INTRODUCTION

Steroids represent an important class of natural as well as synthetic drugs because of their ability to penetrate cells. The steroid system selected by the evolutionary process to perform some of the most fundamental biological functions has become the basis of many important discoveries in medicinal chemistry.

Medicinal chemists have long been intrigued by the fact that minor changes in the steroid molecule by the replacement of one or more carbon atoms can cause extensive changes in biological activity. Naturally occurring steroid nuclei have been modified in several ways with the aim of finding more active compounds, free from undesirable or harmful side effects, and of recognizing the structural and stereochemical features required for the display of specific, selective physiological activity. Among the many known analogues of steroids, compounds containing either heterocyclic rings condensed with the cyclopentanephehydrophenanthrene nucleus or heteroatoms within the steroid nucleus itself, have received much attention in view of their different and interesting biological activities.

The potential of heterosteroids containing heteroatom(s) such as nitrogen, sulphur, or oxygen in the steroidal nucleus in general and azasteroids in particular, has prompted numerous research groups to undertake studies in this field. The literature on biological activity of heterosteroids, particularly azasteroids has been the subject of some reviews. Singh and co-workers and other groups have published comprehensive reviews on biological activity of azasteroids. This is probably due to the fact that an \(-\text{NH}\) group is isosteric with methylene group. Hence the insertion of a nitrogen atom into the steroidal nucleus does not distort the shape of the molecule to a great extent.

The majority of medicinally useful azasteroids are of synthetic origin. Until recently, structure activity relationships of azasteroids were determined largely by empirical methods. However, rational drug design is becoming now increasingly important for the selection of appropriate types of azasteroids as synthetic target molecule for specific medicinal application. For example, many compounds have been synthesized and studied for enzyme inhibition.
Enzymes that normally transform steroid substrates may react irreversibly with reactive mimics of the latter. This can be exploited in the design of enzyme inhibitors that have medicinally useful properties stemming from their ability to block the biosynthesis of physiologically undesirable steroids further along the biosynthetic pathway.

When heteroatom(s) forms part of a fused ring system, attached group or side chain of a steroid nucleus they are known as extra nuclear heterosteroids while when heterosteroids form part of the nucleus they are known as nuclear heterosteroids.

SYNTHETIC HETEROSTEROIDS

There are known hundreds of nucleo-heterosteroids, which are purely of synthetic origin. The reviews by Burbiel, Ibrahim-Ouali, Morand and Gogte cover the relevant literature on the total synthesis of heterosteroids and analogues. For introduction of a heteroatom (or atoms) by total synthesis suitable monocyclic or bicyclic systems have been utilized to construct the heterosteroid skeleton and the other approach is based on construction of heterocyclic component of the steroid nucleus through a sequence of special techniques such as Beckmann rearrangement and the Schmidt reactions. Several synthetic heterosteroids are known in which heteroatom(s) is part of a group or side chain attached to the steroid nucleus as covered in reviews by Martin-Smith and associates.

Several synthetic heterosteroids are known which possess antifungal, antilipaemic, neuromuscular blocking, local anaesthetic, diagnostic, antimicrobial, 5α-reductase inhibitory and antineoplastic activity.

Since the aim of the present investigation has been to synthesize and study some new steroidal 5α-reductase inhibitors to be evaluated for their use in Benign Prostatic Hyperplasia (BPH), it may be in order to give an account of the current medical therapies and research carried out in this area.

BENIGN PROSTATIC HYPERPLASIA

Benign Prostatic Hyperplasia (BPH) alternatively called as Benign Prostatic Hypertrophy is the noncancerous growth of the prostate gland resulting due to over-proliferation of the stromal and glandular elements of the prostate. About 60% of men aged over 50 years have histological evidence of BPH and, after age 70, the
proportion increases to 80%. It is most often associated with prostate enlargement (volume more than 30 ml). Despite the high prevalence of BPH in aged men population, the disease pathogenesis is far from complete understanding. It is a chronic, progressive and highly prevalent disease which clinically manifests as lower urinary tract symptoms (LUTS). The hallmark of LUTS includes frequency, hesitancy, urgency, nocturia, slow urinary stream and incomplete emptying thereby causing socioeconomic burden to the patients. BPH is rarely fatal, but if left untreated, serious life threatening complications, such as acute urinary retention may arise.

In BPH, microscopic foci within specific regions of the prostate grows to form macroscopic nodules which eventually displace the normal prostatic tissue and results into the urethral compression. This compression resulting due to increased cell proliferation and/or impaired apoptosis causes physical enlargement of the prostate gland and is referred to as static component. In addition dynamic component involves sympathetic nerve stimulation causing contraction of prostatic and urethral smooth muscle which results into outflow obstruction. Despite several hypotheses the molecular trigger for BPH remains unknown.

**Prostate gland**

**Anatomy**

The prostate gland is of the size of a walnut in the young post-pubertal male, and gradually enlarges from about the age of 40 years due to BPH. Its immediate relations are the:

- Urinary bladder superiorly
- Rectum posteriorly (separated from the prostate by Denonvilliers’ fascia)
- Striated sphincter wrapped around the apex of the prostate and urethra inferiorly
- Endopelvic fascia and lateral pelvic side walls laterally.

Penetrating its posterior surface are ejaculatory ducts, which enter the prostatic urethra. Being conical in shape, the prostate presents a base above, an apex caudally, and anterior, posterior, and two inferolateral surfaces. The primary source of blood supply is a branch of the inferior vesical artery, from the anterior branch of the internal iliac artery. Venous drainage is into the dorsal venous
complex, the middle rectal veins, and posteriorly into plexiform veins which invest the sacrum and lumbar spine. Lymphatic channels drain into internal and external iliac and obturator lymph node groups.\textsuperscript{35}

**Human Prostate is basically composed of three different cell types:**

- **Glandular cells:** It produces milky fluid that liquefies semen.
- **Smooth muscle cells:** They contract during sex and squeezes the fluid from glandular cells into the urethra where mixes with sperm and other fluids to make semen.
- **Stromal cells:** They form structure of prostate.

The prostate gland is a heterogenous organ consisting of central, peripheral, transition zone (and periurethral) zones and anterior fibromuscular stroma (Figure 1). The central zone and peripheral zone each comprise approximately 25% and 70%, respectively, of the prostate glandular mass. The central zone refers to a wedge of glandular tissue located anterior and posterior to the ejaculatory ducts. Its apex is at the verumontanum, a local projection formed upon ejaculatory ducts’ entry into the prostatic urethra, and the base lies superiorly behind the bladder neck. Ducts of this zone arise from the verumontanum and branch towards the prostatic base along the course of the ejaculatory ducts. The peripheral zone surrounds most of the central zone and partly surrounds the urethra below the verumontanum. From posterolateral recesses of the urethral wall, ducts exit and extend from the verumontanum to the prostatic apex.\textsuperscript{36}

As the prostate cancer usually occurs in the outer area of the prostate i.e. the peripheral zone, BPH is characterized by increase in both glandular and fibromuscular tissue, with periurethral and transition zones of the prostate. Initially, BPH manifests as microscopic nodules in them (with periurethral nodules being mainly glandular and transition zone nodules mainly stromal) and then progressive nodular proliferation leads to bladder outlet obstruction and/or LUTS. BPH is often considered a stromal disease since the ratio of stroma to epithelium increases from 2:1 in normal prostate to 5:1 in BPH.

It has been postulated that localized proliferation of stromal cells in the transition zone may represent the initial event in the pathogenesis of the disease, a
process resembling embryonic de-differentiation, and may be associated with mediators of stromal origin subsequently having paracrine effects on epithelial tissue.\textsuperscript{37}

![Figure 1: Zones of prostate gland](image)

The principal function of the prostate gland is fertility (contributing most of the volume of ejaculate). Prostatic secretion is rich in fructose, providing a substrate for oxidative metabolism of spermatozoa, and also prostate-specific antigen, a serine protease in the human kallikrein family, which is thought to liquefy the viscous seminal fluid, facilitating motility of spermatozoa. Prostate-specific antigen is an easily measured prostate serum marker which is prostate specific, but not cancer.\textsuperscript{34}

**ANDROGEN DEPENDENCE OF PROSTATE GROWTH**

Normal growth, development and maintenance of the prostate is dependent on the testicular androgens.\textsuperscript{38-40} Important precursors to establish the testicular-prostatic relationship include: Berthold’s discovery in 1849 that a blood-borne substance from the testes can act on distant organs (cock’s comb) and the Nobel Prize-winning isolation and synthesis of testosterone by Ruzicka in Switzerland and Butenandt in Germany during 1930s.\textsuperscript{41}

John Hunter, the Scotsman who became known as the “Father of Scientific Surgery” was perhaps the first to study and document findings on the relationship between the prostate and testis in 1786. He described his observations regarding the effect of castration on the prostate gland of bulls as “The prostate and Cowper’s
glands and those of the urethra which in the perfect male are soft and bulky with a secretion salty to the taste, in the castrated animal are small, flabby, tough and ligamentous and have little secretion".42-43

Urologist Charles B. Huggins (1901–1997) first applied the scientific method to the study of androgen-prostate relationships, which earned him the Nobel Prize in 1966.44-45 Huggins most important discovery was that cancers are not always autonomous, but may be under the control of signals, such as hormones, to grow and survive. Huggins and Hodges demonstrated in 1941 that prostatic cancers are androgen-dependent and showed that the deprivation of testosterone slowed the progression of prostate cancer.45 Blocking those signals by orchiectomy or estrogen administration, could restore health of patients even with widespread metastases. Huggins also showed that benign prostatic hyperplasia (BPH) tissue was under control of testicular androgens (Table 1), thus providing scientific basis for the use of castration therapy for advanced BPH, which was used before the turn of the last century.46

Prostate development, differentiation, and maintenance are known to be closely linked to the bioavailability of testosterone and other related sex hormones. Between the 10-20 years of age when serum testosterone levels rise dramatically in males, there is pronounced, exponential growth of the prostate controlled by the balanced agonist and antagonist abilities of androgens to stimulate cell proliferation on the one hand and to inhibit the rate of cell death in prostate tissue on the other. After the age of 20 years, and under the continuing presence of testosterone, the healthy prostate achieves a steady state of self-renewal and maintenance.47 Within the prostate, testosterone is enzymatically converted to an active metabolite, 5α-dihydrotestosterone (DHT), by NADPH dependent 5α-reductase enzyme. Once formed, DHT can bind reversibly to the androgen receptor to regulate prostatic cellular proliferation and survival, or may be further metabolized along a number of alternative pathways including that which yields the endogenous oestrogen 3β-diol. 3β-Diol, in contrast to DHT, has inhibitory effects (mediated via oestrogen receptors) on cell proliferation. It is thought that normally the prostatic level of DHT remains constant even during diurnal and episodic variations in the serum levels of both free and total testosterone.47 The presence of DHT and its binding to androgen receptors can directly up-regulate expression of prostate-specific differentiation markers such
as prostate-specific antigen (PSA) synthesized by the human prostatic epithelial cells and locally active growth factors - the andromedins which stimulates the proliferation of so-called transit amplifying (TA) cells within the prostate.48-49

**Table 1: Functions of the two androgenic hormones**

<table>
<thead>
<tr>
<th></th>
<th>Testosterone</th>
<th>Dihydrotestosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In utero</strong></td>
<td>Wolffian duct derivatives (seminal vesicles, vas deferens, epididymis, ejaculatory ducts)</td>
<td>Urogenital sinus derivatives, (male external genetilia)</td>
</tr>
<tr>
<td><strong>After puberty</strong></td>
<td>Muscle mass, deepening voice, libido, growth of external genitilia, spermatogenesis</td>
<td>Facial acne and beard, male pattern baldness, prostate growth</td>
</tr>
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</table>

Testosterone in serum has approximately 10 times the concentration of DHT, but in the prostate gland, the ratio is more or less reversed. The biologic role of DHT was clarified by the work of Wilson and co-workers at the University of Texas Southwestern Medical Center during the late 1960s.50-51 These investigators demonstrated that, within the prostate, DHT is present in higher concentration and also binds more tightly to the androgen receptor than does testosterone. Thus, DHT remains at high levels in the prostate throughout life, without the age related decline seen in circulating testosterone. For this reason, DHT must exert at least a permissive role in the development of BPH. Other evidence supporting the importance of the biological role of DHT in prostate development and growth is the following:

- DHT accumulates in significantly higher amounts in androgen target tissues such as the prostate, seminal vesicles, and newborn prepuce than in other tissues.52
- DHT is more active in stimulating cell hyperplasia of prostate tissue organ cultures than are other androgens.53
DHT represents 90% or more of the steroid bound in the prostate nucleus.\textsuperscript{54}

Of great importance is the fact that the level of DHT is significantly higher in the BPH prostate compared with the normal prostate and constitutes the major endocrine abnormality.

This high DHT level may represent a key link in the pathogenesis of BPH or may, in itself, represent the pathogenetic mechanism. The continuing probability of increased levels of DHT in BPH versus normal prostate is still very appealing and logical. It has been shown that there is an increase in 5α-reductase enzyme activity in the BPH as compared with the normal prostate.\textsuperscript{55-58} The Figure 2 below summarizes the factors affecting prostatic growth at cellular level.

**Figure 2: Factors regulating prostatic growth at a cellular level.\textsuperscript{59}**

**PATHOPHYSIOLOGY AND STAGES OF BPH**

**Cellular and molecular pathology of BPH**

The prostate gland gets larger with advancing age. That seems strange because most of the body stops growing soon after puberty. However, prostate continues to grow throughout man’s life. It not only expands outwards but can also
grow inward, squeezing the urethra and causing bothersome urinary problems mostly associated with BPH. The pathogenesis of BPH is incompletely understood. Since there is a great variation in macroscopic, microscopic and molecular changes seen in BPH usually a multifactorial pathogenesis is proposed. Any theory must account for hyperplasia of the epithelial and stromal components of the prostate gland. The early growth and development of prostate is regulated by androgens, mainly DHT, via endocrine, paracrine, and autocrine influences (Figure 3).  

**Figure 3: Prostate cell growth and alteration regulated by mainly DHT**

In BPH, there is an increase in the cellular content of the transition zone of the prostate. This growth may be the result of an enhanced number of epithelial stem cell units, or enhanced proliferation by TA cells before they mature to non-proliferating luminal secretory cells, or a decreased ability of androgen receptor to limit the proliferation of luminal secretory cells. The condition is also associated with an increase in the number of stromal cells and enhanced andromedin production from these cells (Figure 4).

An age-related change within the cells has been linked partly to the hyperplasia of the epithelial component of BPH. Cells that are regularly lost are replaced by replication of stem cells hence, with differentiation, identical cell layers are created. Evidence suggests that this process gradually changes with age. This
‘senescent phenotype’ has been shown in BPH epithelial cells, resulting in abnormal cellular response to peptide growth factors and other cellular signalling, allowing the development of BPH.

The rate of apoptosis within BPH epithelium is reduced compared to normal, possibly related to increased expression of bcl-2, a proto-oncogene that is ‘switched on’ in many cancers, and which protects cells against apoptosis signalling.60

At least two paracrine positive feedback loops operate in BPH. Peptide growth factors secreted under the control of circulating androgens is fundamental to the development and maintenance of normal prostate. In BPH it usually becomes dysfunctional thereby stimulating the growth of epithelial and stromal components. The prostatic stroma produces fibroblast growth factor -2, -7 and -10 which exert a stimulatory effect on epithelial growth. The senescent epithelial cell phenotype overexpresses interleukin-1α and -8 which stimulate the production of fibroblast growth factor -2, -7 and possibly -10. Thus, the senescent prostatic epithelium is indirectly responsible for the production of its own stimulatory peptide growth factors. Fibroblast growth factor-2 is also stimulatory to stromal cells, thereby stimulating the growth of the cells that produce the factor.

The antiquated epithelial cell may lose the ability to respond to negative growth signalling such as transforming growth factor-β which otherwise inhibits epithelial growth thereby leading to promotion of differentiation of stromal cells accounting partly for the increased amount of smooth muscle seen in BPH. This tends to increase the resting muscular tone of the prostate, which is one of the factors causing urinary outflow obstruction, and is one of the therapeutic targets of medical management of BPH.

Another factor that has an impact on the development of symptoms is the bladder musculature. Involuntary detrusor contractions are seen in 50% of patients with BPH. The presence of detrusor instability and impaired bladder contractility contributes to the detrusor component. Detrusor instability, however, does not correlate with urethral obstruction. This means that even in the absence of prostatic obstruction, detrusor instability may cause obstructive symptoms.

