Steroid 5α-Reductase Inhibitors

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Abstract: The objective of this study is to synthesize new steroidal compounds based on the progesterone skeleton with a high inhibitory activity for the enzyme 5α-reductase. Presently similar compounds are being used for the treatment of androgen dependent diseases such as: hirsutism, androgenic alopecia, bening prostatic hyperplasia and prostate cancer. Dihydrotestosterone **2** (Fig. (**1**)), a 5α-reduced metabolite of testosterone **1** has been implicated as a causative factor in the progression of these diseases, largely through the clinical evaluation of males who are genetically deficient of steroid 5α-reductase enzyme. As a result of this study, the inhibition of this enzyme has become a pharmacological strategy for the design and synthesis of new antiandrogenic drugs. The advent of finasteride **8** (Fig. (**4**)) a 5α-reductase inhibitor has grately alleviated the symptoms associated with benign prostatic hyperplasia.

In our laboratory we recently synthesized several new 16β-methyl-pregnadiene-3,20-diones derivatives **27** (Fig.(**6**)), **38-42** (Fig. (**11**)), 16β-phenyl-pregnadiene-3,17a-dione derivatives **32-33** (Fig. (**7**)), 16β-phenyl-pregnatriene-3,17a-diones, **30, 31** (Fig. (**7**)) and 16β-methyl-pregnatriene-3,20-diones **43-46** (Fig. (**11**)). These compounds were evaluated as 5α-reductase inhibitors in the following biological models: *Penicillium crustosum* broths, the flank organs of gonadectomized male hamsters, the incorporation of radiolabeled sodium acetate into lipids, the effect of the new steroids on the reduction of the weight of the seminal vesicles and on the *in vitro* metabolism of [^{3}H]T to [^{3}H]DHT in seminal vesicles homogenates of gonadectomized male hamsters. All trienones **30, 31**, and **43-46** in all biological models showed consistently a higher 5α-reductase inhibitory activity than the corresponding dienones **27, 32, 33** and **38-42**. We believe that with these compounds the 5α-reductase enzyme is inactivated by an irreversible Michael type addition of the nucleophilic portion of the enzyme to the conjugated double bond of the steroid. The trienones having a more coplanar structure react faster with the enzyme and thus show a higher inhibitory activity.

INTRODUCTION

The mechanism of action of steroid hormones begins when they migrate from the blood stream to the cell across the cell membrane by a simple diffusion mechanism. Once inside the cells, the steroids form complexes with intracellular binding proteins called receptors which are specific protein tissues. Steroid receptors were discovered by Jensen and Suzuki in 1968 [1] , changing the concept of the mechanism of action of steroid hormones of those days. Latter Gorski [2] demonstrated that hormone receptor binding, triggers a cascade of events that permits the expression of specific genes.

Inside the cells, the uptaken steroid hormones undergo modifications in its molecule producing active metabolites that trigger a cascade of reactions. These molecular changes are due to the presence of enzyme specific tissues. In the prostate for example, the 5α-reductase enzyme type II [3] catalyzes the conversion of testosterone **1** (T) to 5α-dihydrotestosterone **2** (DHT), (Fig. (**1**)). This metabolite is responsible for the induction of some enzymes such as the 3α-hydroxysteroid dehydrogenase and the 17β-hydroxysteroid dehydrogenase [4].

The 17β-hydroxysteroid dehydrogenase has the capacity to convert testosterone to androstanedione and this compound is converted to 5α-androstanedione by the 5α-reductase action. The 5α-androstanedione is transformed to androstanediol by the action of the 3α-hydroxysteroid dehydrogenase enzyme [4-6].

Presently there are several compounds available that can inhibit the mechanism of action of steroid hormones. These inhibitions are carried out at different levels such as: 1) blocking the receptors using an antagonist, 2) inhibiting metabolic enzymes involved in steroidgenesis by chemical agents, 3) inhibiting the mechanism of activation of receptors on molecules that interact with chaperone or Fos and Jun proteins, 4) inhibiting the phosphorylation changes in receptor molecules and 5) modifiyng the hypothalamic-pituitary axis.

Reports in the literature describe a variety of natural or synthetic steroids having antiandrogenic action. Voight *et al.* in 1970 [7] demonstrated that progesterone **3** and deoxycorticosterone **4** inhibit the DHT formation by competing with Δ^4–3-keto site of the testosterone molecule

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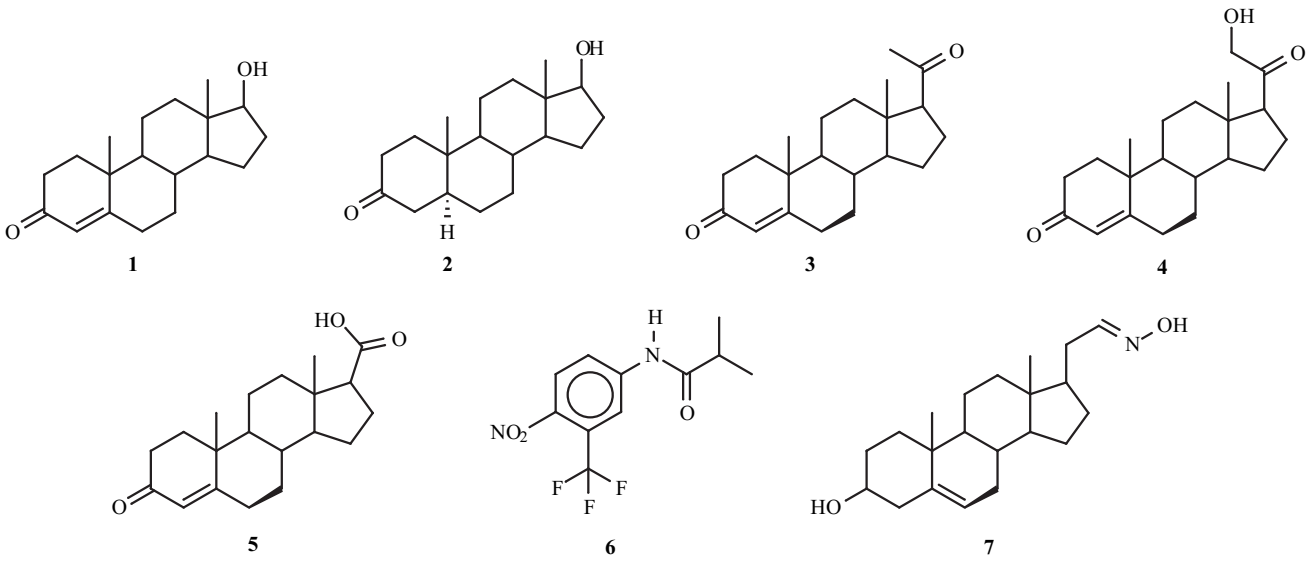

Fig. (1). Structures of testosterone, dihydrotestosterone and several commercially available 5α-reductase inhibitors.

for 5α-reductase enzyme. In 1974 Hsia and Voight [8] also demonstrated that 17β-carboxy-4-androsten-3-one **5**, a product of degradation of deoxycorticosterone is a potent inhibitor of DHT formation. In most of the synthetic and natural steroids, loss of the C-19 methyl group reduces the potency of the steroid to be recognized by 5α-reductase [8].

The effects of the inhibitors on the genoma had been evidenced by the works of Ziboh *et al.* [9], who in 1970 demonstrated that dehydroepiandrosterone inhibited the glucose-6-phosphatedehydrogenase activity and that of Cabeza *et al.* who showed that levonorgestrel changes lipid composition in flank organs of the treated animals [10].

