. Cyproterone acetate added to castration has never been shown in any controlled study to prolong disease-free survival or overall survival in prostate cancer when compared with castration alone [152-155].

Flutamide (89; Table 8)

The first pure antiandrogen was discovered in 1967 by Neri et al. at Schering-Plough. Flutamide, an orally active antiandrogen, is rapidly metabolized to the active compound hydroxyflutamide, which accounts for almost all metabolites of flutamide present in the circulation. For the treatment of cancer, flutamide is administered at the dose of 250 mg every 8 hours. For the treatment of hirsutism and androgenic alopecia in women, a twice daily dose of 250 mg was used [7]. Two large-scale double blind studies have shown that combination of flutamide and medical castration increases the number of responders, and most importantly increases overall survival by an average of 7.3 months when compared with an LHRH agonist and orchiectomy, respectively [110,111]. These studies demonstrate that pure antiandrogens should always be given in combination with medical (LHRH agonist) or surgical (orchiectomy) castration as first treatment at the start of therapy.

Since localized disease provides the only opportunity for cure of prostate cancer, the combination therapy was next administered to patients at earlier stages of the disease. Randomized studies performed in patients have recently demonstrated that combination therapy administered for 3 months before radical prostatectomy increases the proportion of patients having organ-confined disease by about 50% while the same approach associated with radiotherapy has been shown to delay the time to progression [51,156]. Loose bowel movements or diarrhea is observed in 5-9% of cases. No cardiovascular effects are observed. When given in combination, no gynecomastia or breast tenderness is observed.

Anandron (114; Table 8)

Anandron (Nitulamide) is well absorbed after oral administration. Anandron is usually administered at a daily dose of 300 mg per day for one month followed by

the daily maintenance dose of 150 mg. Nitulamide given in association with orchiectomy in advanced prostate cancer has shown, in randomized and prospective studies, a greater proportion of responders, a longer duration of disease-free survival and an increase of an average of 5.4 months and 7.3 months in overall survival compared to orchiectomy alone [157,158]. Other data have shown an improved response and an improved quality of life [159,160]. In analogy with flutamide, the benefits of Anandron are much superior when given as first treatment. Visual adaptation to darkness is impaired in 20-40% of patients. Mild gastrointestinal disturbances were found in a few patients and interstitial lung disease is infrequent.

Casodex (108; Table 8)

Casodex (bicalutamide) is an orally bioavailable and well absorbed antiandrogen. In a randomized, multicenter and open study in 376 patients with metastatic prostate cancer, the effect of Casodex (50 mg/day) was compared with orchiectomy. At 3 months, PSA was reduced by 86% in the Casodex group and by 96% in the orchiectomy group [161].

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