An example is an involuntary contraction that results in urgency and an attempt to void by straining, which is unsuccessful because of small bladder volume.
The resulting symptoms are considered to be obstructive. Decompensating or ageing bladder might cause some of the symptoms associated with BPH rather than symptoms secondary to obstruction.

**Figure 4: Anatomy of BPH**

**Stages of BPH**

Benign Prostatic Hyperplasia has been classified into four different stages by Vahlensieck based on its symptoms (Figure 5).

**Figure 5: Stages of BPH**
Introduction

During the early phase (stage I) hyperplastic nodules develops in the transition zone of the prostate which progressively compress normal prostatic tissue and causes increase of prostate size with predominance of stromal element. This leads to narrowing of prostatic urethra and voiding difficulties.

In stages II and III (moderate BPH) pharmacological treatment may successfully help to control BPH. A surgical intervention is considered indispensable in stage IV of BPH (advanced phase) as obstruction and irritation symptoms lead to a marked worsening of the patient’s quality of life.

Nevertheless, enlargement of prostate alone may be insufficient for development of clinical phase and other factors, such as compliance of prostate capsule and the presence of prostatic, have a role on the onset of clinical BPH.64-65

ETIOLOGY OF BPH

Although ageing represents the central mechanism implicated but a bold new and paradigm changing set of observations have been introduced lately.66 These findings allow far more comprehensive integration of the many dissimilar risk factors that have been reported to be associated with BPH. Novel findings also highlighted the key role of tissue remodeling, hormonal alterations, metabolic syndrome and inflammation.67-75 (Figure 6)

The various possible causes are:

1. **Tissue remodeling in the ageing prostate**: It is considered as the most important factor as with age there is an increase in prostate size which has been related to BPH76-78. Hyperplasia is initially seen in about the fifth decade, and increases with age. About 40% of men aged ≥50 years and >90% of men aged ≥80 years have microscopic histopathological evidence of BPH. In ageing males a significant tissue-remodeling process takes place within the prostate, especially in the transition zone (TZ). Interference in the delicate balance of interacting growth factor signaling pathways occurs, and stromal-epithelial interactions generate an increase in prostate volume. Specifically, the most significant modifications take place in the basal cells which change their intracellular metabolism and become enlarged and hypertrophic. The development of BPH is also accompanied by the occurrence of corpora amylacea and prostatic calculi. These elements typically contain phosphate
salts of calcium, magnesium, potassium, calcium carbonate, or calcium oxalate. Subsequently, the altered secretions of luminal cells and the presence of corpora amylacea and prostatic calculi lead to further calcification, and clogged ducts become visible. Because cell growth is a consequence of either increased cell proliferation or decreased cell death, apoptotic activity was also suggested as a key cofactor in BPH development and progression. Although some authors reported similar levels of apoptosis in the epithelium of BPH relative to normal epithelium, other more recent reports have indicated that abnormal regulation of apoptosis may be associated with BPH. Kyprianou et al. examined the relative expression of two proteins involved in the regulation of prostate apoptosis: transforming growth factor (TGF)-β1 and Bcl-2, a potent apoptosis suppressor. Analysis of balancing the apoptotic versus the proliferative activities revealed a substantial net decrease of apoptosis in both the glandular and basal epithelial cell compartments of the hypertrophic prostate when compared with the normal gland. Also TGF-expressions in the epithelial cells was found to be higher in BPH tissue compared with the normal prostate. Taken together, these results suggest a potential involvement of enhanced expression of apoptotic proteins in the deregulation of the normal apoptotic cell death mechanisms in the human prostate, thus resulting in a growth imbalance in favour of cell proliferation that might ultimately support hyperplasia. Subsequently, the induction of apoptosis and/or necrosis has become more and more appealing in the design and testing of novel therapies for prostatic diseases.

2. Genetic: In studies of inherited factors predisposing to the development of BPH, it has been found that the androgen receptor and the SRD5A2 gene coding for the 5α-reductase enzymes for the conversion of T to DHT appears to cause BPH later.

3. Racial: It has been found that the prevalence of and surgery rates for BPH are lower in Asian men than Caucasian controls. Also a higher prevalence of moderate-to-severe lower urinary tract symptoms in Afro-Caribbean men has been found than the caucasians.
4. **Diet:** There has been found to be associations of BPH risk with total energy and total intake of animal protein. A ‘western’ diet seems to be a risk factor for BPH and prostate cancer.\(^{34}\)

5. **Hydrostatic Pressure:** It was found by Gat and associates that in patients with BPH there was an increase in the hydrostatic pressure that impairs the prostatic venous return and also results in venous back flow from the testicular venous system. The increased venous hydrostatic pressure is the result of damage to the one way valves in the internal spermatic veins that normally protects against back flow and hydrostatic pressure in this system. A major consequence of this venous insufficiency is long-standing exposure of the prostate to the back flow of very high testicular venous testosterone concentrations as well as the increased hydrostatic pressures. Together these two factors contribute to the development of BPH.\(^{35}\)

6. **Obesity:** Ageing, as well as, abdominal obesity in males, is associated with diminished testosterone concentrations and can be viewed as the hypogonadal-obesity cycle.\(^{36}\) The decreased levels of testosterone predispose and allow for the preferential deposition of abdominal/visceral fat. Additionally, increasing obesity is associated with increased aromatase activity which causes further reductions of testosterone, thereby perpetuating the hypogonadal-obesity cycle. For patients with abdominal obesity, each 0.05 increase of waist hip ratio is associated with a 10% increase in incidence of BPH.\(^{37}\)

7. **Hormonal alterations:** Luminal secretory cells require androgens, particularly DHT for terminal differentiation and secretory functions. It has been reported that a higher DHT activity in BPH relative to normal prostate gland tissue has been resulted as a permissive, rather than as a transformative, mediator in the development of BPH. Moreover, in studies based on the analysis of cadaver specimens, an increased accumulation of DHT was observed in BPH tissues.\(^{38-39}\) It has also been demonstrated that androgen signaling is significantly elevated in hyperplastic tissue relative to the adjacent normal prostate. Other studies have also shown that circulating levels of free estradiol remain constant in the ageing man due to an age-related increase in body weight and adipose cells. Indeed, the prevalence of fat tissues is responsible
for the expression of high levels of aromatase, which produces estrogen conversion. The increased estrogenic stimulation of the prostate in the ageing man may lead to the reactivation of prostatic growth. In addition to epithelial effects, estrogens also stimulate stromal cell proliferation.

Figure 6: Relationship between age, metabolic syndrome, inflammation, hormonal alterations, and benign prostatic hyperplasia (BPH)

8. **Metabolic syndrome:** The association between metabolic syndrome and BPH has also been studied recently. Hammarsten et al. were the first to demonstrate that non insulin dependent diabetes mellitus (NIDDM), hypertension, obesity, and low high-density lipoprotein cholesterol (HDL-C) levels constitute risk factors for the development of BPH. It has been shown that patients with BPH may share the same metabolic abnormalities of a defective insulin-mediated glucose uptake and secondary hyperinsulinaemia as patients with metabolic syndrome. These findings support the hypothesis of a causal relationship between high insulin levels and the development of BPH, and they give rise to a hypothesis of increased sympathetic nerve activity in men with BPH. Both BPH and diabetes are indeed associated with LUTS, including a reduced maximum flow rate and an increased post void residual volume. Although the mechanism by which diabetes relates to BPH is unclear, earlier reports have shown that vascular damage induced by Type 2 diabetes can promote BPH. Hypertension has also been suggested to be
involved in the pathophysiology of BPH. Epidemiologic studies have shown that hypertensive men are more likely to develop BPH and hence they undergo medical and surgical therapy than healthy men. These studies have hypothesized that noradrenergic nerves may contribute to the functional component of bladder outlet obstruction due to BPH. 99-100 Various other studies have also suggested involvement of sympathetic nervous system in regulating arterial tone and voiding physiology.101-105

9. Inflammation: BPH has indeed been frequently associated with chronic prostatitis. Chronic inflammation is believed to support the process of fibromuscular growth in BPH.106 Moreover, inflammation was associated with significantly larger prostates, higher prostate specific antigen levels, and a greater risk of acute urinary retention.106-108 The prostate is normally populated by small numbers of T-cells, B-lymphocytes, macrophages, and mast cells.109,110 Interestingly, several studies have shown that the prostatic tissue in BPH patients contains a disseminated infiltration of T and B lymphocytes and numerous colonies of macrophages.109-111 The immune response in the prostate is primarily T-cell mediated, with regulatory T-cells (CD-4) in the stroma and cytotoxic T-cells (CD-8) in the epithelium.112 In this context, by using analyses of T-cell activation marker expression, Steiner et. al. demonstrated that such inflammation mediators remain chronically activated.110 Because local accumulation of activated lymphocytes can cause tissue destruction, high concentrations of cytokines, and consequently tissue rebuilding, might contribute to the pathogenesis of BPH.

CLINICAL SYMPTOMS ASSOCIATED WITH BPH

The clinical symptoms of BPH have a major impact on the daily life of sufferers. These symptoms may be classified as being obstructive (voiding symptoms) or irritative (storage symptoms) in nature.113 They may be summarized as follows:

Voiding symptoms114, 115

- Hesitancy i.e. delay in onset of micturition
- Poor urinary flow and straining. A flow rate of less than 10 mL/s is usually suggestive of obstruction
Introduction

The sensation of incomplete bladder emptying
Terminal or post-micturition dribbling
Prolonged urination

Storage symptoms

Voiding too frequently
Nocturia i.e. having to wake at night to void
Urgency i.e. sudden compelling desire to void, which is difficult to defer
Urge incontinence i.e. involuntary leakage of urine accompanied by urgency

GENERAL GUIDELINES FOR THE MANAGEMENT OF BPH

During the last two decades, it has become clear that the management of lower urinary tract symptoms associated with benign prostatic hyperplasia (LUTS/BPH) is much more than just treating symptoms. It is important to ascertain to what degree signs and symptoms of BPH are interfering with the patients lifestyle. In 2003, American Urological Association (AUA) issued guidelines on the management of BPH which were an update of those produced in 1994 (Figure 7).

Using an evidence-based approach a multidisciplinary panel of experts focused on providing information on available BPH treatment outcomes, to enable physicians to assist their patients in making appropriate treatment decisions. Several interesting new points of distinction worth pointing out:

- Presence of bladder stones (lithotripsy and trial with medical therapy), haematuria (trial with 5α-reductase inhibitors) and other conditions no longer represented an immediate indication for surgery
- Patients with mild or moderate symptoms but not bothersome were recommended for watchful waiting
- 5α-Reductase inhibitors may also be offered to patients with benign prostatic enlargement to reduce the risk of disease progression
- Watchful waiting, medical therapy, minimally invasive therapies and surgical interventions were all represented as treatment options with no preference given and no sequence of treatment recommended
- α-Blockers are recommended for men with moderate-to-severe symptoms, and 5α-reductase inhibitors or combination therapy for men with moderate-to-severe symptoms and demonstrable prostatic enlargement (>30 ml).
In patients with clinically significant prostatic bleeding, a course of a 5 alpha-reductase inhibitor may be used. If bleeding persists, tissue ablative surgery is indicated.

Patients with at least a 10-year life expectancy for whom knowledge of the presence of prostate cancer would change management or patients for whom the PSA measurement may change the management of voiding symptoms.

After exhausting other therapeutic options as discussed in detail in the text.

Some diagnostic tests are used in predicting response to therapy. Pressure-flow studies are most useful in men prior to surgery.

AUA, American Urological Association; DRE, digital rectal exam; IPSS, International Prostate Symptom Score; PE, physical exam; PSA, prostate-specific antigen; PVR, postvoid residual urine; UTI, urinary tract infection.

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**Figure 7: Management of BPH**

**TREATMENTS OF BPH**

Determining the most appropriate treatment option for BPH

The aim of therapy is to improve LUTS and quality of life and to prevent disease progression to AUR or the need for surgery. Overall, medical therapy is indicated for patients with uncomplicated BPH, those with moderate-to-severe symptoms (IPSS ≥ 8), those awaiting surgery, or those unwilling or unsuitable to undertake surgery. Patients with BPH who have complications such as AUR, recurrent urinary tract infections, haematuria, bladder stones, or renal insufficiency/failure secondary to BPH, should be treated surgically. If
assessment reveals mild symptoms (IPSS < 8), then non-pharmacological treatments such as watchful waiting or lifestyle modifications may be adopted (Figure 8).  

\[ 
\text{LUTS} \\
\text{IPSS < 7} \\
\text{PV < 30 ml} \\
\text{Watchful waiting} \\
\text{PV > 30 ml} \\
\text{5-ARI} \\
\]

\[ 
\text{IPSS > 7} \\
\text{PV < 30 ml} \\
\text{5-ARI} \\
\text{PV > 30 ml} \\
\text{\(\alpha\)-blockers} \\
\]

LUTS = lower urinary tract symptoms; IPSS = International Prostate Symptom Score; PV = prostate volume; 5ARI = 5α-reductase inhibitor

Figure 8: Determining most appropriate treatment option for BPH

The various approaches for the management of BPH are:

A. Non-pharmacological Approaches

1. **Watchful waiting:** The American Urological Association (AUA) defines patients having mild symptoms (AUAsi/IPSS ≤ 7) may be managed by watchful waiting which is a well-established and accepted course of action and therapy is implemented only if symptoms become troublesome enough or those with moderate/severe symptoms (AUAsi/IPSS ≥ 8). Watchful waiting although cannot physiologically reduce the present symptoms or the likelihood of future major events and doesn’t have a direct impact on prostate size or growth. Watchful waiting is neither equivalent to placebo treatment because no drug is being given nor when drugs are self-administered or taken under medical instructions can be considered under simple watchful waiting. However, information about the condition of BPH and its relation to prostate cancer is an important part of watchful waiting. Studies have shown that medications are significantly not more effective than placebo in these patients.
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2. **Lifestyle modifications:** Bothersome symptoms can sometimes be sufficiently reduced with minor lifestyle modifications and is particularly useful for men who choose to avoid surgery or drug therapy. Some simple suggested changes to lifestyle include:

- Avoiding drinks that are high in caffeine.
- A diet low in fat and red meat and high in protein and vegetables, as well as regular alcohol consumption, may reduce the risk of symptomatic BPH.\(^{126}\)
- Moderate consumption of alcohol may be beneficial but heavy alcohol consumption may increase LUTS.
- Avoiding drinks after the evening meal is helpful.
- Drinking green tea which contains flavonoids may however, benefit prostatitis.\(^{127,128}\)
- Increase in the amount of fiber and fruit avoid constipation and lower the risk of BPH. A higher risk of BPH is associated with high intake of butter and margarine.
- Low zinc status has been observed in cancer patients, suggesting a possible link between zinc and cancer development. So, zinc may serve an important role in regulating cell growth and apoptosis in prostate cancer and hyperplasia cells.\(^{129}\)
- Genistein, a chemical found in soy, reduce the growth of BPH tissue in laboratory.
- Avoiding medications that can increase obstructive urinary symptoms like tricyclic anti-depressants and anticholinergic drugs, diuretics, narcotics and first-generation antihistamines and decongestants.
- Quitting smoking and altering the nature, volume and timing of fluid intake may help significantly.