Furthermore Sciarra [11] and Griffiths *et al.* [12] reported that flutamide **6** prevents the increase of the epidermal growth factor (EGF) as well as the increase of its receptors in androgen dependent tissues produced by testosterone, dihydrotestosterone and 3α-androstanediol. This action of flutamide on the androgen dependent tissues demonstrates that testosterone and its metabolites as well as the EGF downregulate epidermal growth factor receptor (EGFR) and exhibit a growth-promoting effect by androgens in the prostate gland, mediated by peptide growth factors [11, 12]. This mechanism is in agreement with that reported earlier for other tissues, that require the participation of cAMP as a

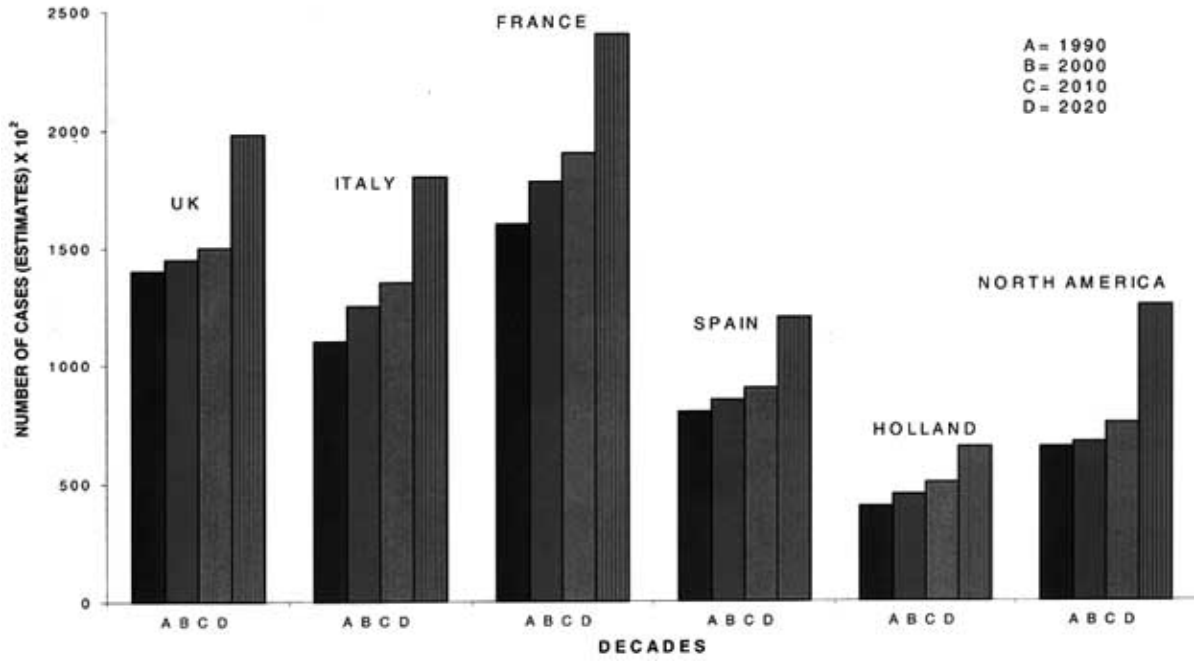

Fig. (2). Diagrammatic representation of cancer increase.

signal molecule for its growth and differentiation, suggesting that in this case β adrenoceptors are involved. It is well known fact that testosterone increases β adrenoceptors in prostate cell membranes. Collins *et al.* [13] and Guthrie *et al.* [14] demonstrated that testosterone, but not dihydrotestosterone increases β receptors in prostate gland, which in addition increases the prostate binding, essential for the prostate differentiation [15].

An analysis of these concepts leads to an alternative hypothesis that androgens and β adrenergic pathways may be involved indirectly in the growths of local growth factors or other intracelular mediators which regulate the growth and differentiation programs displayed by specialized target cells as is the case with the prostate gland.

ANDROGEN ANTAGONISTS

Androgen antagonists offer a potentially useful treatment for androgen mediated diseases such as: prostate cancer, benign prostatic hyperplasia, polycistic ovary sindrome, hirsutism in women , seborrhea, androgenic alopecia and precocious puberty [16]. A recent report from the World Health Organization [17] revealed that carcinoma of the prostate is the second most commonly diagnosed cancer after skin cancer in the male population of the USA and the second most common cause of death from cancer after that of lung.

The age adjusted mortality rates per 100,000 vary in different regions of the United States from 18.9 for white males in Arkansas to 55.5 for black men in North Caroline, probably the highest mortality rate in the world [17, 18]. Overall, around the world, the incidence of prostatic cancer is increasing at an annual rate of approximately 2% to 3% (Fig. (**2**)), with the lifetime risk of the disease developing in North American men, being nearly 10 % [19]. Most of the antiandrogens are synthetic substances with exception of progesterone **3** (Fig. (**1**)), which has a moderate antiandrogenic activity. No ailment is more common among middle aged males or cause more apprehension than prostate troubles or a prostatic operation; it is the operation men fear most. Although surgery presently represents the most acceptable treatment for prostatic cancer, about 400,000 resections are performed annually in USA [17, 18]; there are also several other modalities available for the treatment of both benign prostatic hyperplasia and prostate cancer. The most important therapeutic methods available today are: inhibition of androgen production by luteinizing hormone-releasing hormone (LHRH) agonist [18], inhibition of androgen action by androgen receptor antagonists [19], inhibition of testosterone **1** conversion to dihydrotestosterone **2** by 5α-reductase enzyme inhibitors [19, 20], β adrenoreceptors antagonists [21, 22] and several other less common therapies [3].

5α-REDUCTASE INHIBITORS

Enzymes involved in the biosynthesis and metabolism of testosterone **1** are attractive target for drug design and drug development. The phenotypic consequences of genetic steroid 5α-reductase deficiency however do suggest a strategy for the development of drugs that may be useful in the treatment of benign prostatic hyperplasia, acne, male pattern baldness and prostate cancer.

At birth male with steroid 5α-reductase deficiency have ambiguous external genitalia. They are common raised as a girls. At puberty, there is a striking change with the development of a typical male phenotype including enlargement of the phallus to become a functional penis. Such male pseudohermaphrodites, initially identified in the Dominican Republic by Imperato [23], as steroid 5α-reductase deficient, were refered by local townspeople as guevedoces (penis at age 12). Post puberty affected individuals demonstrated a tiny prostate, no temporal recession of the harline and no acne. The deficiency in steroidal 5α-reductase, the NADPH-dependent enzime that converts testosterone to 5α-dihydrotestosterone, results in very low circulating dihydrotestosterone levels and a much elevated testosterone: dihydrotestosterone ratio compared with normal men [23].

The study of these individuals allows roles for those two androgens at puberty to be assigned.

Testosterone Induced

Male sex drive performance

Muscle mass increase

Penis enlargement

Scrotum enlargement

Vocal cord enlargement

Spermatogenesis

Dihydrotestosterone Induced

Increase facial , body hair

Acne

Scalp hair recession

Prostate enlargement

Since testoterone **1** and dihydrotestosterone **2** share a common receptor, it appears surprising that the effect of dihydrotestosterone is not mimicked by testosterone. Dihydrotestosterone amplifies the androgen signal (compared with that of testosterone) as a result of its severalfold higher affinity for the androgen receptor. It has also been suggested that the dihydrotestosterone-receptor complex has a higher affinity for acceptor site in the nuclear chromatin than does the testosterone-receptor complex [24].

The local conversion of testosterone to dihydrotestosterone by 5α-reductase enzyme probably serves as an androgen amplification mechanism. Such amplification

may be less critical in tissues where testosterone suffices for the androgen effect (anabolic development), but essential for dihydrotestosterone dependent tissues (prostate, seminal vesicles and sebaceous glands).

Dihydrotestosterone **2** is therefore essential for differentiation of male external genitalia including the prostate, during embryogenesis. It is not yet certain whether dihydrotestosterone is required for the growth of the differentiated prostate, although dihydrotestosterone propionate administration to an affected subject for one year did result in an increase in prostate size [24]. Dihydrotestosterone depletion should have positive therapeutic consequences without having adverse effects on the desirable masculine characteristics mediated by testosterone.

The 5α-reductase enzyme 3-oxo-steroid-4-ene-dehydrogenase is an hydrophobic protein formed by 259 amino acids. Its molecular weight is 29,462 and is located in the microsomal fraction on the target cell. [3-6].

Species differences between the prostatic steroid 5α-reductase of rat, dog and human have been demonstrated with a series of 4–aza-steroidal inhibitors containing varying substituent at position 17 [25, 26]. These compounds with a 17β-dialkylcarbamoyl group are approximately equipotent inhibitors of the rat and the human enzyme, but are only 0.1-15 % as potent inhibitors of the dog enzyme. Inhibitors with a b-spiroether substituents are most potent with the rat enzyme being only 15–50 % and 0.2–0.4 % as effective against the activity derived from dog and human respectively. Appropriate substitution at C-17 is therefore of particular importance in inhibitor design. Furthermore, the pH optimum of the enzyme activity varies between the human (pH 4.8-5.2), dog (pH 6.2-6.8) and rat (pH 6.5- 7.0).

A mechanism of action (Fig. (**3**)) consistent with the known regio and stereochemistry of reduction involves direct hydride donation from NADPH to the 5-position of testosterone, thus leading to enolate formation at C-3, C-4. The enolate presumably would be stabilized by some electrophilic residue (E^+) in the active site. This process may be viewed alternatively as activation of the enone by (E^+) leading to a positively polarized species which accepts a hydride from NADPH at C-5. Enzyme mediated tautomerism then leads to the product dihydrotestosterone with the release of $NADP^+$.

The enzyme 5α-reductase responsible for the reduction of testosterone to dihydrotestosterone exists in two forms: 5α-reductase isozymes type 1 and 2. Type 1 occurs in the skin, liver, ventral prostate and acts in acidic and basic medium. The type 2 enzyme is expressed in ventral prostate, epididymis and other reproductive tissues. It acts in acidic medium. Humans, monkeys and rats have both types of the enzyme that can be distinguished on the basis of substrate affinity, pH optimum and inhibition to certain 4-azasteroids. This enzyme serves as an important regulator of hormone action in androgen sensitive cells [27], and is absent in some androgen sensitive tissues such as muscles and testes of old human males.

The most extensively studied class of 5α-reductase inhibitors are the 4-azasteroids [28] which includes the drug finasteride **8** (Fig. (**4**)). Finasteride is the first 5α-reductase inhibitor approved in the USA for the treatment of benign prostatic hyperplasia (BPH). This drug has approximately a 100-fold greater affinity for type 2 5α-reductase enzyme than for the type 1 enzyme demonstrating an IC_{50} value of 4.2 nM for type 2 enzyme [28]. In humans, finasteride decreases prostatic DHT levels by 70-90 % and reduces prostate size [29], while testosterone tissue levels remain constant. The use of finasteride **8** demonstrated a sustained improvement in the treatment of BPH and a reduction in prostate specific antigen (PSA) levels [30, 31]. Related analogs **9**, **10** and **11** have also demonstrated effectiveness *in vitro* and *in vivo* [32-35]. A new 6-azasteroid **12** has also shown potential inhibition of 5α-reductase [36].

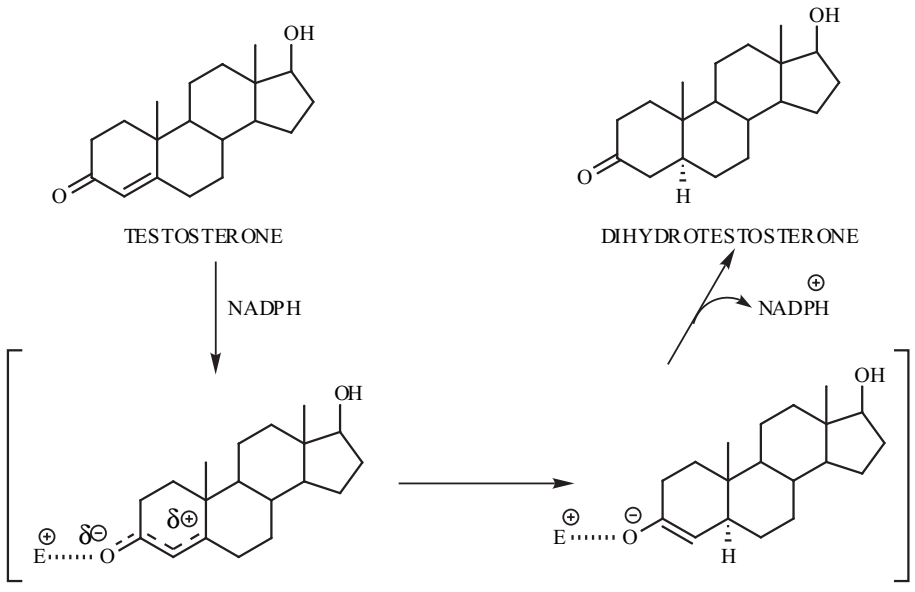

Fig. (3). Mechanism of testosterone reduction.

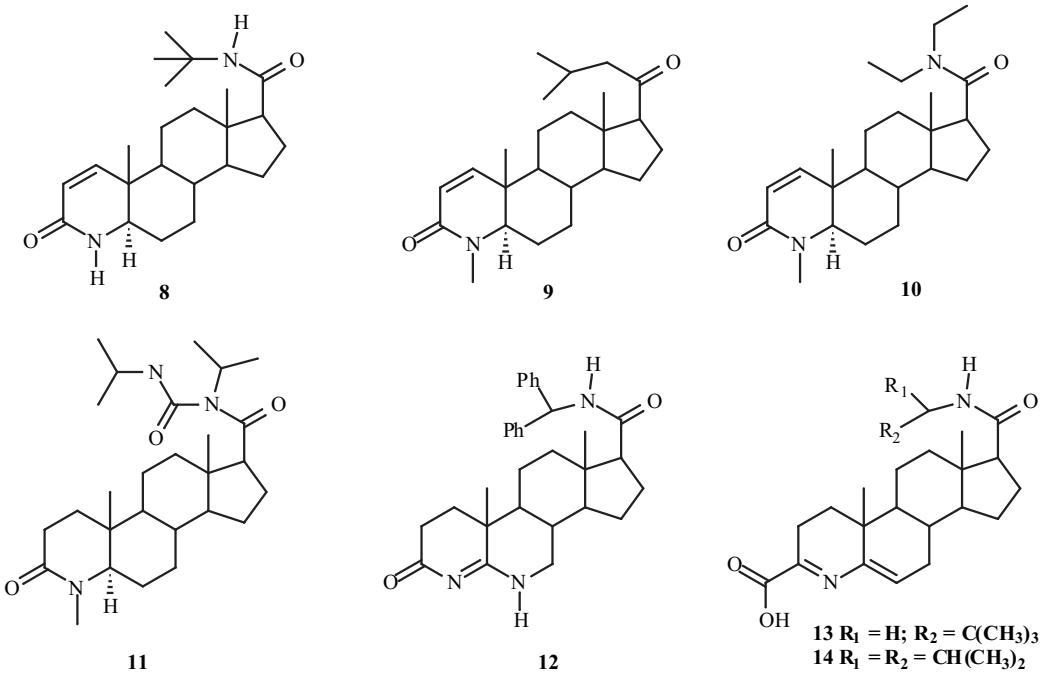

Fig. (4). Structures of finasteride and analogs steroidal 5α-reductase inhibitors.

Androstandiene-3-carboxylic acids **13** and **14** (Fig. (**4**)) were recently synthesized and have shown a potent uncompetitive inhibition of type 2 5α-reductase [37, 38]. Epristeride **13** [39] has exhibited the ability to lower serum DHT levels by 50% in clinical trials [40, 41]. Other analogs with acidic funcionality at the C-3 position include **15** and **16** (Fig. (**5**)) and the estratriene carboxylic acid **17** have also demonstrated a high inhibitory activity [42]. Finally the allenic secosteroid **18** and compound **19** have shown a potent irreversible inhibitory action to 5α-reductase, even though it was originally developed as an irreversible inhibitor of 3β-hydroxy-steroid dehydrogenase-D-4,5-isomerase [42-44].