3. **Urinary catheterization:** Urinary catheterization may be used for some men when medicines are not suitable as treatment choice or those who cannot have surgery. In this a catheter may be placed via the urethra or via the
abdominal wall. This allows the urine to drain continuously with minimal inconvenience. But men with catheter are more susceptible to infection and may find it interfering with their sex life.113

4. Surgical treatment: Surgical intervention is recommended for complication of LUTS such as, acute urinary retention, gross haematurea secondary to BPH, renal failure or bladder calculi secondary to BPO and for severe symptoms.130 Surgery can also be initial treatment in patients with high AUA symptom scores.

a. Transurethral Resection of prostate (TURP)

For decades, transurethral resection of the prostate (TURP) has been the gold-standard therapy for severe benign prostatic obstruction (BPO).131 It involves stripping away the enlarged prostate via the urethra, under anesthetic. So no incision is required. It uses diathermy current through a fine metal cutting loop. Efficacy is proven in terms of symptom relief and removal of obstruction. During prolonged resection, significant blood loss can occur and absorption of irrigation fluid may lead to hyponatraemia and confusion ('TURP syndrome'), but major complications are rare.132

b. Transurethral incision of prostate (TUIP)

It is used in patients with small prostate glands and primarily a bladder neck muscular obstruction. In sexually active men, it can be done by adjusting the endoscopic incision to reduce the incidence of retrograde ejaculation.34 In this procedure only one or two incisions are required to reduce constriction of the urethra without removing any of the prostate gland. An electrical knife is used to make incision(s) from inside the bladder neck down to the verumontanum. These incisions should be deep enough to penetrate the prostate tissue down to the prostate capsule.133 The presence of fat tissue at the bottom of the incision indicates that the incision is of the correct depth.134

c. Suprapubic or retropubic prostatectomy

It is carried out in men with very large prostate glands. The inner portion of the prostate gland is removed through an incision in lower abdomen using suprapubic or retropubic approach. It is a major operation and requires
Introduction

hospital stay of 7 or more days. It has been seen that bladder function alteration presenting as reduction in capacity, contractility and sphincteric activity most significantly occurs immediately after surgery and remains relatively unchanged thereafter. After 3 years of radical prostatectomy persistent or de novo detrusor over activity is observed in majority of the patients resulting in deterioration of the storage symptoms despite improvement in the voiding symptoms.¹³⁵

5. **Minimally invasive surgical therapy (MIT):** It usually involves heating the prostate gland by various means (electrical, microwave, laser). Insertion can be directly into the prostate via a needle or into the urethra via a catheter or probe. Transurethral microwave thermotherapy (TUMT), transurethral needle ablation (TUNA), and laser prostatectomy (including holmium laser enucleation of the prostate [HoLEP] and potassium titanyl phosphate [KTP] laser) represent the best studied and most accepted minimally invasive surgical treatments (MITs). TUNA and TUMT are simple and safe techniques that can be performed under local anaesthesia in a significant number of patients.¹³⁶ The AUA has recommended use of MIT for men with obstructive BPH symptoms, lateral lobe enlargement, and prostates of 60g or less.¹³⁷ MIT has been gaining ground as an alternative to both surgical and medical therapies.¹³⁸

a. **Transurethral Microwave Therapy (TUMT)**

TUMT or thermotherapy, is the destruction of enlarged prostate tissue by heating it to around 45-55°C. Microwave heat is delivered via a probe inserted via the urethra with a temperature regulator placed in the rectum. TUMT has been established as a standard in the minimally invasive management of benign prostatic hyperplasia (BPH) with good objective and subjective results and low morbidity. Application of TUMT has also been expanded to patients with urinary retention, indicating a potential alternative to surgery.¹³⁹

b. **Transurethral Needle Ablation (TUNA)**

Transurethral needle ablation of the prostate has demonstrated safety and a short-term symptomatic efficacy similar to TURP. Destruction of enlarged prostate tissue is achieved by using radio-frequency energy
applied to the centre of prostate gland at a temperature of 120°C. It is applied to the core of prostate to avoid damage to urethra and is reported to be more effective on moderately enlarged prostates.\textsuperscript{140}

c. Transurethral Electrovaporisation (TUEVP)
Photoselective vaporisation (PVP) with the Green Light HPS 120-W laser (GLL) was recently introduced for treatment of benign prostatic hyperplasia (BPH). Compared with TURP, 120-W GLL PVP is safe and effective in treatment of BPH.\textsuperscript{141} In this technique the prostate tissue is vaporized by a high electric current rather than cut away. The electric current also seals any bleeding.

d. Laser Surgery
Laser surgery is used for removal of prostate tissue. It requires the incision of a laser probe or a wire into the urethra to rest on the surface of prostate. Now-a-days in contrast to all other laser techniques currently used for the surgical treatment of benign prostatic obstruction Holmium Laser Enucleation of the Prostate (HoLEP) allows a complete pathological assessment of the retrieved tissue, since thermal or vaporisation effects on the HoLEP specimens are minimal.\textsuperscript{142} Laser techniques consist of coagulation, vaporisation, resection, and dissection, depending on the wavelength, power, and type of laser emission (continuous or pulsed).

e. Prostatic Stenting
Patients who cannot undergo general anaesthesia mechanical stenting is applied in their cases. The obstruction caused by enlarged prostate can be held back by an incision, under a local anesthetic, of a cylinder shaped mesh structure, called a stent. This is made of flexible metal such as titanium alloy. Stents reduce the symptoms of BPH without any of the side effects of surgery.\textsuperscript{143}

B. Pharmacological Approaches

I. Existing Treatments

1. \(\alpha\)-Adrenergic blockers

The adrenergic receptor belongs to the superfamily of G-protein coupled receptors which transduce signals across the cell membrane and are classified into three principal families: \(\alpha-1\), \(\alpha-2\) and \(\beta\).\textsuperscript{32,144} Three subtypes of the \(\alpha-1\) receptors in
the prostate (α-1α, α-1β, and α-1δ-AR) have been characterized pharmacologically, and their expressions have been reported in the prostate with the α-1α being the most predominant i.e. about 70% of the α-1 receptors. The prostate capsule, stroma and the bladder necks are densely populated with α-1-adrenoceptors. α-1α Receptor is believed to be involved in smooth muscle contraction in prostate and bladder neck causing obstruction and voiding problems. Although α-1α is major subtype in the prostate tissue but the activation of α-1α- adrenergic subtype may be responsible for ischemia-induced cardiac arrhythmia.

Recently, the role of α-1β-adrenergic receptor subtype in the regulation of blood pressure has been researched, whereas potential therapeutic use of the α-1δ-adrenergic receptor subtype has not been firmly established. Some evidence suggests that α-1δ-adrenergic receptor may play a role in the control of blood pressure because of their involvement in the contraction of vessels. Due to the α-1δ-adrenergic receptor being predominant in the destrusor muscle, their relevant role in the control of the symptoms associated with BPH is also postulated.

The α-blockers work on the dynamic aspect of obstruction which causes narrowing of the bladder neck and includes the hyperplasia of the prostatic fibromuscular stroma that can be responsible for up to 40% of bladder outflow obstruction. α-1-Adrenergic receptor antagonists improve LUTS by relaxing prostatic and urethral smooth muscle tone, which decreases outlet resistance.

Several α-blockers are available on the market with slight differences in action, side effects and dosing. Phenoxylbenzamine (1) was the first non-selective α-blocker with both α-1 and α-2 activity but is no longer used for LUTS/BPO due to cardiovascular and CNS side effects with hypotension, nasal congestion, fatigue and dizziness reported in 30% of those treated. Prazosin [1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylsulfonyl)piperazine hydrochloride] (2) emerged as the first α-1 specific antagonist.

The next advancement in drug therapy was the advent of the α-1 selective drugs such as Terazosin [(±)-4-amino-2-[4-(tetrahydro-2-furoyl)-1-piperaziny]-6,7-dimethoxy -quinazoline hydrochloride dehydrate] (3) and Doxazosin [(±)-1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-ylcarbonyl) piperazine methanesulfonate] (4), with their longer half-lives allowing for once daily dosing but
side effects such as dizziness, fatigue, somnolence and hypotension were
reported.\textsuperscript{152}

Alfuzosin[(R,S)-N-[3-{(4-amino-6,7-dimethoxy-2-quinazolinyl) methylamino ]
propyl] tetrahydro-2-furancarboxamide hydrochloride] (5), is a selective and
competitive $\alpha$-1 antagonist and is touted as a clinically uroselective $\alpha$-blocker,
although it has no \textit{in vitro} specificity to any of the $\alpha$-1 receptor subtype, but when
administered orally is found to be concentrated in the prostate. The drug also
demonstrated a lower incidence of vasodilatory side effects with the once daily
versus three times daily dosing.\textsuperscript{153}

Tamsulosin [5-{(2R)-2-[2-(2-ethoxyphenoxy)ethylamino]propyl}-2-methoxy-
benzenesulfonamide monohydrochloride] (6), a sulfonal derivative, is the most
potent $\alpha$-1 antagonist investigated and has been used for the $\alpha$-1$_a$ and $\alpha$-1$_d$
receptors, which are thought to be localized to the prostatic stroma but side effects
such as dizziness and rhinitis were somewhat higher.\textsuperscript{154}

Naftopidil[(\pm)-1-[4-(2-methoxyphenyl)piperazinyl]-3-(1-naphthyloxy)propan-2-
ol] (7), is the novel $\alpha$-1 receptors antagonist studied extensively in Japan and China
for hypertension, BPH and dysuria. It is in phase III clinical trials. The drug is
selective for the $\alpha$-1$_d$ receptor with 3- and 17- fold higher affinity than that of the $\alpha$-1$_a$
and $\alpha$-1$_b$ receptors, respectively. It has been suggested in limited information from
Japan that treatment with naftopidil provides short-term improvement in urinary
symptom scale scores: total IPSS, QoL score and urinary symptoms from baseline
comparable to low-dose tamsulosin. It also displays calcium-channel antagonism
and serotonin agonistic effects all leading to relaxation of smooth muscle in the
prostate, proximal urethra and bladder trigone.\textsuperscript{149, 151, 153}

The most recent alpha-blocker is Silodosin [(-)-1-(3-Hydroxypropyl)-5-{(2R)-2-
(2-[2,2,2-trifluoroethoxy]phenoxy)ethyl]amino)propyl]-2,3-dihydro-1H-indole-carbo-
xamide] (8), which improved IPSS, QoL and maximum flow rate (Qmax) with
retrograde ejaculation being the most common side effect and is in phase II clinical
trials.\textsuperscript{155-157}

GYKI-16084 is a combined $\alpha$-1 and postsynaptic selective $\alpha$-2 blocker that
has also undergone phase II clinical studies. It significantly improved the American
Urological Association Symptoms Index (AUA-SI) score and increased Q$_{\text{max}}$ in
patients with BPO.\textsuperscript{158}
**Introduction**

RBx 6198, (2-{3-[4-(2-isopropoxy-phenyl)-piperazin-1-yl]-propyl}-3a,4,7,7a-tetrahydroisoindole-1,3-dione (9), is the novel and potent (nanomolar affinity) \( \alpha_{1_a} \)-adrenoceptor antagonist.\(^{159}\)

A number of \( \alpha_{1_a} \)-subtype selective antagonists belonging to different structural classes of compounds have been disclosed recently such as SNAP 5089 and SNAP 5150 which are niguldipine analogues possessing high selectivity for \( \alpha_{1_a} \)-receptors. In a dog model, uroselectivity was obtained with SNAP 5089.\(^{37}\) GG 818 (oxazole) and RS 100975 also proved to be very selective for \( \alpha_{1_a} \)-versus \( \alpha_{1_b} \)- and \( \alpha_{1_d} \)-receptors and they underwent clinical trials recently. SNAP 6021 (dihydropyrimidinone).\(^{160}\) SNAP 7915 (oxazolindinone) and phenylpiperazine analogues have also been disclosed recently.\(^{161}\) The various \( \alpha \)-adrenergic blockers along with their selectivity and structures are summarized in *Table 2* below.

**Table 2: \( \alpha \)-Adrenergic blockers along with their selectivity**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Selectivity</th>
<th>Duration</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Phenoxbenzamine</td>
<td>( \alpha_{1}=\alpha_{2} )</td>
<td>Long acting</td>
<td><img src="image" alt="Phenoxbenzamine" /></td>
</tr>
<tr>
<td>2 Prazosin</td>
<td>( \alpha_{1_{a}}=\alpha_{-} )</td>
<td>Short acting</td>
<td><img src="image" alt="Prazosin" /></td>
</tr>
<tr>
<td></td>
<td>( \alpha_{1_{b}}=\alpha_{1_{d}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Terazosin</td>
<td>( \alpha_{1_{a}}=\alpha_{1_{b}}=\alpha_{1_{d}} )</td>
<td>Long acting</td>
<td><img src="image" alt="Terazosin" /></td>
</tr>
</tbody>
</table>

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*University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh*
<table>
<thead>
<tr>
<th>No.</th>
<th>Drug</th>
<th>Effect</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Doxazosin</td>
<td>Long</td>
<td><img src="image" alt="Doxazosin" /></td>
</tr>
<tr>
<td>5</td>
<td>Alfuzosin</td>
<td>Short</td>
<td><img src="image" alt="Alfuzosin" /></td>
</tr>
<tr>
<td>6</td>
<td>Tamsulosin</td>
<td>Long</td>
<td><img src="image" alt="Tamsulosin" /></td>
</tr>
<tr>
<td>7</td>
<td>Naftodipil</td>
<td>Long</td>
<td><img src="image" alt="Naftodipil" /></td>
</tr>
<tr>
<td>8</td>
<td>Silodosin</td>
<td>Long</td>
<td><img src="image" alt="Silodosin" /></td>
</tr>
<tr>
<td>9</td>
<td>RBX6198</td>
<td>Long</td>
<td><img src="image" alt="RBX6198" /></td>
</tr>
</tbody>
</table>

*University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh*
2. Cytotoxic Agents

Any imbalance between the physiological process of cell proliferation and cell death may lead to change in prostate size with the subsequent development of abnormalities in the gland. So it is reasonable to assume that cytotoxic agents are able to induce apoptosis, causes significant decrease in proliferation rate and are useful for treatment of disease that cause abnormal or uncontrolled cell proliferation. Camptothecin (10) identified as first member of cytotoxic agents acts upon eukaryote topoisomerase I to stabilize these transient intermediates. The presence of persistent DNA breaks triggers apoptosis cascade in cancer cells. Non-camptothecin topo I agents lead to identification of novel indolecarbazole class of compounds represented by rebeccamycin (11).162

Some antimitotic agents such as vinblastin (12) and doxorubicin (13) are only marginally effective due to dose limiting systemic toxicity. They exploit the proteolysis activity of PSA within prostate cancer tissue to facilitate release of cytotoxic agent selectively into the microenvironment of tumor cells.163
Introduction

Thapsigargin (14) is a sesquiterpene lactone isolated from seeds and roots of Umbelliferone. *Thapsia garganica* inhibit the ubiquitous sarcoplasmic and endoplasmic reticulum Ca\(^{2+}\) dependent ATPase. They have ability to kill proliferatively quiescent G\(_0\) cell.\(^{164}\) A series of cardenolides and related compounds have been isolated from aerial parts and roots of ornamental milkweed, *Asclepias curassavica*. Calopropin isolated from this plant family has been reported as potent cytotoxic agent against KB cells.\(^{165}\) Significant cytotoxic activity against T-lymphoblastic cells was found in diosphenols and seco-anhydrides. Betulinic acid (15) a pentacyclic triterpenoid has anti-HIV, cytotoxic and antitumor property.\(^{166}\)

N-alkylated analogues of the natural polyamines (spermine, spermidine and putrescine) also exhibited strong cytotoxic activity against human tumor cell lines.\(^{167}\) Various other cytotoxic agents are *Paclitaxel*, macrocyclic polyamides, certain derivatives of quinolines, quinazolines and pyrrole.

These agents have the potential to be explored as possible drugs in BPH and prostate cancer.

3. Phytotherapeutic Agents

Phytotherapy or the use of plant extracts for treatment of lower urinary tract symptoms (LUTS) associated with benign prostatic hyperplasia (BPH) was first described in Egypt in the 15th century BC.\(^{168}\) Phytotherapeutic products containing inherently vast structural diversity than synthetic compounds have been used traditionally in developing countries while the use of them as complementary alternative medicine (CAM) is increasing rapidly in developed countries such as Europe particularly in France, Germany and USA for the treatment of BPH. In USA alone about 33 % of those who opt for nonsurgical therapy for BPH use herbal
preparation alone or in combination with prescription medicines as these preparations are available as nonprescription dietary supplements. 169-170

Phytotherapeutic products are not the actual plant, but are extracts derived from the roots, seeds, bark, or fruits of the various plants used.

Numerous plants have shown to improve uncontrolled growth of the prostate gland and improve urinary tract symptoms associated with benign prostatic hyperplasia. 171 Also, these agents do not require prescriptions in the United States so patients frequently begin taking these medications proactively to maintain prostate health or when they initially become symptomatic as there are widespread beliefs that these products are “natural” and, therefore, better for you; are “inherently safe,” without side effects; and are “good for you” in both preventing and treating diseases. 172-175

Although mono preparations (single plant only) are available, many companies manufacture combination products (plant extracts) in an attempt to provide enhanced efficacy to improve marketability, and to provide their own “unique” product that can be registered, because these products have no patent protection. The composition of plant extracts is very complex. 171 They contain a wide variety of chemical compounds including phytosterols, plant oils, fatty acids, and phytoestrogens (Table 3). It is unclear which of these compounds the “active” component is.