Recently Hartman *et al.* [45] synthesized novel steroidal oxime inhibitors for 17α-hydroxylase/C-17,20 lyases and 5α-reductase types 1 and 2 having the basic structure as shown in **7** (Fig. (**1**)). This compound showed an excellent inhibitory activity toward the human 5α-reductase types 1 and 2 and a reasonable activity toward the rat enzyme

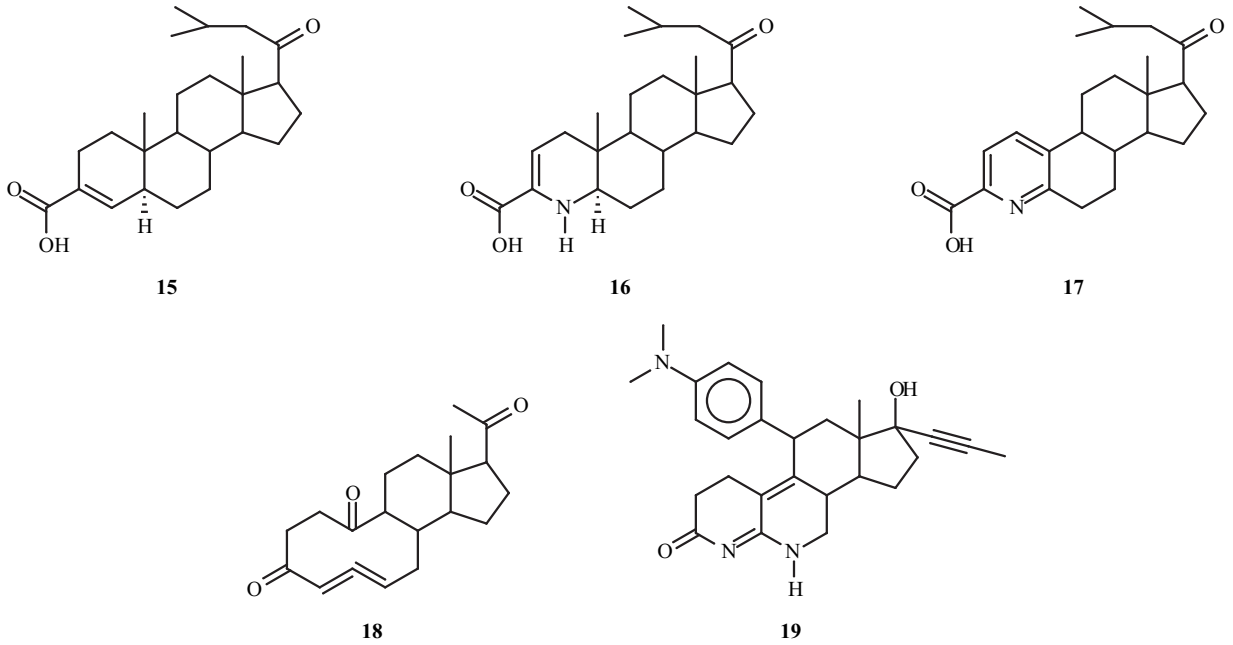

Fig. (5). Commercially available steroidal 5α-reductase inhibitors.

Synthesis and Pharmacological Evaluation

In this paper we describe the synthesis and pharmacological evaluation of several new 16-methyl substituted pregnane compounds (Fig. (**6**)) and phenyl substituted D-homo-pregnane steroidal derivatives (Fig. (**7**)). The biologically active compounds **21, 26** and **30-33** were prepared from the commercially available 16-

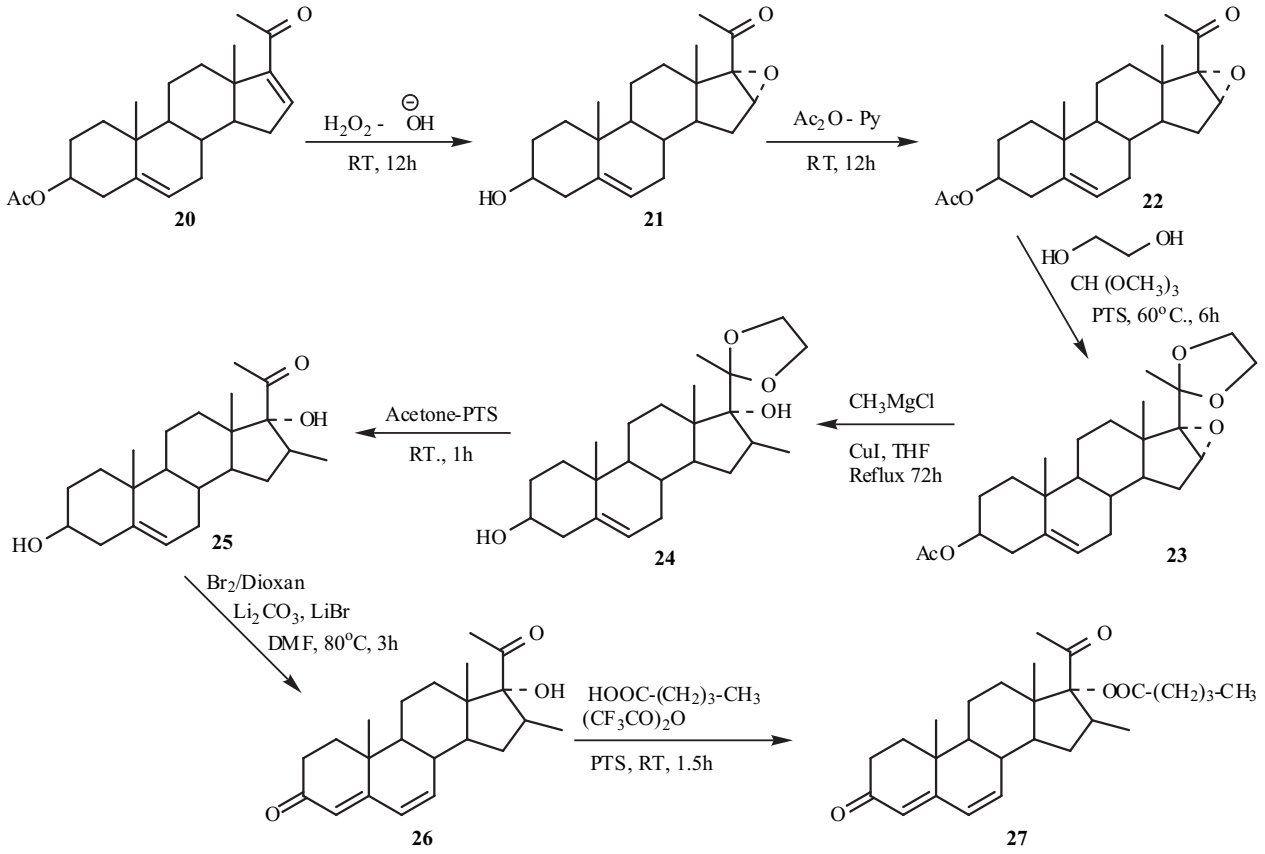

Fig. (6). Reaction sequence for the synthesis of the biologically active compounds.

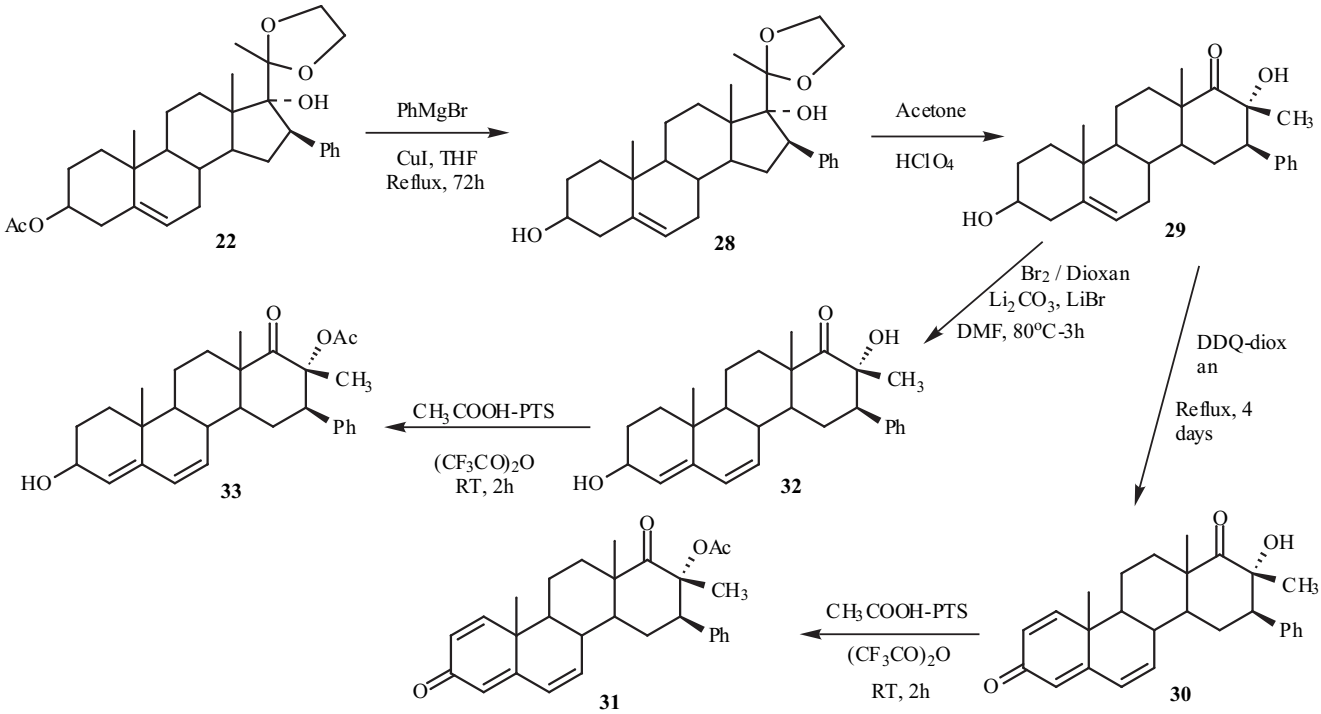

Fig. (7). Reaction sequence for the synthesis of the biologically active compounds.