Table 3: Major chemical constituents of plant extracts 171

<table>
<thead>
<tr>
<th>Phytosterols</th>
<th>Lupenone</th>
<th>Polysaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Sitosterol</td>
<td>Lupeol</td>
<td>Flavanoids</td>
</tr>
<tr>
<td>Δ-5-Sterols</td>
<td>Terpenoids</td>
<td>Phytoestrogens</td>
</tr>
<tr>
<td>Δ-7-Sterols</td>
<td>Fatty acids</td>
<td>Coumestrol</td>
</tr>
<tr>
<td>Campesterol</td>
<td>Lectins</td>
<td>Genistein</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>Plant oils</td>
<td></td>
</tr>
</tbody>
</table>

The mechanism of action of the phytotherapeutic agents is uncertain as many in vitro experimental studies have proposed numerous mechanisms of action. Almost all of these studies use supraphysiologic doses, which are many times higher.
than the standard doses used clinically and are mainly performed in tissue culture, which might not accurately reflect in vivo effects.\textsuperscript{175} The main mechanisms of action that have received the greatest attention are anti-inflammatory, 5α-reductase inhibition, and more recently growth factor alteration. The effects of these extracts, therefore, are not related to the activity of one component, but are more likely based on the additive and/or synergic action of all their components.

Due to the uncertainty regarding their efficacy and safety many practitioners, despite the popularity of herbal preparations with the public have been reluctant to recommend these products. Most phytotherapeutic compounds are unlicensed and do not require evidence of efficacy, safety or purity. A significant limitation in the evaluation of phytotherapeutic agents for men with BPH is the paucity of studies that have been conducted using rigorous scientific methods. Most of the older studies did not use standard objective measures of treatment results.\textsuperscript{176,177} In recent years, however, there have been an increasing number of well-performed studies conducted by European companies who own proprietary rights to these products as these companies will benefit economically, if effectiveness is demonstrated.\textsuperscript{178}

The various phytotherapeutic plants that are being used for the treatment of BPH are:

I. \textit{Serenoa repens} (Saw Palmetto Berry extract)

The extracts of the dried ripe fruit of \textit{Serenoa repens} (american dwarf palm, palmetto scrub, \textit{Sabal serrulata}, or \textit{Sabalis serrulatae}) is the most widely used herbal product in Europe and USA by patients for prostate health and management of LUTS caused by BPH.\textsuperscript{175} Numerous medications such as Permixon, Prostagalen, Prostaselect, PAI09, Curbicin, Prostavigol and Strogen forte contains saw palmetto berry as one of their principal ingredients.\textsuperscript{179} Saw palmetto is as an approved herb by the German Commission E for use of urination problems associated with BPH and prostate adenoma.\textsuperscript{180} Its clinical effectiveness has been shown in a number of trials lasting 6 months to 3 years although the conclusive proof is not yet there. No significant side effects have been observed except minor cases of stomach problems.\textsuperscript{181-182}

It has been postulated that the liposterolic extract (LSESr, Permixon\textsuperscript{®}) obtained by hexane extraction of \textit{Serenoa repens} has antiandrogenic, antiestrogenic
Introduction

and anti-inflammatory effects. It also inhibits the type I and type II isoenzymes of 5α-reductase, inhibits prolactin and growth factor-induced cell proliferation. The lipid soluble portion consists of 85-90% of fatty acids and sterols. The steroid fraction consists of β-sitosterols, stigmasterols, cycloartenol, lupeol, lupenone and methylcycloartenol. Also present in the extracts are long chain fatty acid but the steroidal portion is pharmacologically active. However, most of these in vitro studies were performed in cell cultures or used supraphysiologic dosages of SPB extract. Therefore, the significance of these mechanism studies is uncertain.

LSESr inhibits in vitro DHT formation in rat prostate and human foreskin and in stromal and epithelial cells of human BPH either separated or co-cultured saw palmetto review. LSESr was shown to be a non-competitive inhibitor of 5α-reductase I and an uncompetitive inhibitor of 5α-reductase II. Inhibition of 5α-reductase (type I) activity by extract of Serenoa repens has also been demonstrated in DU 145 cells, a metastatic human prostate carcinoma cell line.190

Saw palmetto ethanol extract, SPET-085, concentration-dependently inhibited 5α-reductase type II in a cell-free assay using cell homogenates isolated from stably transfected HEK293 cells with an IC50 value of 2.88±0.45 μg/mL. Saw palmetto extract is an effective dual inhibitor of 5α-reductase isoenzyme activity in the prostate and induces its effects without interfering with the cellular capacity to secrete prostate-specific antigen (PSA).191

Although most of the research has been focused on 5α-reductase inhibitory activity as saw palmetto’s most important mechanism of action, other mechanisms have been investigated. In some trials it has been found that men treated with saw palmetto had a marked drop in estrogen receptor activity within the prostate gland compared with men receiving placebo. This finding led to the suggestion that saw palmetto leads to a decrease in estrogen-mediated prostate growth due to competitive inhibition of cytosolic estrogen receptors. Recent studies have demonstrated that saw palmetto induces histological changes such as increase in prostatic epithelial contraction and the percentage of atrophic glands within the prostate gland although its precise mechanism of action remains unclear.192

II. Pygeum africanum

Pygeum africanum (Tadenan) is an extract from the bark of the African plum tree and is widely used in France and throughout Europe since 1969. It is the second
Introduction

most popular herbal product used by many americans for BPH.\textsuperscript{193} Other formulations like Prostata are also available but almost all the research and clinical trials of this product have been done using the product Tadenan. The extract is characterized by the presence of phytosterols, pentacyclic triterpenes and ferulic acid esters.

It is used to treat prostate cancer, prostatitis and especially BPH. The phytosterols, especially $\beta$-sitosterol, have antinflammatory properties which inhibits the formation of prostaglandins responsible for the swelling in the prostate gland. The pentacyclic triterpenoids (oleanolic and ursolic acids) are believed to inhibit the activity of glucosyl-transferase associated with inflammation and swelling in the prostate while ferulic esters ($n$-docosanol and $n$-tetracosanol) helps prostate to get rid of cholesterol deposits which accompany BPH.\textsuperscript{170,194} $n$-Docosanol (16) has been found to reduce prolactin levels in the body. Prolactin increases the uptake of testosterone and increases the conversion of testosterone to DHT in the body. The sterolic portion of pygeum helps in preventing the accumulation of testosterone in prostate.\textsuperscript{195,196}

\begin{equation}
\text{(16)}
\end{equation}

\textit{In vitro} studies using Tadenan (\textit{Pygeum africanum} extract) indicated that it inhibited cell proliferation and enhanced apoptotic cell death of prostate stromal cells, specifically targeting fibroblast hyperproliferation induced by basic fibroblast growth factors. Myofibroblasts also played a major role in BPH progression, when they proliferated and increased their numbers.\textsuperscript{197} In this regard, incubation with growth factors and hormones confirmed the link between \textit{pygeum africanum} action and the proliferative status of cells mediated by blocking specific mechanisms linked to FGF2 activity.\textsuperscript{198} Moreover cultured differentiated smooth muscle cells were resistant to PA effects. It was also shown that in rabbits pretreatment with Tadenan prevented the contractile dysfunction induced by partial bladder outlet obstruction but the significance of dosages remains clear due to being supraphysiologic as they were in range of 100 mg/kg.\textsuperscript{199}

Tadenan extract when administered intraperitoneally in castrated rat model antagonizes the testosterone in the prostate and seminal vesicles. More recently, it was reported that \textit{Pygeum} extract can prevent the inflammatory cells that appear to
be involved in the BPH development from infiltrating into the prostate as it inhibited the production, induced by the 5-lipoxygenase enzyme, of such metabolites as chemotactic leukotriens by human polymorphonuclear cells stimulated by means of calcium ionophore A 23187.59,200

Recently, the ethanolic extracts of *P. africanum* were shown to inhibit human PCa cell growth, induce apoptosis and alter cell kinetics. TRAMP mice, a mouse model to investigate PCa, exhibited a reduction of PCa incidence compared to the control group after feeding with *P. africanum* extract.201

A standardized preparation of *P. africanum*, may be a useful treatment option, at least in the short term, for men with lower urinary symptoms consistent with benign prostatic hyperplasia. However, inadequacies in the reporting of outcomes limit the ability to estimate its safety and efficacy.195

**III. Urtica dioica (Stinging Nettle)**

Stinging nettle (*Urtica dioica L.*) root extract also has a long history of traditional use as a medical treatment for symptomatic BPH and LUTS.202,203 *Urtica dioica* (stinging nettle) and *Urtica urens* (dwarf nettle) are members of the Urticaceae family native to Eurasia, and are considered therapeutically interchangeable. The extracts from the roots contain a mixture of water- and alcohol- soluble compounds including lectins, phenols, β-sitosterol, polysaccharides and lignins with extraction procedures varying from company to company.168,204 Many different possible mechanisms of action have been suggested for stinging nettle including inhibition of prostatic growth factor interaction by blocking of the conversion of testosterone to dihydrostersterone , inhibition of membrane sodium and potassium-adenosine triphosphatase in the prostate, with resulting suppression of prostate cell metabolism and growth.205,206 Limited clinical trials also suggested that nettle extract Bazoton is beneficial for men with milder forms of BPH.207

The hydrophilic components of nettle, including lectins and polysaccharides, appear to be important, particularly in prostate disease. The importance of nettle root lignans such as (-)-3,4-divanillyltetrahydrofuran in BPH and other androgen and estrogen-sensitive conditions may be due to interference with binding of sex hormone binding globulin (SHBG) to testosterone, the testosterone receptor and/or the SHBG receptor.
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A botanical formula consisting of whole herb *Urtica dioica* and *Pygeum africanum* bark was found to inhibit both aromatase and 5α-reductase. Studies also suggest that the active compounds show a cyclooxygenase inhibition. Mild gastro-intestinal upset has been seen in some patients as adverse reaction.

IV. *Secale cereale* (Rye Pollen)

The commercial preparation "Cernilton" is a pollen extract prepared from several plants found growing in countries such as Sweden. This drug is available across Europe and is manufactured by microbial digestion of the pollen followed by extraction with both water and organic solvents. Thus, the total extract consists of both a water-soluble and an acetone-soluble fraction. One dose of Cernilton contains 60 mg of Cernitin T60, a water soluble pollen extract fraction, and 3 mg of Cernitin GBX, acetone soluble pollen extract fraction. The acetone fraction is known to contain β-sterols. The mechanism of action of Cernilton remains unknown.

Water soluble fraction was found to inhibit the growth of immortal prostate cancer cell lines as well as epithelial and stromal cells derived from BPH tissue in cell culture *in vitro* but *in vivo* action is uncertain. A decrease in prostate size has also been seen in Wistar rats treated with Cernilton. In addition, smooth muscle relaxation in mouse and pig urethra, along with increased contraction of bladder muscle, has been reported as a dose-dependent effect of Cernilton.

The acetone soluble fraction was found to contain β-sterols. Several *in vitro* studies undertaken to investigate the mechanism of action suggest Cernilton has anti-androgenic effects, may relax urethral smooth muscle tone and increase bladder muscle contraction, or may act on the α-adrenergic receptors and relax the internal and external sphincter muscles. But the long term efficacy and safety of Cernilton as well as their effectiveness in preventing complications of BPH such as acute urinary retention or the need for surgical interventions is not known.

V. *Cuban Royal Palm* (*Roystonea regia*)

D-004, a lipid extract of the fruit of the Cuban royal palm (*Roystonea regia*), contains free fatty acids, with oleic, lauric, palmitic, and myristic acids being the most abundant components which have been found to inhibit prostate 5α-reductase activity. Oral treatment with D-004 reduced prostatic hyperplasia (PH) induced with testosterone but not PH induced with DHT in rodents, suggesting an inhibitory effect on prostate 5α-reductase activity. D-004 inhibited 5α-reductase in a
noncompetitive manner because it decreased the $V_{\text{max}}$ of the enzyme reaction without changing the $K_m$, i.e., it does not involve the binding of the active component(s) of the extract to the site of enzyme activity. Although further in vivo studies are required to fully assess the inhibitory effects of D-004,\textsuperscript{215, 216}

**VI. Echinacea purpurea**

Easter purple coneflower (*Echinacea purpurea*) has been found to be rich in polysaccharides, phytosterols, phenolic compounds and caffeic acid derivatives. It is reported to possess multifunctional effects such as immunostimulating, antiinflammatory, antivirus, anticancer, radioprotective as well as possible antiandrogenic activities.\textsuperscript{217-219} Roots of coneflower also contain some other active substances that could affect the prostate function. The administration of *E. purpurea* L. Moench extract to rats with hyperplasia for 4 and 8 weeks gradually and significantly reduced the prostate mass and reversed the degenerative changes in the structure of the prostate gland (squeezing of epithelium of prostate gland, containing low columnar epithelial cells without any intracellular vacuoles, appearance of fragments of degenerating cells in the lumen of glands). This investigation suggests that extract of purple cone flower prevents the development of BPH.\textsuperscript{220}

**VII. Cucurbita pepo**

Since the end of the 19\textsuperscript{th} century Pumpkin seed (*Cucurbita pepo* L.) oil has been used to treat urinary tract problems. It has been found to be effective alone or when given in combination with saw palmetto to treat BPH. Pumpkin seed extract was found to improve the functioning of the bladder and urethra. Pumpkin seeds are extremely safe but may lead to minor stomach upset.\textsuperscript{170} *C. pepo* seeds have been mentioned in many pharmacopoeias including the British Herbal (4th Ed.) and the USP Pharmacopoeias (10th Ed.). In 1985 the Commission E for Phytotherapeutic Substances of the German Federal Health Office published a positive monograph on *Cucurbitae peponis semen* (seeds of *C. pepo* and related cultivars), with clinical indications for the micturition disorders associated with BPH. The seeds of this plant contain 30% to 51% oil. Main fatty acids are linoleic acid (43-55%) and oleic acid (27-38%). Other substances described in the seeds of *C. pepo* include tocopherols and sterols in free and glucosidic forms. $\beta$- and gamma tocopherols are present (0.03%). Minor amounts of $\Delta^5$-sterols are also found in the seeds, as opposed to
their higher content in the rest of the plant. Δ7-sterols are present in variable quantities. The sterols constitute 55-60% of the oil non-saponifiable content. β-sitosterol, present in C. pepo oil, has also shown to be a strong inhibitor of prostaglandin biosynthesis in prostatic tissue of patients with BPH and also to exert a marked anti-inflammatory action.221

VIII. Hypoxis rooperi

Phytosterol extracts derived from the South African star grass, Hypoxis rooperi, are sold under the trade name of Harzol. Hot aqueous extracts of dried or fresh corns are also used to treat BPH and prostate adenoma. The extract has activity on prostate adenoma mainly due to β-sitosterol while anti-inflammatory effects are due to interference with prostaglandin metabolism which is attributed to the presence of rooperol222. β-Sitosterol contains a mixture of phytosterols, with smaller amounts of other sterols, bonded with glucosides. β-sitosterol-β-D-glucoside also have been reported to be present.168

Harzol was also found to enhance the production and secretion of plasminogen activators in isolated and epithelial cells. Additionally, prostate stromal cell cultures when conditioned with β-sitosterol were found to have elevated levels of transforming growth factor (TGF)-β1 which is an important differentiation factor and apoptosis inducer but none of these effects have been shown to occur in vivo or to be clinically relevant.223

Azuprostat (Azupharma GmbH, Gerlinger, Germany) primarily contains β-sitosterols from Hypoxis rooperi as well as from Pinus (pine) and Picea (spruce) and is a combination preparation of extracts.

However, β-sitosterol is common to all 3 plants.224 The existing evidence of Harzol and Azuprostat being effective is limited to trials of short duration, relatively few patients studied and lack of standardized β-sitosterol preparations. Their long term effectiveness, safety and ability to prevent BPH complications are not known.178

XI. Opuntia

The Prickly pear plant, of the genus Opuntia with more than 400 species, is native to the Americas from Chile to Canada. A number of different forms of betacyanins are known to exist and co-exist in the Opuntia species. Betanin, one of the major betacyanin pigments, is reported in O. ficus indica. It is a potent antioxidant with anti-inflammatory and anti-cancer properties. Oral administration of
cactus flower extract revealed improvement in symptoms of BPH, with decrease in urgency to urinate and feeling of fullness in the bladder.225-226

X. Phellodendron

Traditional Chinese medicines to treat urinary disorders often contain the herb Phellodendron which is reputed to have properties which are useful in the treatment of urinary tract symptoms. Phellodendron or cork tree is a genus of deciduous trees in the family Rutaceae. Extracts from the bark of Phellodendron appear to act on the urogenital system. Relaxation of prostatic smooth muscle is the most effective mechanism of action to relieve urinary symptoms caused by urethral obstruction due to BPH. Phellodendron acts on a non-specific part of the contractile mechanism of the prostatic smooth muscle to produce its effects and not by interfering with the neuromuscular transmission process. Phellodendron is known to contain phytoestrogens which acts by inhibiting prostate growth thus being able to relieve the lower urinary tract symptoms caused by BPH.227

XI. Ganoderma luciderm

Extract from the fruiting body of *Ganoderma luciderm* in ethanol has significantly inhibited the growth of ventral prostate induced by testosterone. It has also shown antiestrogenic activity i.e. significant effect on proliferation rate of MCF-7 cells.228

XII. Piper nigrum

Testosterone 5α-reductase inhibitory activity of aqueous ethanolic extracts obtained from several different parts of six piper species, namely *P. nigrum*, *P. betle*, *P. methysticum*, *P. kadsura*, *P. longum* and *P. cubeba* have been examined. Among them the extracts of *P. nigrum* leaf, *P. nigrum* fruit and *P. cubeba* fruit showed potent 5α-reductase inhibitory activity. Extract of *P. nigrum* leaf and its lignan (17) exhibited both testosterone 5α-reductase inhibitory activity and melanogenesis stimulation activity. It was further found that piperine (18) a major alkaloid amide is responsible for potent inhibitory activity. Topical application of 5% solution of the methanolic extract of *P. nigrum* leaf showed a significant anti-androgenic activity.229 Extract of *P. nigrum* leaf can be a desirable hair-care cosmetic material for prevention of gray hair and alopecia. Inhibitory activity of *P. nigrum* fruit extract may be attributed to its major constituents piperine and fatty acids such as linoleic, oleic and palmitic acids.

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Evidence suggests that the consumption of soy isoflavones is related to lower rates of histological BPH among elderly Chinese men. Isoflavones are polyphenolic compounds found predominantly in legumes (soy, lentils, beans) and in red clover. The main isoflavones are formonetin, its dimethylated product diadzein, biochanin, and its desmethylated product genistein. Genistein and biochanin A are effective inhibitors of 5α-reductase activity in genital skin fibroblasts and in BPH homogenates.\textsuperscript{230} In some studies it has been shown that soy extract was more effective in inducing cell cycle arrest and apoptosis than soy isoflavones in both early stage (LnCap) and late-stage (PC3) prostate cancer cells however, future \textit{in vivo} studies are needed to evaluate and compare the effects of soy and soy isoflavones on prostate cancer.\textsuperscript{231}

Apigenin (19) is a member of the flavanoid family, and is a naturally occurring phytoestrogen widely distributed in many fruits and vegetables. It has shown \textit{in vitro} antitumour activity in carcinoma of the thyroid, colon, breast and prostate. Previous studies have shown that Apigenin (19) induces apoptosis in prostate epithelial cells through mitochondrial mediated cell death. The drug is currently in the discovery phase for the treatment of BPH. It was also shown that apigenin treatment resulted in inhibition of proliferation and increased apoptosis in cultured prostatic stromal cells. Although preliminary results shows possible beneficial effects in the prevention and treatment of BPH.\textsuperscript{232}
XIV. Willow herbs (*Epilobium* sp.)

Ethanolic extracts of the fresh aerial parts of *Epilobium* (Onagraceae) have been used in folk medicine for the treatment of BPH and for inflammation of the prostate gland. The constituents of *Epilobium* are not well established but the presence of sterols, triterpenes, fatty acids, macrocyclic tannins, flavonoid glycosides and a few other compounds has been reported. It has also been used to treat all the prostate disorders like cancer, hypertrophy, prostatitis and also found to possess anti-inflammatory, analgesic and anti-androgenic activity *in-vivo*.

Oenothein B has been isolated from the pink willow herb (*E. parviflorum*) and has been identified as the active compound responsible for inhibiting 5α-reductase activity. Rose bay willow herb (*E. angustifolium*) extracts have also been shown to induce a marked inhibition of growth on prostatic epithelial cells.

XV. *Brassica rapa* L. Pollen

*Brassica rapa* pollen was found to reduce the volume of the prostate in rat model. SFE-CO$_2$ (supercritical CO$_2$ fluid extract) extract of the *Brassica rapa* exhibited remarkable bioactivity than other solvent extracts. Also, SFE-CO$_2$ extract showed potent 5α-reductase and aromatase inhibitory activity.

XVI. *Agathosma betulina* (Berg.) Pillans

*Agathosma betulina* (Berg.) Pillans leaf preparations (buchu) have traditionally been used as urinary tract disinfectant and diuretic. It is used in Africa to treat urinary tract infections as well as inflammation of the prostate. The urinary tract antiseptic actions of buchu are thought to be due to volatile oils and the anti-inflammatory effects due to flavonoids. The German Commission E monograph on buchu concludes that there is insufficient evidence to support the modern use of buchu for the treatment of urinary tract infections or inflammation as clinical trials using buchu remain to be performed.
**XVII. Pinus species**

Ethanolic extracts (50%) of Resina Pini of *Pinus* sp. showed potent 5α-reductase inhibitory against rat enzyme. The active constituent from fraction responsible for activity has been identified as abietic acid (20) which is a diterpene resin acid. Neoabietic acid (21) and Pimaric acid (22) were also found to be active against 5α-reductase enzyme suggesting that negatively charged anionic carboxyl group on the molecule is responsible for inhibitory activity.

**XVIII. Cimicifuga racemosa**

Aqueous/ethanolic extract of *Cimicifuga racemosa* (CR BNO1055) was found to inhibit proliferation of the human prostate cancer cell line LNCaP. Treatment of male rats with this extract resulted in significantly lower prostate weights while no effect was seen in orchidectomized (orx) animals thereby exhibiting potent 5α-reductase inhibitory activity. It might be useful in ageing men to prevent BPH as well as the development of prostate cancer.

![Chemical structures of abietic acid, neoabietic acid, and pimaric acid](image)

**XIX. Botulinum neurotoxin A (BoNT/A)**

Botulinum neurotoxin A (BoNT/A) intraprostatic injections reduces the prostate volume by the promotion of apoptosis and causes downregulation of α1<sub>adreno</sub>receptors thereby affecting both the static and dynamic component of BPH-related LUTS. Limited clinical trials demonstrated an easy and minimally invasive intraprostatic application of BoNT/A with a favourable safety profile and marked improvement in urinary flow rate and reduction in postvoid residual, prostate volume, and also prostate-specific antigen in some studies. However, the level of evidence is still low and further randomized controlled studies are mandatory.
XX. Coconut Oil

Coconut oil (CO), extracted from coconut (Cocos nucifera), is mainly composed of medium-chain fatty acids. Although the exact composition of coconut oil can vary according to the source, lauric acid has been shown to be its most abundant component.\textsuperscript{242} Coconut oil (400 and 800 mg kg\textsuperscript{-1}) administered orally for 14 days dose-dependently inhibited prostate enlargement induced by testosterone in rats. Higher concentrations of lauric and myristic acids in coconut oil have been attributed to its 5α-reductase inhibitory activity. Further experimental studies are required to explore coconut oil in men with BPH.\textsuperscript{243}

XXI. Red clover

The extract of \textit{Trifolium pratense} (red clover) has been used historically by Asians and Europeans as a medicinal herb. The formulation of red clover, extracted from red clover leaves and flowers, is well established and is a rich source of all four isoflavones: genistein, formononetin, daidzein and biochanin A. In a clinical trial it was found that urinary flow rates increased by 9.8%, International Prostate Symptom Score decreased by 23.3%, and quality-of-life score improved by 17%. It can be used in BPH but more trials are required.\textsuperscript{244}

XXII. Miscellaneous Formulations

i. Himplasia

\textit{Himplasia} is a polyherbal formulation which has proved beneficial in reducing the symptoms of prostatic hyperplasia. Himplasia contains \textit{Tribulus terrestris}, \textit{Caesalpinia bonducella}, \textit{Crataeva nurvala}, \textit{Areca catechu}, \textit{Asparagus racemosus} and \textit{Akika pishti}. It possesses both α-adrenoceptor antagonistic and 5α-reductase enzyme inhibitory activities. It relieves the symptoms of benign prostatic hyperplasia and reduces prostate weight. Himplasia also improved the urinary flow rate while reducing post-void residual urine. It was also found to inhibit prostatic stromal proliferation, reduction in the IPSS score and improvement in urodynamics associated with BPH.\textsuperscript{245}

ii. Chinese Zi-Shen Pill (ZSP)

\textit{Chinese Zi-Shen Pill} is an inventive preparation that can be used for preventing and relieving BPH and urination disturbances. It consists of three kinds of medicinal plants: \textit{Anemarrhena asphodeloides} Bge (Liliaceae, rhizome),
Introduction

Phellodendron amurense Rup. (Rutaceae, bark) and Cinnamomum cassia Presl (camphoraceae, bark) with a ratio of 10:10:1 in weight. ZSP is traditionally being used to treat BPH, prostatitis or frequent urination by the kidney-tonifying and pass dredging method and has got good effect.\(^{246}\)

Diethyl ether extract of common anemarrhena rhizome has testosterone 5α-reductase inhibitory activity. Moreover, Mangiferin, the major bioactive constituent of Anemarrhena asphodeloides Bge, has stimulant effect on TGF-β1, a cytokine inhibitor of angiogenesis.\(^{247}\) Hence, the inhibitory effects of ZSPE on BPH are the results of multitarget protective effects such as 5α-reductase, VEGF, bFGF and TGF-β1. Each of the major active chemical components of ZSPE such as mangiferin, neo-mangiferin, berberine and cinnamic acid should contribute to protective effects of ZSP on BPH.\(^{246}\)

iii. PC-SPES

It is an eight-herb Chinese formulation that consists of isatis (Isatis indigotica), liquorice (Glycyrrhiza glabra) or Gancoa (G. uralensis), Chinese skull cap (Scutellaria baicalensis), reishi (Ganoderma lucidum), saw palmetto, Asiaginseng (Panax ginseng), denodrantherm (Denodranthera morifolium) and rabdosia (Rabdosia rubescens). It is recommended as a food supplement for patients suffering from prostate disorders. It has been found to decrease the blood levels of prostate-specific antigen (PSA) in men. It has significant oestrogenic and antiandrogen effects, leading to an inhibitory action in the prostate.\(^{248,249}\) It has been found to cause symptoms of oestrogen excess including gynecomastia, nipple tenderness, loss of libido and impotency due to the significant content of warfarin and diethylstilbestrol. This product was recalled in 2002.

iv. Prostagutt forte

This is the combination product of saw palmetto and stinging nettle that has been tested against finasteride in a large scale clinical trial. Although it had no effect on prostate volume; finasteride reduced prostate volume by 15%. Both products were well tolerated, erectile dysfunction (2.8%) and reduced ejaculate volume (2%), however, were more frequent under Finasteride. The same combination (Prostaguttl forte) has been compared to tamsulosin in a 12-month prospective, randomized trial. The IPSS improved in both study arms by 9 points.\(^{250,251}\)
v. Bodyprost

Bodyprost is a 40% ethyl alcohol herbal extraction of *Echinacea purpurea*, *Rumex confertos* and *Corylus avellana*. The principal active substances of the formula are flavonoids (cempherole, quercetine, hyperizide and nipodine), free and bound amino acids, fatty acids, tannic substances (polycathechine and pirogalol groups), ether oil and tannins etc. Herbal formula Bodyprost is recommended for medical treatment of BPH stage I-II.252

vi. Eviprostat

Eviprostat is among the most widely used phytotherapeutic agent for lower urinary tract symptoms in BPH. Eviprostat consists of five components; four are extracted from *Chimaphila umbellata*, *Populus tremula*, *Pulsatilla pratensis* and *Equisetum arvense* and the fifth is germ oil from *Triticum aestivum*. *Chimaphila umbellata* has been found to contain hydroquinone glycosides such as isohomoarbutin, homoarbutin and naphthoquinone derivatives such as chimaphilin, which are known to have urinary antiseptic effects. *Populus tremula* contains salicylate glycosides such as salicin, which is converted in the body into salicylic acid with anti-inflammatory activities.

*Pulsatilla pratensis* has protoanemonin forming agents which changes to protoanemonine and anemonin. These compounds are known to have antipyretic, motility-inhibiting, and antibiotic effects.253 *Equisetum arvense* contains flavonoids such as ornitin, kaempferol-3-O-glucoside and quercetin-3-O-glucoside, and sterols such as β-sitosterol and campestester, which contribute to anti-inflammatory activities.254 Germ oil from *Triticum aestivum* contains high level of polyunsaturated fatty acids and 0.2–0.3% (w/w) of tocopherols (vitamin E). Several reports suggest that polyunsaturated fatty acids and tocopherols have beneficial effects in some inflammatory animal models. The precise mechanisms of action of eviprostat remain to be elucidated. Combination therapy with eviprostat and the α1-adrenergic blocker tamsulosin is even more effective, because of the combined effect of their different modes of action. The suppression of reactive oxygen species by components except germ oil may partly contribute to the anti-inflammatory action of eviprostat and this action may be helpful in its therapeutic effect on BPH.253
4. 5α-Reductase Inhibitors

History and Development of 5α-Reductase Inhibitors

Revolution in prostate treatments was achieved in 1954 following the development of drugs that act as competitive inhibitors of 5α-reductase, when steroid 5α-reductase inhibitory activity was demonstrated in rabbit liver homogenate by paper chromatography.

Two years later, 17β-hydroxy-5α-androstan-3-one, or 5α-dihydro-testosterone (DHT), was first identified as the major metabolite of the incubation of testosterone (T) with rat liver homogenate. Both independent experiments indicated the presence of 5α-reductase (NADPH: 4-en-3-oxosteroid 5α-oxidoreductase, EC 1.3.99.5; 5α-Reductase) in the liver tissues of rabbits and rats.255

In 1968, 5α-reductase activity was measured in rat prostate which was also identified as a target of dihydrotestosterone (DHT) stimulation. Subsequently 5α-reductase activity was measured and DHT was identified as predominant androgen in prostate.

In 1974, male pseudohermaphroditism, an autosomal recessive form was shown to result from 5α-reductase deficiency. The discovery of the Dominican “guevedoces” helped lead the transformation of urology from a purely surgical specialty into a discipline centered on effective drug therapy and minimally invasive treatments.256 “Guevedoces” is the term local folk applied to the biological males born in an isolated village of the Dominican Republic with female appearing external genitalia, who then develop typical male genitalia at puberty. The explanation is a congenital deficiency of the enzyme 5α-reductase.257

Throughout life, the prostate in the guevedoces remains small. The chief legacy of the guevedoces is a class of drugs known as 5α-reductase inhibitors (5ARIs), the first of the “prostate pills”.258 This disorder, also known as pseudovaginal perineoscrotal hypospadias, or type 2 familial male pseudohermaphrodites, is characterized by a 46XY karyotype, female phenotype at birth, and virilization at puberty. The affected males are born with a blind-ending vaginal pouch, a clitoral phallus, inguinal testes, and are reared as females. At puberty, they develop a deep voice, increased muscle mass, enlargement of the phallus, and do not develop menses or breast enlargement. Later in adulthood, the prostate remains small and beard is absent. Acne, male pattern baldness, and
prostatic enlargement are not observed. Biochemical investigations revealed normal serum testosterone, markedly diminished serum DHT, decreased 5α-reduction of serum testosterone to DHT and deficient 5α-reductase activity in external genital tissues. Investigators concluded that testosterone is required for differentiation of embryonic mesonephric duct structures and that DHT is required for differentiation of the embryonic urogenital sinus and tubercle into male external genitalia. Furthermore, male psychosexual development and virilization at puberty are mediated by testosterone, whereas adult prostatic growth and male pattern baldness are mediated by DHT.\textsuperscript{257}

In normal male development and growth the DHT target tissues convert testosterone to DHT by microsomal 5α-reductase. Adult features of type 2 familial male pseudohermaphroditism, such as lack of prostate growth and male pattern baldness, provided a rational model for development of 5α-reductase inhibitors to treat these conditions.\textsuperscript{259-260}

**Enzyme 5α-Reductase**

A significant correlation between the androgens and prostate is well known. Testicular androgens constitute the most important mitogenic factor \textit{in vivo} for the prostate.\textsuperscript{261} Normal circulating levels of androgens are required for the maintenance of structural function, growth and integrity of the prostate tissue. However, androgens have no direct effect on prostatic epithelial cells in culture.\textsuperscript{262} Androgens enhance the production of many growth factors in the prostate tissue \textit{in vivo} through a complex cell to cell interaction involving both epithelial and stromal prostatic cells.\textsuperscript{263} Androgen signalling cascade involves the synthesis of T (23) in testes and adrenal glands which gets peripherally converted to DHT (24). DHT formed gets transferred to the target tissues and binds to the target receptor with consequent modulation of gene expression.\textsuperscript{264}

Both T and DHT bind to and activate the androgen receptor (AR), but DHT shows a higher affinity leading to different kinetic processes. DHT dissociates from AR protein much more slowly than its precursor. Therefore, at a given time ARs are occupied by DHT much more than by testosterone. T is converted to DHT by a steroid 5α-reductase enzyme (3-oxo-steroid-4-ene dehydrogenase \{E.C. 1.3.99.5\}) which is a system of two membrane bound nicotinamide dinucleotide phosphate (NADPH) dependent enzymes at the level of prostatic stromal and basal cells. This
has led to the development of steroidal and non steroidal 5α-reductase inhibitors as they inhibit the conversion of \( T \) (23) to DHT (24) as shown in Figure 9.\(^{265-267} \) Thus 5α-reductase dictates the cellular availability of DHT to prostatic epithelial cells and consequently modulates its growth.