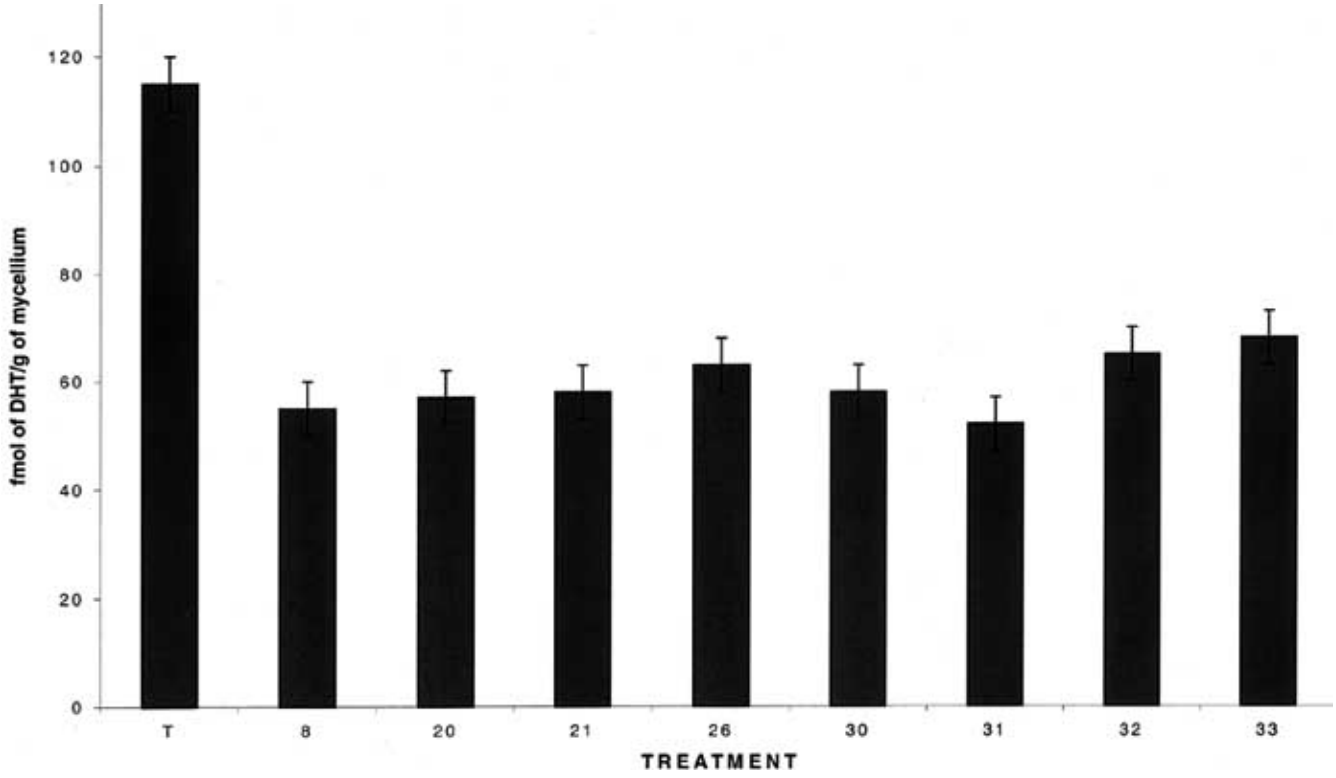

Fig. (8). Effect of different synthetic steroids on the conversion of [^{3}H]T to [^{3}H]DHT in *P. crustosum*.

dehydropregnenolone acetate **20**; surprisingly this compound showed a small pharmacological activity (Fig. (**6**)) [46-48]. The *in vitro* biological activity of these compounds was determined by following the transformation of testosterone (T) (**1**) to dihydrotestosterone (DHT) (**2**) produced by 5α-reductase enzyme in *P. crustosum* broths [49, 50], by the *in vivo* steroid action upon the flank organs where the DHT is bound to its cognate receptor [47, 51] and also by the incorporation of radiolabeled sodium acetate into lipids in these glands [52, 53].

Conversion of T to DHT has been demonstrated in *P. decumbens* and *P. crustosum* broths obtained from fermented pistachios, lemons and corn tortillas [49,50]. For the determination of the biological activity of the new steroids **20, 21, 26** and **30-33** as 5α-reductase inhibitors, we used the conversion of T to DHT in *P. crustosum* broths as shown in (Fig. (**8**)). Radiolabeled T in the incubated medium significantly increased its conversion to DHT as compared to T plus finasteride **8** or a combination of steroids **20, 21, 26** and **30-33** with testosterone (T). Finasteride **8** decreased the conversion of [^{3}H]T to [^{3}H]DHT ($p<0.005$) effected by the fungi. These data indicate that **8** as well as the above mentioned steroids are good inhibitors for the conversion of T to DHT in this model.

In another experiment, we used the flank organs of gonadectomized male hamsters to test the 5α-reductase inhibitory activity of these steroids [47, 52]. Hamster flank organs are dorsal spots on the skin that are composed of pilosebaceous tissue. These glands are androgen-dependent and upon castration, the diameter of the spot decreases; daily injection of T restores the original size. The flank organs can convert T to DHT and are used for the screening of new antiandrogenic drugs [51,54]

The flank organs are larger in males than in females and are capable of synthesizing lipids, furthermore they can modify the sebum lipid composition under T or progesterone stimuli [52,53]. In this experiment, the diameter of the pigmented spot on the glands 15 days after castration significantly decreased ($p<0.005$) as compared to that of the uncastrated animals. Subcutaneous injection of the vehicle alone did not change this condition. However treatment with T restored the original diameter of the spot.

Finasteride **8** [54, 55] as well as steroids **20, 21, 26** and **30-33** significantly decreased ($p<0.005$) the diameter of the pigmented spot on the flank organs as compared to that of the T treated animals Table **1**. The most effective compounds in these experiments were steroids **20** and **31** which reduced the diameter of the pigmented spot to 1.5 mm, followed by finasteride, 2.5 mm and steroids **30, 32** and **33**. These data suggest that compounds **20** and **30-33** are strong inhibitors of the conversion of T to DHT and this confirms the validity of this model for the determination of 5α-reductase inhibitory activity. Table **1** also shows the percentage of [1,2-^{14}C] sodium acetate incorporation into lipids as well as the rate of this incorporation. Compounds **20, 30** and **31** exhibited a much lower percentage of sodium acetate incorporation than finasteride **8**. These date which are the result of two different experiments performed in duplicate confirm the importance of sex hormones in lipid metabolism in the flank organs [52]. The vehicle treated control sample showed a higher percentage of sodium acetate incorporation although it is much lower than that of T

Table 1. Effect of Different Compounds on Male hamster Flank Organ Diameter (mm ± S.D.) and *In vitro* Incorporation of [^{14}C] Acetate in Lipids

	Treatment(mg)	Diameter of the pigment spot (mm)	Percentage of incorporation	Incorporation 1,2-^{14}C acetate into lipids (nmol/gland)
CONTROL		2.5 ± 0.9	0.46	0.125 ± 0.004
T	0.2	6.0 ± 2.0	0.96	0.255 ± 0.002
T + 8	0.2	2.5 ± 0.9	0.31	0.080 ± 0.005
T + 20	0.2	1.5 ± 0.4	0.07	0.055 ± 0.001
T + 21	0.2	3.0 ± 1.0	0.35	0.090 ± 0.004
T + 26	0.2	4.3 ± 1.0	0.76	0.115 ± 0.004
T + 30	0.2	2.0 ± 0.5	0.15	0.080 ± 0.009
T + 31	0.2	1.5 ± 0.5	0.07	0.056 ± 0.001
T + 32	0.2	2.0 ± 0.3	0.78	0.115 ± 0.008
T + 33	0.2	2.0 ± 1.0	0.70	0.100 ± 0.004