![Diagram of testosterone and dihydrotestosterone conversion](image)

**Figure 9: Site of action of 5α-reductase inhibitors**

**Types of 5α-Reductase Enzymes**

Modern methods of molecular biology had assisted in identifying two types of 5α-reductase enzyme - type I and type II from human and rat prostatic complimentary deoxyribonucleic acid (cDNA) libraries and the structures of both genes were elucidated at the beginning of this decade.\(^{268-269} \) The type I enzyme is not the major species expressed in the prostate and is present mainly in the hair follicles and peripheral skin whereas type II 5α-reductase is the major isozyme in genital tissues and a deletion in the gene leads to male pseudohermaphroditism.\(^{270-271} \) Type I enzyme is constitutively expressed in the brain and in adulthood appears mainly localized in the myelin membranes and has a catabolic rather than an activating role in the brain while type II enzyme is transiently expressed in the prenatal period and in males its expression is controlled by androgens and appears to be confined in the hypothalamus and in the hippocampus after stress hence type II enzyme might participate in the perinatal differentiation of brain towards a male pattern.\(^{272} \) The two isozymes differ in the constitution of amino acids as well as molecular weight. The type I isozymes is active at pH 6.0-8.5 while type II is active at pH 5.0-5.5 (Table 4). The two isozymes also differ in the location of the gene structure while type I is located at 5p15 while type II is located at 2p22 although they had same gene structure.\(^{273-274} \)
### Table 4: Comparison of properties of 5α-reductase isozymes

<table>
<thead>
<tr>
<th>Properties</th>
<th>Type I 5α-reductase</th>
<th>Type II 5α-reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>259 amino acids</td>
<td>245 amino acids</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>29,462 Daltons</td>
<td>27,000 Daltons</td>
</tr>
<tr>
<td>Optimal pH</td>
<td>6.0-8.5</td>
<td>5.0-5.5</td>
</tr>
<tr>
<td>Biochemical properties</td>
<td>Hydrophobic</td>
<td>Hydrophobic</td>
</tr>
<tr>
<td>Gene location</td>
<td>SRD5A1, 5p15</td>
<td>SRD5A2, 2p23</td>
</tr>
<tr>
<td>Gene properties</td>
<td>5 exons, 4 introns</td>
<td>5 exons, 4 introns</td>
</tr>
<tr>
<td><em>In vitro</em> inhibition by Finasteride</td>
<td>$K_i \geq 300 \text{ nM}$</td>
<td>$K_i = 3-5 \text{ nM}$</td>
</tr>
<tr>
<td>Localization (in tissues)</td>
<td>Sebaceous glands of the skin, sweat glands, dermal papilla cells fibroblasts from all areas</td>
<td>Prostate, genital skin, epididymis, seminal vesicles</td>
</tr>
<tr>
<td>Selectivity to the inhibitors</td>
<td>Inhibitors with 4-methyl-4-aza functionality are very potent.</td>
<td>4-aza, 6-aza and charged 3-substituents derivatives are highly selective.</td>
</tr>
</tbody>
</table>

More recently with the development of genome-wide gene expression profile analyses a third type of 5α-reductase enzyme (type III) has been identified in hormone-refractory prostate cancer cells (HRPC). This enzyme also converts T to DHT in HRPC cells in a similar way to type I enzyme and was found to be active at pH 6.9.

Type III isozyme has been recognized as a ubiquitous enzyme in mammals. Northern blot and real time RT-PCR analyses have identified this enzyme in androgen and non-androgen target human tissues such as pancreas, brain, prostate cancer cell lines, skin and adipose tissues.
Mechanism of Action of 5α-Reductase

Chemical Mechanism

The proposed chemical mechanism of T (23) reduction to DHT (24) by 5α-reductase catalysis, based on the known regio and stereochemistry of reduction involves the formation of a binary complex between the enzyme and NADPH, followed by the formation of a ternary complex with the substrate T (23). A delocalized carbocation is formed due to the activation of the enone system by a strong interaction with an electrophilic residue (E+) present in the active site. Enolate of DHT is formed by the direct hydride transfer from NADPH to α face of the delocalized carbocation leading to a selective reduction at C-5. This intermediate, which is presumably coordinated with NADP⁺ on the α-face, is attacked by the proton on the β-face at C-4 giving the ternary complex E-NADP⁺-DHT. Then the departure of DHT gives binary NADP⁺-Enzyme complex and finally the release of NADP⁺ leaves the enzyme free for future catalytic cycles.274 (Figure 10)

Kinetic Mechanism

The kinetic mechanism of testosterone reduction is presented in the Figure 11. It involves the formation of a complex between enzyme and NADPH, followed by complex with substrate testosterone.32,278,279

There are three types of inhibitors interacting with enzyme complexes:

a) Type A: Inhibitors that compete with cofactor NADPH and the substrate testosterone i.e. bisubstrate

b) Type B: Compounds that bind reversibly to NADPH enzyme complex by being competitive with natural substrate testosterone i.e. competitive inhibitors and fails to turn over rapidly

c) Type C: Inhibitors fitting the enzyme-NADP⁺ complex should be uncompetitive versus the substrate
Enz = enzyme; E' = electrophile or electrophilic site; Nu = nucleophile or nucleophilic site; R = adenine dinucleotide phosphate (ADP).

**Figure 10: Chemical mechanism of 5α-reductase enzyme**

**Figure 11: Kinetic mechanism of 5α-reductase catalysis**

The design of new 5α-reductase inhibitors of type-B is based on the concept of transition state (T.S.) of the enzymatic process. The enzyme binding should be greater for compounds that mimic the transition state of the enzymatic process, and thus result in higher inhibition. Accordingly, two possible transition states have been postulated (Figure 12):
Introduction

DHT (24)

Figure 12: Transition States of 5α-reductase Inhibitors

The "substrate-like" TS in which the hybridization of C-3, C-4 and C-5 are similar to those of intermediate, and "product like" TS in which the hybridization of C-3, C-4 and C-5 are similar to those of the enol form of DHT.278, 279

More potent inhibitors of steroid 5α-reductase have been found among the transition state analogues as molecules mimicking the transition state of the enzymatic processes exhibit a greater binding to the enzyme and hence produce greater inhibition. The enzyme 5α-reductase binds the 3-keto-Δ^4 steroids in such a way that the carbonyl group is brought into vicinity of a positively charged centre on the enzyme whereby the conjugated ketone becomes activated as shown in Figure 13.

Figure 13: 5α-Reduction of 3-keto-Δ^4-steroids
A hydride ion can then be transferred from the coenzyme NADPH to the 5α-position of the steroid. The resulting enolate is protonated at the axial 4β-position by the solvent and the product is released. This evidence for protonation was based on the model studies with the *Penicillium decumbens* 5α-reductase enzyme.280

**Purification of 5α-Reductase**

Purification of 5α-reductase directly from any mammalian species has not succeeded as the enzyme had to be stabilized by various cofactors and solubilized by detergents, which inactivate 5α-reductase in most cases. However, some scientists like Moore and Wilson had partially purified enzyme from rat prostate and activity was maintained for four days by either glycerol or NADPH and the NADPH-stabilized enzyme was purified 90-fold.281

In 1990, Levy *et al.* prepared rat liver 5α-reductase, the supernatant aliquots of which contained more than 80% of the microsomal enzyme activity and could be stored at -80°C without loss of enzymatic activity for several months.279

5α-Reductase inhibitors are discussed below as steroidal and nonsteroidal inhibitors:

**Steroidal 5α-Reductase Inhibitors**

As the only information available about the 5α-reductase isozymes is their primary sequence estimated from c-DNAs the design of novel inhibitors is affected. Due to the unstable nature of enzyme during purification its crystal structure is not known. The first inhibitors have been therefore, designed by modifying the structure of natural substrates, including the substitution of one carbon atom of the rings of the steroids by a heteroatom such as nitrogen thereby forming azasteroids. Singh and co-workers6,7 as well as other groups8 have published comprehensive reviews on biological activity of azasteroids.

Azasteroidal compounds having nitrogens at various positions have also been covered in this review. However, their 5α-reductase inhibitory activity has either not been done or they are devoid of activity. Some azasteroids have been found to be 5α-reductase inhibitors. In the following section azasteroidal inhibitors have been discussed depending upon the position of nitrogen in the steroidal nucleus i.e. nuclear azasteroids.
2- and 3-Azasteroids

Doorenbos and Wu\textsuperscript{282} and Mazur\textsuperscript{283} in the early sixties synthesized some of the 3-azasteroids but Anderson and Liao in 1968 reported for the first time that steroidal N-oxido-3-aza-1,3,5(10)-triene is a good inhibitor of enzyme 5α-reductase.\textsuperscript{284} Haffner in 1994, reported the synthesis of some novel 3-pyridyl-N-oxide steroids (25, 26) which mimic the enolate or enol like transition state of the enzyme-substrate complex.\textsuperscript{285}

![Chemical Structures (25-32)]

N-Oxide steroids (25) and (26) were assayed against both type I and type II 5α-reductase and proved to be potent inhibitors of type II 5α-reductase with the Ki (μM) being 0.031 and 0.104, respectively.

In 2003, Robinson and co-workers reported the synthesis of various 2- and 3-azasteroidal derivatives (27-32) as effective and stable transition state 5α-reductase inhibitors.\textsuperscript{286}
Introduction

All the synthesised 2- and 3-azasteroids (27-32) were evaluated for human 5α-reductase inhibition. Amines (28 and 32) showed poor inhibitory activity against both type I and type II isozymes whereas lactams (27 and 31) displayed only marginal improvement against type II isozymes. However, nitrones (29 and 30) showed significant enhancement in biological activity.

4-Azasteroids

One of the extensively studied and clinically used classes of azasteroidal 5α-reductase inhibitors is that of 4-Azasteroids. Voigt et al. in 1970, screened a large number of steroids including 23 steroidal hormones for their ability to inhibit the conversion of T (23) into DHT (24) by a crude cell free enzyme system isolated from rat ventral prostate. In 1973, series of effective 5α-reductase inhibitors were synthesized and evaluated. The key structural requirements for the 5α-reductase inhibitory activity were found out to be the presence of 4-en-3-one function and 17β-side chain having one or more oxygen functionalities. Molecules possessing these features act as competitive inhibitors of testosterone 5α-reductase, therefore, all of them could be regarded as a substrate of the enzyme 4-en-3-one steroids. 4-Androsten-3-one-17-carboxylic acid (33) was identified as a potent inhibitor of 5α-reductase.

![Structure of 4-Androsten-3-one-17-carboxylic acid (33)](image)

It has been reported to be a competitive inhibitor of the enzyme and showed 87.7% inhibition for the microsomal enzyme of human skin. None of the compounds from this series could be shown to interfere with in vivo conversion of the dihydrotestosterone, because of their rapid conversion into the inactive 4,5-dihydro form by the enzyme.

In 1980, Merck and Co. while searching for a nonreducible inhibitor of 5α-reductase, reported series of 4-azasteroids where C-4 of 3-oxo-5α-steroids was replaced by nitrogen. The studies showed that there was not only an increase in the
5α- reductase inhibitory activity but also retention of the in vivo activity. Therefore, azasteroids were designed to mimic the putative enzyme bound enolate intermediate by incorporating sp²-hybridized center at C-3 and C-4. Thus a lactam was introduced in the ring A of the steroids to mimic the enol transition state of the enzyme-NADPH-substrate (E.NADPH.S) complex. Substitution at C-17 has been found to enhance potency by binding to a lipophilic pocket on the enzyme. These competitive inhibitors strongly interact with the enzyme at the active site and on other hand unlike the substrate cannot be further reduced to 5α-metabolites thus have in vivo inhibitory activity. The steroidal pharmacophore provides an anchor between the key A-ring lactam and the C-17 substituent while the former acts as a transition state mimic of intermediate enolate, the latter significantly enhances potency via binding at a pocket largely lipophilic in nature. The key 4-aza-3-oxo-5α-androstane pharmacophore and the basic structure activity relationship (SAR) is outlined below (Figure 14)  

Taking into consideration that substitution at C-17β- could dramatically affect the potency; a large number of modifications were carried out to find potent inhibitors. 4-MA {17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one} (34) was found to be a potent dual inhibitor of both human 5α-reductase isozymes having IC₅₀ value of 1.9 nM against human 5α-reductase II and 1.7 nM against human 5α-reductase I, however, it was withdrawn from the clinical developments due to hepatic toxicity and lack of selectivity over 3β-hydroxy steroid dehydrogenase enzyme. Out of the series its unsaturated analogue 17β-(N-tert-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one, MK-906, Finasteride (35) was found to be the best and extensively studied. Finasteride (35) was a potent inhibitor of 5α-reductase type II with only
weak in vitro activity versus 5α-reductase type I having IC\textsubscript{50} value of 9.4 and 410 nM, respectively. At clinical dose, 5 mg/day, it caused 65-80% lowering of plasma DHT levels.\textsuperscript{299} Finasteride (35) was the first drug to be approved in U.S. for BPH.

![Chemical structures of 4-MA (34) and MK-906(35) Finasteride]

Long-term studies have demonstrated that there is a sustained improvement in BPH disease and reduction in the prostate specific antigen (PSA) level.\textsuperscript{300}

Merck as well as Glaxo reported in 1996 that Finasteride (35) and close analogues are mechanism based inactivators of 5α-reductase II. Although it is accepted as an alternate substrate and is ultimately reduced to dihydrofinasteride (37), this proceeds through an enzyme-bound NADP-dihydrofinasteride adduct. Initially it was believed that Finasteride (35) act as a transition state mimic whereby confirmation of the A-ring lactam closely mimics the enol form of transition state of 5α-reduced testosterone but now it is understood that the most likely cause of the slow offset inhibition is rate-limiting hydride transfer from NADPH to the Δ\textsuperscript{1}-double bond of Finasteride (35). In the case of the 1,2-ene-containing Finasteride (35), reduction of C-1 enables the nucleophilic attack of C-2 on the nicotinamide C-4. This aberrant reduction results in the formation of lactam enolate which is not positioned for efficient protonation by the enzyme. Instead the enolate is trapped by the electrophilic pyridinium cation of the NADP, yielding a covalent adduct to the co-factor and to the protein (Figure 15).

This dihydrofinasteride-NADP adduct is a remarkably potent bisubstrate analog inhibitor and it binds to the free enzyme with a second-order rate constant equal to \( k_{cat}/K_m \) for turnover of T (23) and has a dissociation constant \( K_i \leq 1 \times 10^{-13} \) M. Finasteride (35) is also a mechanism-based inhibitor of the human skin (type I) isozyme, but it is processed with a much smaller second-order rate constant, \( k/K_i = 3 \times 10^3 \text{ M}^{-1} \text{ s}^{-1} \), which attenuates its activity against this isozyme in vivo. Indeed,
Merck has demonstrated the presence of (36c) in the inhibited form of 5α-reductase type I. \(^{301-303}\)

Weintraub et al. in 1985 reported 20-(hydroxymethyl)-4-methyl-4-aza-2-oxa-5α-pregnan-3-ones and their corresponding 3-thiones (38-41). These compounds were tested \textit{in vitro} for inhibition of testosterone 5α-reductase and were found to be weak inhibitors with \(K_i\)'s in the \(10^{-7}\) range. It was argued that replacement of C-2 in the steroid nucleus by oxygen in the case of 4-aza-3-oxo-steroids would convert it to a urethane (39) from a lactam (38), respectively, thereby enhancing the polarity at C-3 carbonyl, and its affinity for the enzyme active site. \(^{304}\)

![Figure 15: Mechanism of Finasteride inhibition of 5α-Reductase](image-url)

\(P=\text{Phosphoadenosine diphosphoribose}\)

\(R_1=\text{CONHC(CH}_3\text{)3}\)

Ref: Bull et al. JACS 1996
Bakshi and co-workers reported dual inhibitors of human type I and type II steroid 5α-reductases in the form of a series of 4-aza-3-oxo-5α-androstene-17β-N-aryl-carboxamides. Some of these compounds were found to be potent inhibitors of both isozymes. Variation of the C-17 amide substituent on the 4-aza-3-androstane skeleton has resulted into a fruitful search of the potent dual azasteroid inhibitors (42-48).

\[
\begin{align*}
(42) & \quad R_1=C_6H_5, \quad R_2=H \\
(43) & \quad R_1=C_6H_5, \quad R_2=CH_3 \\
(44) & \quad R_1=C_6H_5, \quad R_2=C_6H_5 \\
(45) & \quad R_1, \quad R_2= 1\text{-indoliny}l \\
(46) & \quad R_1=\text{2-CF}_3C_6H_4, \quad R_2=H \\
(47) & \quad R_1=3-C_6H_5C_6H_4, \quad R_2=H \\
(48) & \quad R_1=1\text{-naphthy}l, \quad R_2=H
\end{align*}
\]

Dutasteride, (GG745), 17β-N-{2,5-bis(trifluoromethyl)phenyl})-3-oxo-4-aza-5α-androst-1-ene-17-carboxamide (49) had emerged as the most potent dual inhibitor from this group (Table 5). It has been approved by U.S. FDA in 2002, for the symptomatic treatment of BPH. Unlike Finasteride (35), Dutasteride (49) is a competitive inhibitor of both 5α-reductase type I and 5α-reductase type II isozymes, reduced dihydrotestosterone levels >90% following one year oral administration. It is also a time dependent inhibitor as Finasteride (35) and it forms a stable complex with a slow rate of dissociation constant and does not bind to the androgen receptor. By reducing DHT level, it reduces the size of enlarged prostate, so improving the urinary flow rate. It is about 60 times more potent than Finasteride (35) and has been shown to decrease the risk of acute urinary retention.

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh
and BPH related surgery.\textsuperscript{311,312} This greater degree of suppression of serum DHT has been found to correlate with the intraprostatic DHT suppression. Dual inhibition of 5α-reductase is more beneficial than selective type II inhibition as dual inhibition doesn’t allow the escape of DHT which can formed through type I mediated synthesis thus providing greater efficacy as DHT levels are suppressed to a great extent.

Table 5: \textit{In vitro} screening of compounds 42-49 against type I and II human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Human 5α-reductase type I IC\textsubscript{50} (nM)</th>
<th>Human 5α-reductase type II IC\textsubscript{50} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>20</td>
<td>0.2</td>
</tr>
<tr>
<td>43</td>
<td>350</td>
<td>24.6</td>
</tr>
<tr>
<td>44</td>
<td>&gt;1000</td>
<td>25.2</td>
</tr>
<tr>
<td>45</td>
<td>120.2</td>
<td>0.4</td>
</tr>
<tr>
<td>46</td>
<td>5.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>47</td>
<td>14.0</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>48</td>
<td>8.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Dutasteride (49)</td>
<td>2.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>52</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

Long term studies have shown that Dutasteride (49) a dual inhibitor is well tolerated during daily use for up to 2 years. It had a tolerability profile comparable to that of placebo with the exception of a modestly elevated incidence of impotence and decreased libido compared with placebo.

Also Dutasteride did not clinically significantly impact bone metabolism markers, bone mineral density or lipid levels.\textsuperscript{313}
A series of C-17-acylurea-substituted 4-azasteroids (50-55) (Table 6) were synthesized by Di Salle et al. in early 1990’s in a programme aimed at searching for novel 5α-reductase inhibitors exploiting the tolerance of functionality at this position. Significantly greater potency was found with the derivative containing C-4 methyl group and a saturated A ring.314

(50) \( R_1 = \text{C}_6\text{H}_{11}, R_2 = \text{C}_6\text{H}_{11}, R_3 = \text{CH}_3 \)

(51) \( R_1 = t\text{-Bu}, R_2 = t\text{-Bu}, R_3 = \text{CH}_3 \)

(52) (Turosteride) \( R_1 = i\text{-Pr}, R_2 = i\text{-Pr}, R_3 = \text{CH}_3 \)

(53) \( R_1 = i\text{-Pr}, R_2 = i\text{-Pr}, R_3 = \text{H} \)

(54) \( R_1 = i\text{-Pr}, R_2 = i\text{-Pr}, R_3 = \text{CH}_3, \Delta^{1(2)} \)

(55) \( R_1 = i\text{-Pr}, R_2 = i\text{-Pr}, R_3 = \text{H}, \Delta^{1(2)} \)

One of the most potent compound of this group, Turosteride, (52), a close analogue of 4-MA (34), but unlike 4-MA was found to be devoid of binding at the rat androgen receptor and a weak inhibitor of 3β-hydroxy steroid dehydrogenase. 315

Table 6: In vitro screening of compounds 50-55 against human and rat prostatic 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Human 5α-reductase</th>
<th>Rat 5α-reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC_{50} (nM)</td>
<td>IC_{50} (nM)</td>
</tr>
<tr>
<td>50</td>
<td>41</td>
<td>83</td>
</tr>
<tr>
<td>51</td>
<td>212</td>
<td>-</td>
</tr>
<tr>
<td>Turosteride (52)</td>
<td>55</td>
<td>53</td>
</tr>
<tr>
<td>53</td>
<td>381</td>
<td>227</td>
</tr>
<tr>
<td>54</td>
<td>1218</td>
<td>1611</td>
</tr>
<tr>
<td>55</td>
<td>1553</td>
<td>1154</td>
</tr>
<tr>
<td>4-MA (34)</td>
<td>28</td>
<td>37</td>
</tr>
</tbody>
</table>
Introduction

Other azasteroids which retained 5α-reductase inhibitory activity are 2-substituted (56), A-homo- (57) and 19-nor- (58) analogues.

Various 17β-(N-ureylene-N,N’-disubstituted)-4-methyl-4-aza-3-one as potent 5α-reductase derivatives were synthesized based on the observation that selectivity of inhibitors can be increased against type I isozyme by making correct choice of hydrophobic substituent at C-17 position led to development of Table 7 (59-64) as 5α-reductase inhibitors as they have potent selectivity against 5α-reductase type I enzyme.

Azasteroids with N-cyclopropyl ring exhibit potent inhibitory activity against type I 5α-reductase. Increase in the chain length from N’-ethyl to N’-butyl the compound showed strong inhibitory activity while branching of alkyl chain decreased potency of compounds and introduction of 1,2-double bond significantly reduced the activity. Replacement of N’-alkyl chain with phenyl moiety gave the most active compound (62) of the series.

(59) R₁= Cyclopropyl, R₂=C₂H₅
(60) R₁= Cyclopropyl, R₂=C₄H₉
(61) R₁= Cyclopropyl, R₂=C₄H₉, Δ¹(2)
(62) R₁= Cyclopropyl, R₂=C₆H₅
(63) R₁= CH₃, R₂= Cyclohexyl
(64) R₁= C₄H₉, R₂= Phenyl
Table 7: *In vitro* screening of compounds 59-64 against type I and II human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC$_{50}$(nM) or % inhibition at 100nM (given in parenthesis)</th>
<th>Human 5α-reductase type I (transfected 293 cells)</th>
<th>Human 5α-reductase type II (transfected SW-13 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
<td>27.3±3.4 (20±3.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>5.3±1.1</td>
<td>(46.3±1.3)</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>24.2±1</td>
<td>(4.9±0.2)</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>1.3±0.64</td>
<td>(56.3±4.5)</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>31.5±6.0</td>
<td>(48.7±4.2)</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>11.5±1.9</td>
<td>(39.7±0.4)</td>
<td></td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>262±43.2</td>
<td>8.5±0.4</td>
<td></td>
</tr>
</tbody>
</table>

A number of 17β-(N-alkyl/aryl formamido) (65-70) and 17β-[(N-alkyl/aryl)alkyl amido]-3-oxo-4-aza-5α-steroids (71-75) were prepared and evaluated as 5α-reductase inhibitors by Li et al. from 17β-hydroxy-4-aza-steroids on the basis of the studies that 17β-carboxamides at C-17 position have pronounced effect on the activity of 5α-reductase and molecules possess androgen receptor activities (Tables 8 and 9). Structure activity relationship indicated that 5α-reductase type I enzyme has preference for N-substituted linear alkyl side chain of 4-5 carbon atoms. N-Amyl substituted 17β-formamide (76) was found to be one of the most promising inhibitor of 5α-reductase type I while N-heptyl (69) and N-octyl (70) showed dual inhibition of both isozymes of 5α-reductase (Table 8).

![Chemical structures](image-url)
### Table 8: In vitro screening of compounds 65-70 against type I and II human 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I (IC₅₀(nM))</th>
<th>Type II (IC₅₀(nM))</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>3.05±0.296</td>
<td>&gt;100</td>
</tr>
<tr>
<td>66</td>
<td>0.91±0.236</td>
<td>&gt;100</td>
</tr>
<tr>
<td>67</td>
<td>2.19±0.476</td>
<td>&gt;100</td>
</tr>
<tr>
<td>68</td>
<td>2.35±0.421</td>
<td>&gt;100</td>
</tr>
<tr>
<td>69</td>
<td>9.57±1.745</td>
<td>14±1.11</td>
</tr>
<tr>
<td>70</td>
<td>16.9±3.911</td>
<td>18.4±1.541</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>26.3±4.784</td>
<td>4.53±0.96</td>
</tr>
</tbody>
</table>

Similarly in series of 17β-[(N-alkyl/aryl) alkyl] arylamido] derivatives (Table 9) exhibited highly potent inhibitory activity for human 5α-reductase type I.\(^{317}\)

![Chemical structure](image)

(71) \(R_1= (\text{CH}_2)_4\text{CH}_3, R_2= (\text{CH}_2)_3\text{CH}_3\)  
(72) \(R_1= (\text{CH}_2)_4\text{CH}_3, R_2= (\text{CH}_2)_3\text{CH}_2\text{Br}\)  
(73) \(R_1= \text{C}_6\text{H}_5, R_2= \text{C}_6\text{H}_5\)  
(74) \(R_1= \text{C}_6\text{H}_5, R_2= \text{CH} (\text{CH}_3)_2\)  
(75) \(R_1= 4-\text{CH}_3\text{OC}_6\text{H}_4, R_2= \text{CH} (\text{CH}_3)_2\)

Table 9: In vitro screening of compounds 71-75 against type I and II human 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I (IC₅₀(nM))</th>
<th>Type II (IC₅₀(nM))</th>
</tr>
</thead>
<tbody>
<tr>
<td>71</td>
<td>1.77±0.343</td>
<td>1000&gt;IC₅₀&gt;100</td>
</tr>
<tr>
<td>72</td>
<td>2.42±0.409</td>
<td>1000</td>
</tr>
<tr>
<td>73</td>
<td>2.93±2.158</td>
<td>3.75±1.977</td>
</tr>
<tr>
<td>74</td>
<td>10.5±2.739</td>
<td>582</td>
</tr>
<tr>
<td>75</td>
<td>5.44±1.067</td>
<td>1000&gt;IC₅₀&gt;100</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>26.3±4.784</td>
<td>4.53±0.96</td>
</tr>
</tbody>
</table>

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Various 7β-substituted derivatives (76-81) have also been prepared. Preliminary screening of the compounds as inhibitors of 5α-reductase from human scalp and prostate revealed that the presence of 7β-methyl substitution in ring B, presence of cholesterol type side chain at C-17 and ketone functionalities at C-3 in 4-azasteroids resulted in potent selective inhibitor against 5α-reductase type I.318,4,7β-Dimethyl-4-aza-5α-cholestan-3-one (MK 386) (82) emerged as one of the most potent inhibitor of type I 5α-reductase (Table 10).319

![Chemical structures](image)

(76-81) \hspace{1cm} MK 386 (82)

(76) \( R_1 = \text{CH}_3, R_2 = \text{H} \) \hspace{1cm} (77) \( R_1 = \text{CH}_3, R_2 = \text{C}_2\text{H}_5 \)
(78) \( R_1 = \text{H}, R_2 = \text{CH}_3, \Delta^{1(2)} \) \hspace{1cm} (79) \( R_1 = \text{CH}_3, R_2 = \text{CH}_3, \Delta^{1(2)} \)
(80) \( R_1 = \text{CH}_3, R_2 = \text{CH}_3, \Delta^{5(6)} \) \hspace{1cm} (81) \( R_1 = \text{CH}_3, R_2 = \text{n-Pr}, \Delta^{5(6)} \)
(82) \( R_1 = \text{CH}_3, R_2 = \text{CH}_3 \)

**Table 10: In vitro screening of compounds 76-82 against type I and II human 5α-reductase**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC_{50}(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type 1</td>
</tr>
<tr>
<td>76</td>
<td>1.7</td>
</tr>
<tr>
<td>77</td>
<td>5.7</td>
</tr>
<tr>
<td>78</td>
<td>1.6</td>
</tr>
<tr>
<td>79</td>
<td>2.0</td>
</tr>
<tr>
<td>80</td>
<td>0.6</td>
</tr>
<tr>
<td>81</td>
<td>8.4</td>
</tr>
<tr>
<td>MK-386 (82)</td>
<td>0.9</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>52</td>
</tr>
</tbody>
</table>

*University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh*
During 1995, some 17β-hydroxy-17α-(ω-hydroxy/haloalkynyl)-4-methyl-4-aza-3-oxo-5α-androst-1-ene-3-ones were synthesised by X. Li et al. and their antiandrogenic activity was reported. Salle and co-workers have reported synthesis and 5α-reductase inhibitory properties of various 4-azasteroids with fluoro substituted 17β amidic side chains and further investigated FCE 27837 (N-[1,1,1-trifluoro-2-oxobut-3-yl]-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamid-e) (83), for its endocrinological properties in comparison with those of Finasteride (35) (Table 11).

![FCE 27837 (83)](image)

**Table 11: In vitro screening of compound 83 against human and rat 5α-reductase inhibition**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC_{50} (nM)</th>
<th>Human</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCE 27837 (83)</td>
<td>51(3)</td>
<td></td>
<td>60(3)</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>51(6)</td>
<td></td>
<td>32(5)</td>
</tr>
</tbody>
</table>

*Number of assays in parentheses

Labrie and associates synthesized several steroids having lactam in ring A and substitution at 17β position in order to have specific and dual inhibitors of 5α-reductase. Several of the compounds were found active (Tables 12 and 13).
### Table 12: *In vitro* screening of compounds 84-87 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Human 5α-reductase inhibition Ki(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>84</td>
<td>2.6</td>
</tr>
<tr>
<td>85</td>
<td>1.8</td>
</tr>
<tr>
<td>86</td>
<td>2.2</td>
</tr>
<tr>
<td>87</td>
<td>5.1</td>
</tr>
</tbody>
</table>

(88) $R_1=\text{Cyclo C}_3\text{H}_5$, $R_2=\text{CH}_3$

(89) $R_1=\text{Cyclo C}_3\text{H}_5$, $R_2=\text{C}_6\text{H}_5$

(90) $R_1=\text{Cyclo C}_3\text{H}_5$, $R_2=\text{Cyclo C}_6\text{H}_{11}$

### Table 13: *In vitro* screening of compounds 88-90 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Human 5α-reductase inhibition Ki(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>88</td>
<td>0.5</td>
</tr>
<tr>
<td>89</td>
<td>2.3</td>
</tr>
<tr>
<td>90</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Several 17β-substituted 4-aza-5α-androstan-3-one carboxamides with unsaturation between C-1 and C-2 were synthesized by Panzeri and co-workers (91-93). These were found to be highly potent against human 5α-reductase enzyme (Table 14).
(91) $R_1 = H$, $R_2 = CH_3$, $R_3 = H$, $Z = CF_3$  
(92) $R_1 = H$, $R_2 =$ Isopropyl, $R_3 = H$, $Z = CH_3$  
(93) $R_1 = H$, $R_2 = CH_3$, $R_3 = CH_3$, $Z = CF_3$

Table 14: *In vitro* screening of compounds 91-93 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Human 5α-reductase IC$_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>91</td>
<td>16</td>
</tr>
<tr>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>93</td>
<td>14</td>
</tr>
</tbody>
</table>

In 1996, Giudici et al. reported the synthesis of FCE 28260 (94) [(22R,S)-N-(1,1,1-trifluoro-2-phenylprop-2-yl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide] (Table 15) as a potent dual inhibitor of both 5α-reductase isozymes and it was found to cause 74% reduction in the DHT levels.  