Results are expressed as percentage of nmol/per gland ± S.D. Significant difference was observed between the diameter of gland, as well as in the incorporation of [^{14}C] acetate in lipids ($p<0.005$) from castrated control animals (control) and T-treated (T). Finasteride (**8**) decreased both the diameter of the glands as well as the incorporation of [^{14}C] acetate in lipids ($p<0.005$). New steroids (**20, 21, 30-33**) significantly inhibited ($p<0.005$) the diameter of the flank organs as well as the incorporation of [^{14}C] acetate in lipids.

treated hamsters. These experiments indicate that *P. crustosum* broths can be used as a model for the evaluation of 5α-reductase inhibitory effect of a variety of steroidal compounds and thus replace the animal model which involves the sacrifice of dozens of hamsters. In the *P. crustosum* broths, finasteride as well as compounds **20, 21, 26** and **30-33** inhibited the conversion of T to DHT, thus confirming the presence of 5α-reductase enzyme in the fungal culture [47].

As we expected, the decrease of the diameter of the pigmented spot on the flank organs produced by compounds

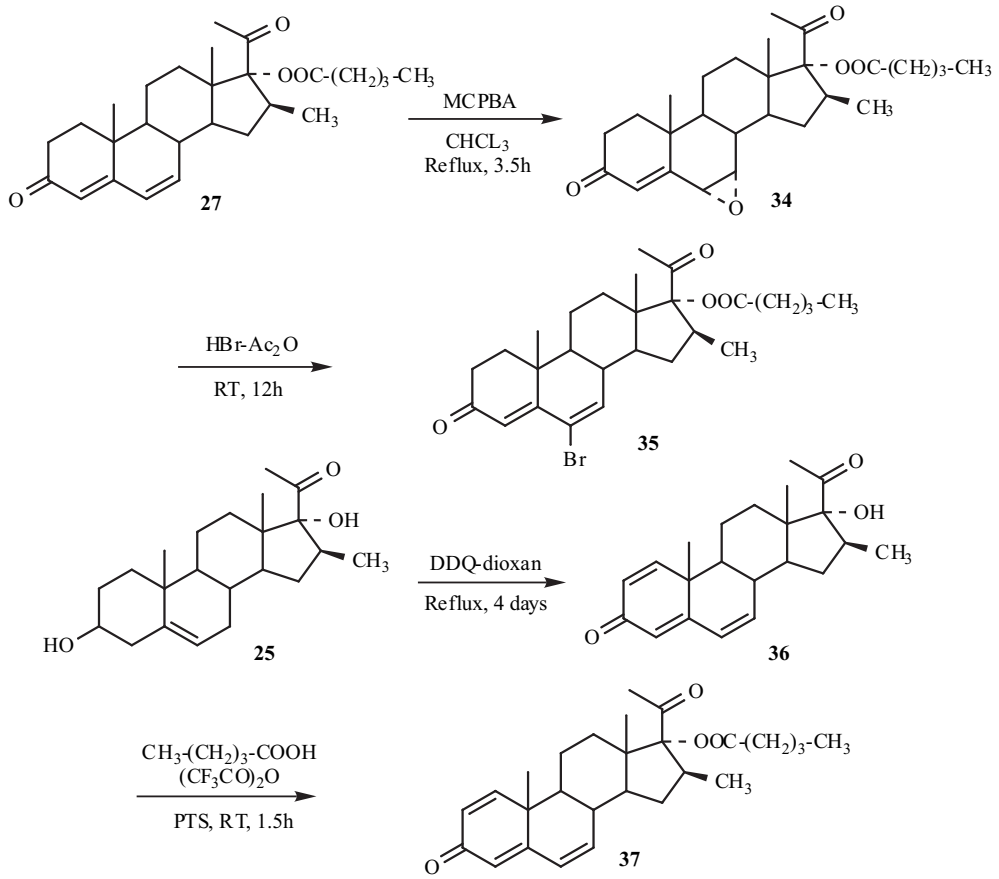

Fig. (9). Reaction sequence for the synthesis of the biologically active compounds.

20 and **30-33** correlates well with the reduction of the labeled sodium acetate incorporation into lipids. This corroborates the fact that the 5α-reductase inhibitory effect is related to the labeled sodium acetate incorporation. However the observed effect could also be the result of the antagonistic activity of the novel steroids on the androgen receptor. It is important to emphasize that the results from the three experiments correlate very well, thus confirming the fact that steroids **20** and **30-33** are powerful 5α-reductase inhibitors.

In another study we determined the 5α-reductase inhibitory activity of several 16-methyl substituted pregnane derivatives such as: the dienones **27, 35**, the epoxy compound **34** and the trienones **36** and **37** all synthesized in our laboratory (Fig. (**9**)).The pharmacological evaluation of these compounds was carried out in flank organs, seminal vesicles and the effect of the new steroidal compounds on the *in vitro* metabolism of [^{3}H]T to [^{3}H]DHT in seminal vesicles homogenates of gonadectomized male hamsters [47].

In the flank organs model Table **2** the diameter of the pigmented spot on the glands 15 days after castration significantly decreased ($p<0.005$) as compared to that of the uncastrated animals. The new steroids **27** and **34-37** decreased the diameter of the pigmented spot as compared to that of testosterone (T), thus suggesting an inhibitory effect on the enzyme 5α-reductase. The most effective compound in this model was steroid **37** which reduced the diameter of the pigmented spot to 1.8 mm. Table **2** also shows the 5α-reductase inhibitory effect of steroids **27** and **34-37** related to

Table 2. The Diameter of Flank Organs and Weight of the Seminal Vesicles were Measured from Animals that Received Sc Treatments of C-16 Substituted Steroids. The Results are Given ± Standard Deviation

	Treatment(mg)	Diameter of the pigment spot (mm)	Weigtht of seminal vesicles (mg)
CONTROL		2.75 ± 0.05	177.45 ± 30.7
T	0.2	4.25 ± 0.50	317.83 ± 30.1
T + 8	0.2	3.00 ± 0.02	216.00 ± 27.8
T + 27	0.2	3.00 ± 0.00	265.15 ± 30.4
T + 34	0.2	2.00 ± 0.00	208.75 ± 24.5
T + 35	0.2	3.00 ± 0.50	246.00 ± 49.1
T + 36	0.2	3.00 ± 0.50	231.25 ± 35.2
T + 37	0.2	1.80 ± 0.50	202.00 ± 30.1

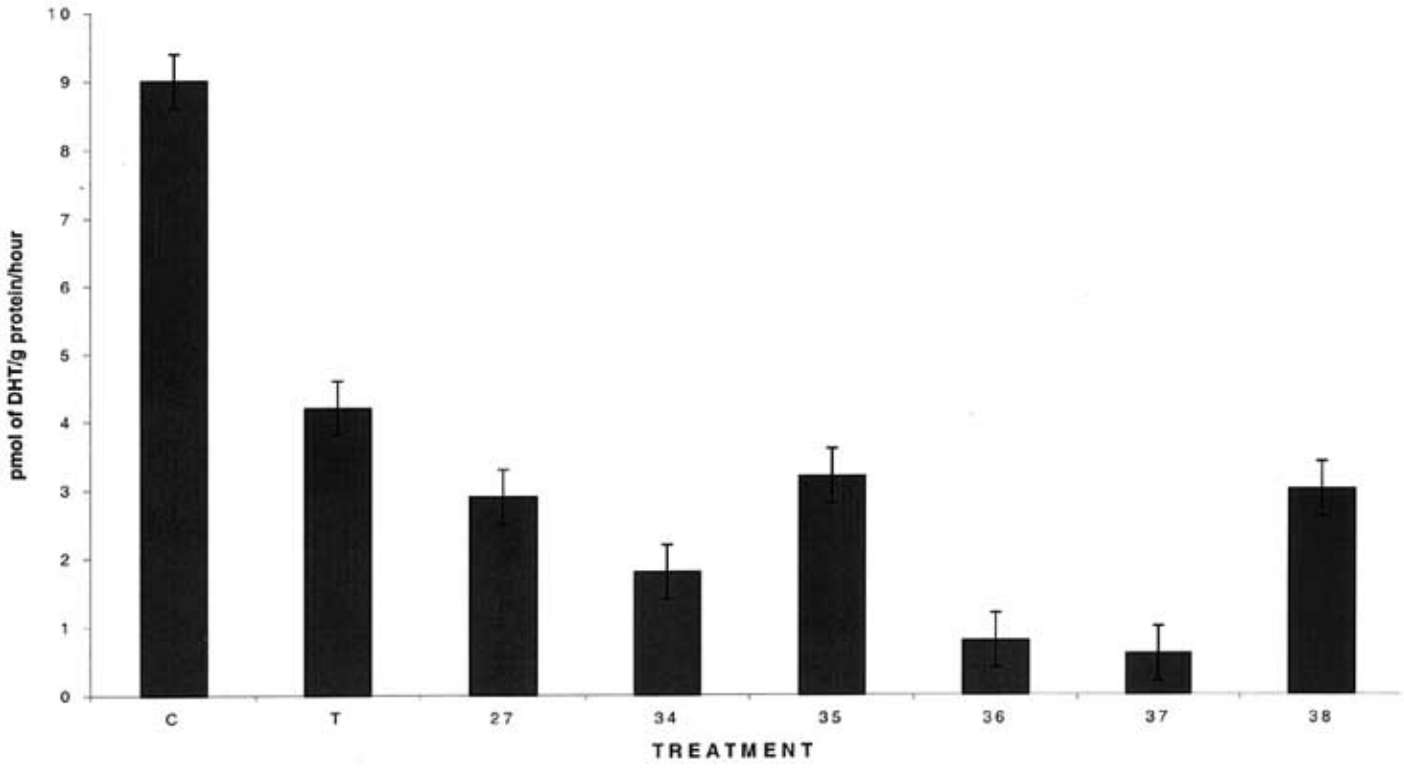

Fig. (10). Effect of different synthetic steroids on *in vitro* conversion of [^{3}H]T to [^{3}H]DHT in castrated male hamster flank organs.

the weight of the seminal vesicles of castrated male hamsters treated with T, a combination of T plus finasteride **8** and T plus steroids **27** and **34-37**. These data show that the new steroidal compounds decreased the weight of the seminal vesicles as compared to the T treated hamsters, thus indicating a high 5α-reductase inhibitory effect. Steroids **34** and **37** exhibited a higher biological activity than compounds **27, 35** and **36**. As can be seen from (Fig. (**10**)), (DHT formation expressed as pmol of DHT/g of protein per hour) the trienones **36, 37** and the epoxy compound **34** showed a higher biological activity than the corresponding dienone **27** (Fig. (**9**)), [47, 55-58].

Tables **1** and **2** exhibit the 5α-reductase inhibitory activity of several dienone and trienone steroidal compounds all showing a high biological activity. In order to extend the scope of this study and obtain a greater variety of data from steroidal compounds , we prepared several new dienones **38-42** and trienones **43-46** (Fig. (**11**)). The pharmacological activity of these compounds as 5α-reductase type 2 inhibitors were determined by measuring the amount of dihydrotestosterone produced, expressed as pmoles of protein per hour. Compounds **38-42** and **43-46** (Fig. **12**)) were administered to castrated hamsters at a dose of 200 μg 15 days after castration and the DHT formation was measured. These data show very convincingly that the trienones **43, 44** and **45** inhibited more effectively the DHT synthesis than the control sample finasteride **8**, the drug of choice today for the treatment of benign prostatic hyperplasia. On the other hand, the steroidal dienones **38-42** exhibited a lower 5α-reductase inhibitory activity [59]. As previously observed [47], compounds having the 4,6-diene-3-one moiety **27** (Fig. (**6**)), **32, 33** (Fig. (**7**)) and **38-42**, (Fig. (**11**)) always exhibited a lower 5α-reductase inhibitory activity than the 1,4,6-trien-3-one system, compounds **30, 31**, (Fig. (**7**)) and **43-46**, (Fig. (**11**)).

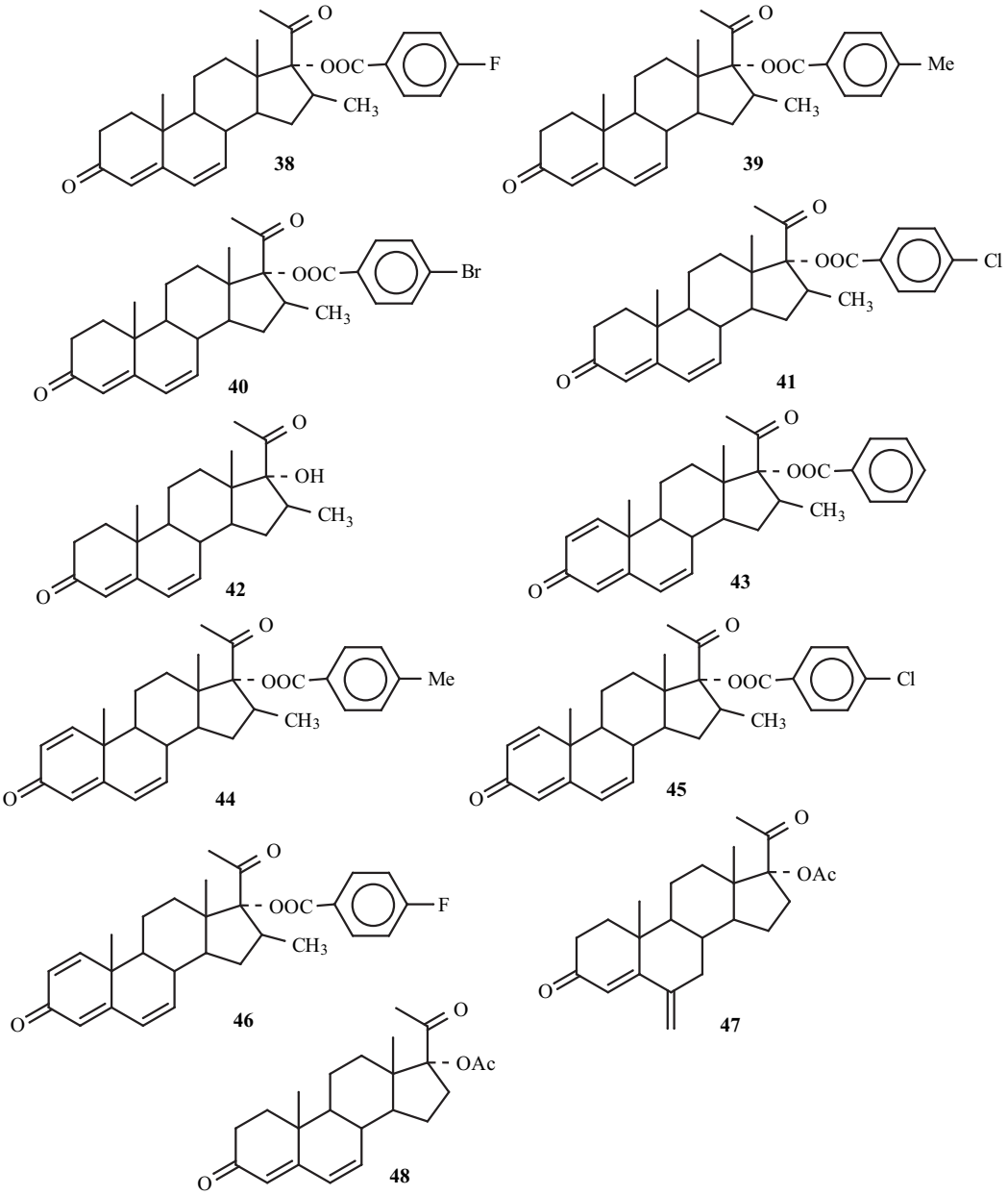

Fig. (11). Structure of biologically active dienones and trienones.

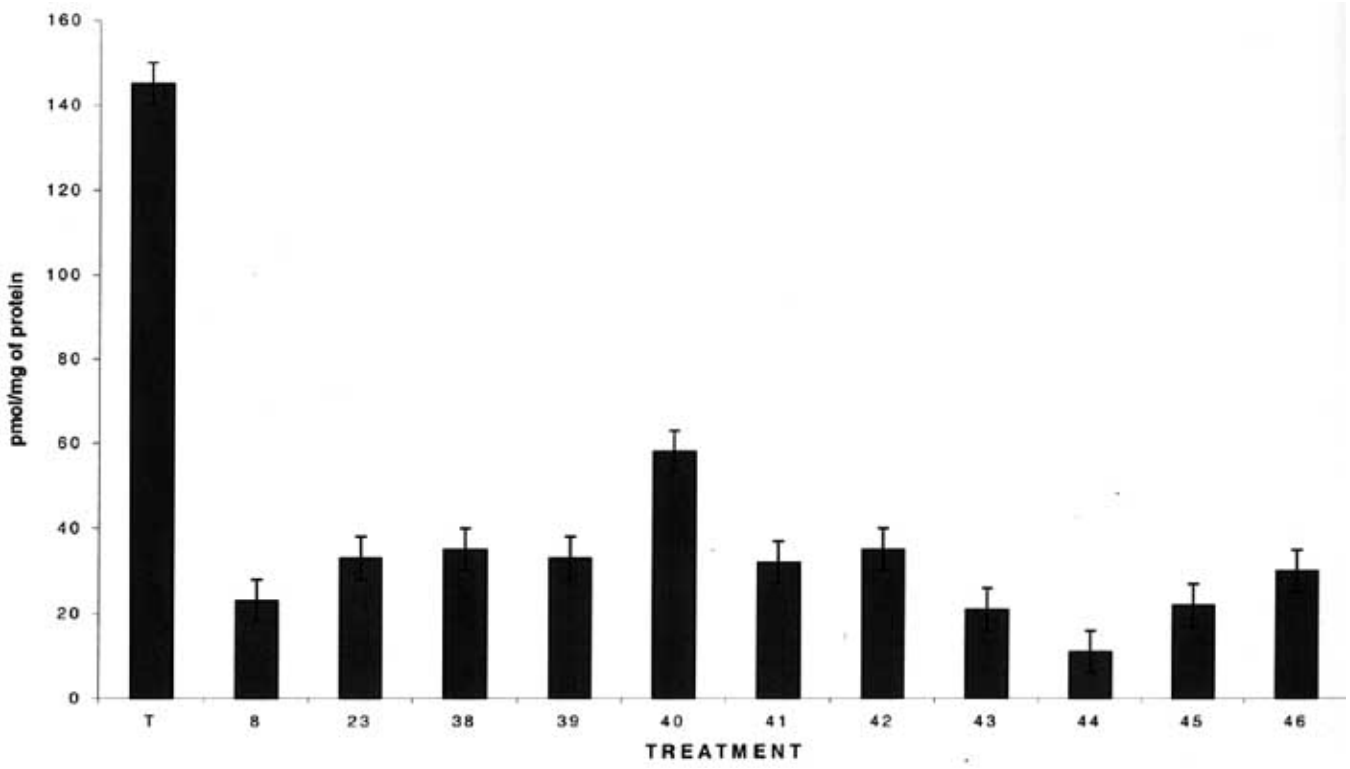

Fig. (12). Effect of different synthetic steroids on *in vitro* conversion of [^{3}H]T to [^{3}H]DHT in castrated male hamster seminal vesicles.

Several years ago we carried out a theoretical computer assisted study [58] with similar compounds. The results from this work indicated that the first step in the inhibition of the enzyme 5α-reductase consists in the formation of an enzyme-steroid activated complex. In a subsequent step, the nucleophilic portion of the enzyme (amino group) adds to the conjugated double bond of the steroid in a Michael type addition reaction to form an irreversible adduct with a concomitant inhibition of the enzyme 5α-reductase. This fact explains very well the higher biological activity of the trienones **30, 31** and **43-46** as compared to the dienones **27, 32, 33** and **38-42**. The trienones having a more coplanar structure react faster with the nucleophilic portion of the enzyme in a Michael type addition reaction than the dienones [59].

This hypothesis explains also the fact that 17α-acetoxy-6-methylenepregn-4-ene-3,20-dione **47** has much higher 5α-reductase inhibitory activity than the corresponding 17α-acetoxypregna-4,6-diene-3,20-dione **48**, (Fig. (**11**)), both compounds previously synthesized and evaluated by Petrow [60]. The 6-methylene compound **47** having an exocyclic double bond can react much easier with the enzyme in a Michael type addition reaction than the corresponding endocyclic diene **48**.

In the case of the active epoxy compound **34** (Fig. (**9**)) having a flatter B-ring, it is also possible that the nucleophilic portion of the enzyme reacts with the electrophilic carbon atom C-7 of the oxiran ring with a subsequent opening of the epoxy ring thus forming a stable steroid-enzyme adduct [59]. As a result of this, the enzyme 5α-reductase is inhibited and this explain the high pharmacological activity of the epoxy compound **34**.

At the present time, we are synthesizing several new exocyclic and endocyclic dienones and trienones with the purpose of enlarging the scope of this hypothesis. The intermediate compounds obtained in this synthesis **21-25** and **26** showed a low pharmacological activity.

CONCLUSION

This study reports the synthesis and pharmacological evaluation of several new 16-methyl substituted pregnadiene, pregnatriene and also 16-phenyl substituted D-homopregnane steroidal derivatives (Figs. (**6, 7**)). As can be seen in (Fig. (**8**)) compounds **20** and **30-33** decreased the conversion of [^{3}H]T to [^{3}H]DHT in *P. crustosum* broths [49, 50]. These data show very convincingly that the *P. crustosum* broths can be used as a model for the evaluation of 5α-reductase inhibitory effect and thus can replace the animal model which involves the sacrifice of many hamsters. The same steroids decreased the diameter of the flank organs and also reduced the incorporation of radiolabeled sodium acetate into lipids (Table **1**). The results from the three experiments correlate very well and thus confirm the fact that steroids **20** and **30-33** are powerful 5α-reductase inhibitors.

In another study, the 5α-reductase inhibitory activity of several dienones **27, 35** (Figs. (**6** and **9**)) and trienones **36, 37** (Fig**.** (**9**)) was determined. In this experiment, these steroids were evaluated in three different models: the flank organs, seminal vesicles and the effect of these steroidal compounds on the *in vitro* metabolism of [^{3}H]T to [^{3}H]DHT in seminal vesicles homogenates of gonadectomized male hamsters [47].

In the flank organs model, Table **2**, the steroidal derivatives **27** and **34-37** decreased the diameter of the pigmented spot as compared to testosterone treated animals thus suggesting an inhibitory effect for the enzyme 5α-reductase. The same steroidal compounds exhibited also a high biological activity in the seminal vesicles model (Fig. (**10**)).

In order to increase the scope of this study we synthesized several new dienones **38-42** and trienones **43-46** (Fig. (**11**)). These compounds were evaluated as 5α-reductase inhibitors by measuring the amount of dihydrotestosterone produced (Fig. (**12**)). These data show very convincingly that the trienones **43, 44** and **45** inhibited more effectively the DHT synthesis than the control sample finasteride **8**. On the other hand the dienones **38-42** exhibited a lower 5α-reductase inhibitory activity [47, 59]. Computer assisted theoretical studies carried out in our laboratory [58] indicated that the first step in the inhibition of the enzyme 5α-reductase consists in the formation of an enzyme-steroid activated complex. In a subsequent step, the nucleophilic portion of the enzyme (amino group) adds to the conjugated double bond of the steroid in a Michael type addition reaction to form a irreversible adduct with a concomitant inhibition of the enzyme 5α-reductase. This hypothesis explains very well the higher biological activity of the trienones **30, 31** and **43-46** as compared to the dienones **27, 32,33** and **38-42**.

The trienones having a more coplanar structure react faster with the nucleophilic portion of the enzyme in a Michael type addition reaction than the dienones [59].This hypothesis was corroborated very well by Petrow [60] who determined that 17α-acetoxypregna-4,6-diene-3,20-dione **48** has a lower 5α-reductase inhibitory activity than the corresponding exocyclic diene **47**. The 6-methylene compound **47** reacts much faster with the nucleophilic portion of the enzyme to form an enzyme-steroid activated complex and thus shows a higher inhibitory activity for the enzyme 5α-reductase.

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