(94)

Table 15: *In vitro* screening of compound 94 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compound</th>
<th>Type I (IC$_{50}$ (nM))</th>
<th>Type II (IC$_{50}$ (nM))</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>36±9</td>
<td>3.3±1.2</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>470±41</td>
<td>8.5±1.2</td>
</tr>
</tbody>
</table>
CIBA-GEIGY Ltd. reported the synthesis of CGP53153 (N-(2-cyano-2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide) (95) (Table 16), a novel inhibitor of 5α-reductase and structurally related to Finasteride (35) was found to be 10 times more potent than Finasteride in reducing prostate weight of rat.326

![Diagram of compound 95]

Table 16: *In vitro* screening of compound 95 against rat and human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rat prostate 5α-reductase (nM)</th>
<th>Human prostate 5α-reductase (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>36</td>
<td>262</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>11</td>
<td>18</td>
</tr>
</tbody>
</table>

In 1996, Ishibashi and co-workers reported the synthesis and evaluation of various 11α-acetoxy, 11α-hydroxy, 11β-hydroxy and 11-oxo substituted 4aza-5α-androstane analogues (96-100) with a diphenylmethylcarbamoyl moiety at C-17. Compounds with an 11β-hydroxy or 11-oxo showed inhibitory activities comparable to Finasteride (35). The 4-methyl 11β-hydroxy-4-aza-5α-androstane derivative (98) was found to be most potent against rat and human enzyme and more active than Finasteride (35) (Table 17).327

![Diagram of compounds 96-100]

(96) R₁= OH, R₂= H, R₃= H, Δ¹(2)
(97) R₁, R₂= (=O), R₃= H, Δ¹(2)
(98) R₁= OH, R₂= H, R₃= CH₃, Δ¹(2)
(99) R₁, R₂= (=O), R₃= H
(100) R₁, R₂= (=O), R₃= CH₃
Introduction

Table 17: *In vitro* screening of compounds 96-100 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rat 5α-reductase %inhibition at 10⁻⁸ M</th>
<th>Human 5α-reductase relative inhibitory potency to MK-906 (MK-906=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>50</td>
<td>0.55</td>
</tr>
<tr>
<td>97</td>
<td>74</td>
<td>1.6</td>
</tr>
<tr>
<td>98</td>
<td>74</td>
<td>2.9</td>
</tr>
<tr>
<td>99</td>
<td>39</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>100</td>
<td>33</td>
<td>1.0</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>28</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Merck in 1997 reported several 4-aza 5α-androstan-3-one 17β-(N-substituted carboxamides) (101-106) as potent human type-II 5α-reductase inhibitors. From the studies it was indicated that the 17-amide N substituent included aromatic residue potent dual inhibitors of type I and type II 5α-reductase were obtained (Table 18).

![Chemical structure](image)

(101) \( R_1 = H, R_2 = H, \Delta^{(2)} \)
(102) \( R_1 = H, R_2 = CH_3, \Delta^{(2)} \)
(103) \( R_1 = CH_3, R_2 = H, \Delta^{(2)} \)
(104) \( R_1 = CH_3, R_2 = CH_3, \Delta^{(2)} \)
(105) \( R_1 = H, R_2 = H \)
(106) \( R_1 = CH_3, R_2 = H \)

Table 18: *In vitro* screening of compounds 101-106 against human steroid 5α-reductase

| Compounds | IC<sub>50</sub>(nM) |
|-----------|----------------|----------------|
|           | Type I  | Type II |
| 101       | 13      | 0.2     |
| 102       | 420     | 20      |
| 103       | 30      | 210     |
| 104       | 120     | 50      |
| 105       | 410     | 15      |
| 106       | 5       | 11      |
| Finasteride (35) | 52 | <0.1 |
The addition of N\textsuperscript{4}-methyl substituent in A ring increases human androgen receptor affinity while addition of unsaturation to the A ring (\(\Delta\text{I}\)) increased human androgen receptor binding. The unsubstituted carbanilides in the \(\Delta\text{I}\)-N\textsuperscript{4}-methyl series showed some selectivity for type I 5\(\alpha\)-reductase over type II enzyme. Whereas addition of aryl substitution at the 2-position increased type II 5\(\alpha\)-reductase binding, thus providing dual inhibitors with excellent human androgen receptor binding. Compound (108) was found to be the most potent inhibitor from this series (Table 19).\textsuperscript{328}

![Chemical Structure](image)

Table 19: *In vitro* screening of compounds 107-110 against human steroid 5\(\alpha\)-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I (IC\textsubscript{50} (nM))</th>
<th>Type II (IC\textsubscript{50} (nM))</th>
</tr>
</thead>
<tbody>
<tr>
<td>107</td>
<td>30</td>
<td>210</td>
</tr>
<tr>
<td>108</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>109</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>110</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>52</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

In 1998, Salle *et. al.* reported synthesis of a novel compound PNU 157706 [N-(1, 1, 1, 3, 3, 3-hexafluorophenylpropyl)-3-oxo-4-aza-5\(\alpha\)-androst-1-ene-17\(\beta\)-carboxamide] (111) as a potent dual type I and II 5\(\alpha\)-reductase inhibitor. PNU 157706 (111) was found to reduce prostate weight 16 fold than Finasteride (35) while the ED\textsubscript{50} values being 0.12 and 1.9 mg/kg/day, respectively (Table 20).\textsuperscript{329}

---

*University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh*
Table 20: *In vitro* screening of compound 111 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>111</td>
<td>3.9±0.1</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>313±74</td>
<td>11.3±2.6</td>
</tr>
</tbody>
</table>

Results are the mean±SE of 3 separate assays. Incubations were performed in the presence of 3 or 1 μM [3H] testosterone, for type I or II isozyme, respectively.

In 2000, Lerner and co-workers reported the synthesis of haptens 112(a, b) and 113 (a, b) which belongs to 4-aza steroids. The resulting 5α-dihydrotestosterone was shown to be the more potent intracellular hormone.330
Menzenbach and associates reported the synthesis of several 17-methylene-4-azasteroids as inhibitors of 5α-reductase. Azaestrone II (114) was found to be inhibitor of 5α-reductase with IC₅₀ = 34 x 10⁻¹⁰ (for prostate) and IC₅₀ = 25 x 10⁻¹⁰ (for seminal vesicle).³³¹

![Structure of 114](image)

### 6-Azasteroids

6-azasteroidal inhibitors were designed by the Glaxo based upon the 3-keto-4-en-6-amine functionality to mimic the structural and charge polarization features of the transition state for the enzyme catalyzed transfer of hydride from NADPH to testosterone. The higher reduction potential of ketoenamine compared to that of α,β-unsaturated ketone prevents these compounds from acting as substrates for 5α-reductase and they show slow offset inhibition instead of irreversible as shown by 4-azasteroids.³⁰²

Structure activity relationship has also been reported by Frye and associates at C-4, N-6 and C-17 carbamoyl (Figure 16).³⁰³,³³²,³³³

![Figure 16: SAR of 6-azasteroids](image)
Initially a set of N-6, C-1, C-2, C-4 substituted derivatives of 6-aza-androst-4-en-3-ones (115-122) were prepared to explore the structure activity relationship of A and B rings versus type I and II 5α-reductase (Table 21).

\[ \text{(115)} \quad R_1 = H, \ R_2 = H \]
\[ \text{(116)} \quad R_1 = H, \ R_2 = \text{CH}_3 \]
\[ \text{(117)} \quad R_1 = \text{Cl}, \ R_2 = H \]
\[ \text{(118)} \quad R_1 = \text{Br}, \ R_2 = H \]
\[ \text{(119)} \quad R_1 = \text{CH}_3, \ R_2 = H \]
\[ \text{(120)} \quad R_1 = H, \ R_2 = \text{CH}_3, \ \Delta^1 \]
\[ \text{(121)} \quad R_1 = H, \ R_2 = H, \ 1, 2-\alpha\text{-methano} \]
\[ \text{(122)} \quad R_1 = H, \ R_2 = H, \ 2 \alpha, \beta\text{-CH}_3 \]

Methylation at N-6 (116) and substitution of C-4 with small lipophilic groups such as Cl (117), Br (118) and \text{CH}_3 (119) increases type I 5α-reductase activity selectivity 4 fold while type I 5α-reductase activity was decreased by unsaturation (120), 1,2 cyclopropanation (121) and C-2-methylation (122).

Table 21: In vitro screening of compounds 115-122 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase IC$_{50}$(nM)</th>
<th>Type II 5α-reductase IC$_{50}$(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>115</td>
<td>750</td>
<td>1.5</td>
</tr>
<tr>
<td>116</td>
<td>180</td>
<td>2.3</td>
</tr>
<tr>
<td>117</td>
<td>51</td>
<td>9</td>
</tr>
<tr>
<td>118</td>
<td>97</td>
<td>2.1</td>
</tr>
<tr>
<td>119</td>
<td>40</td>
<td>3.9</td>
</tr>
<tr>
<td>120</td>
<td>1300</td>
<td>5.7</td>
</tr>
<tr>
<td>121</td>
<td>14000</td>
<td>1.8</td>
</tr>
<tr>
<td>122</td>
<td>3500</td>
<td>3.4</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>150</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Introduction

By careful optimization of the C-17 substituent along with combining A- and B-ring substitutions, potent dual inhibitors of both isozymes of 5α-reductase were obtained (123-135) (Table 22).

(123) \(R_1= CH_3, \ R_2= H, \ R_3 = NH\text{-t-Bu}\)
(124) \(R_1= H, \ R_2= H, \ R_3 = i\text{-Bu}\)
(125) \(R_1= Br, \ R_2=H, \ R_3 = \text{NH-1-Ad}\)
(126) \(R_1= H, \ R_2= H, \ R_3 = \text{NHCH(Ph)2}\)
(127) \(R_1= H, \ R_2=H, \ R_3 = OCH_3\)
(128) \(R_1= CH_3, \ R_2= H, \ R_3 = \text{NHCH(Ph)2}\)
(129) \(R_1= H, \ R_2= H, \ R_3 = O\text{-2-Adamantyl}\)
(130) \(R_1= H, \ R_2= H, \ R_3 = \text{NHCH (4-fluorophenyl)2}\)
(131) \(R_1= H, \ R_2= H, \ R_3 = \text{NHCH (4-chlorophenyl)2}\)
(132) \(R_1= H, \ R_2= H, \ R_3 = n\text{-Pr}\)
(133) \(R_1= H, \ R_2= H, \ R_3 = \text{NH (cyclohexyl)2}\)
(134) \(R_1= H, \ R_2= H, \ R_3 = n\text{-octyl}\)
(135) \(R_1= H, \ R_2= H, \ R_3 = CH_2 (\text{cyclohexyl})\)

It was found that optimizing C-17 group resulted in 5α-reductase type I inhibitory activity in (124) with 5-7 fold increase of activity in (126). Swapping a methyl ester (127) for an admantyl ester (129) provides selectivity towards 5α-reductase-I. Preparation of analogues of 124 resulted in compounds (130,131,132) which were found to be 16-200 fold selectivity towards type I 5α-reductase. Compounds (124,132,134,135) having ketone at C-17 proved extremely potent inhibitors of type I 5α-reductase. Out of the group, compound (126) demonstrated efficacy equivalent to Finasteride (35) in a castrated rat model of DHT dependent prostate growth. In general, large lipophilic groups at C-17 provide selectivity against 5α-reductase I.

A variety of C-17 amide-substituted 6-aza-androst-4-en-3-ones were prepared and evaluated against human type I and II steroid 5α-reductase in order to optimize potency versus both isozymes of 5α-reductase. Out of the study two series of potent and selective C-17 amides were discovered, 2, 5-disubstituted anilides and (arylcycloalkyl) amides (136-142) (Table 23).
Table 22: *In vitro* screening of compounds 123-135 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase IC₅₀(nM)</th>
<th>Type II 5α-reductase IC₅₀(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>123</td>
<td>12</td>
<td>1.4</td>
</tr>
<tr>
<td>124</td>
<td>9</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>125</td>
<td>4.5</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>126</td>
<td>30</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>127</td>
<td>150</td>
<td>3.2</td>
</tr>
<tr>
<td>128</td>
<td>3.6</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>129</td>
<td>6.9</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>130</td>
<td>20</td>
<td>0.16</td>
</tr>
<tr>
<td>131</td>
<td>20</td>
<td>0.12</td>
</tr>
<tr>
<td>132</td>
<td>12</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>133</td>
<td>20</td>
<td>0.40</td>
</tr>
<tr>
<td>134</td>
<td>1.0</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>135</td>
<td>4.0</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>150</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Evaluation of some optimal compounds from this series in a chronic castrated rat model of 5α-reductase inhibitor induced prostate involution, and pharmacokinetic measurements identified compounds (138, 139, 140 and 141) with good *in vivo* efficacy and half-life in the dog.\(^{333}\)

(136) R=5-bromo, 2-tert-butyl
(137) R= 2-tert-butyl, 5-phenyl
(138) R=2-tert-butyl,5-trifluoromethyl
(139) R = 2-tert-butyl,5-(4-tert-butylphenyl)
(140) R= 2,5-bis(trifluoromethyl)
Table 23: *In vitro* screening of compounds 136-142 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase IC$_{50}$ (nM)</th>
<th>Type II 5α-reductase IC$_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>136</td>
<td>4.2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>137</td>
<td>4.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>138</td>
<td>8.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>139</td>
<td>1.3</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>140</td>
<td>4.0</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>141</td>
<td>6.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>142</td>
<td>0.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>150</td>
<td>0.18</td>
</tr>
</tbody>
</table>

B-Homologated analogue of 17β-N, N'-diethylcarboxy-6-aza-androst-4-en-3-one (143) has also been found to be potent inhibitor of 5α-reductase inhibitor with IC$_{50}$ = 318 nM.  

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Bergmann and co-workers synthesised 7-substituted 5-4-6-azasteroid derivatives (144) as 5α-reductase inhibitors.\textsuperscript{335} But activity data was not reported for these compounds.

\[ R_1 = \text{H or CH}_3, R_2 = \text{CH}_3 \]
\[ R_3 = \text{hydrogen, Alk-R}_4, X-\text{Alk, XCO-Alk, Co-Ar, CO-NH-Ar, CO-NH-Het etc.} \]
\[ \text{where Alk is C}_{1-12} \text{ straight or branched alkyl, Ar is phenyl, X is O, N or S,} \]
\[ \text{Het is piperidinyl, piperizinyl, piperolidinyl, pyrrolyl etc.} \]

6-Azacholest-3-ones (\textbf{Table 24}) were assayed against both type I and type II 5α-reductase by Haffner.\textsuperscript{336} All three compounds were found to be potent dual inhibitors of 5α-reductase. Unlike the 4-azasteroids the cholesterol side chain imparts very little selectivity between type I and type II 5α-reductase.

It was also found that C-7 methyl group might provide a potent 5α-reductase I selective compound. The \( \alpha \)-C-7 methyl diastereomer (145) proved to be 7-fold more active 5α-reductase I inhibitor than \( \beta \)-diastereomer (146).

\[ (145) = \alpha-\text{CH}_3, \]
\[ (146) = \beta-\text{CH}_3 \]
Table 24: *In vitro* screening of compounds 145-147 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase $k_i$ (nM)</th>
<th>Type II 5α-reductase $k_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>145</td>
<td>0.8</td>
<td>7.9</td>
</tr>
<tr>
<td>146</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>147</td>
<td>1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Fang and Sharp in 1996 synthesized several 6-azaandrostrenones of the general structure (148) as 5α-reductase inhibitors.\(^{337}\)

\[ \text{16}\beta\text{-Aryloxy, -alkoxy and heteroaryloxy 6-azasteroids of the general formula} \]

as given below were synthesised by Aster and co-workers and the compounds were found to be potent inhibitors of 5α-reductase.\(^{338}\) Compound (149) was found to be potent inhibitor of human 5α-reductase type I with IC\(_{50}\) in the range of 0.1-1000 nM. Several novel 6-aza-B-homosteroids were reported by Rahier and Taton but they were not tested for 5α-reductase inhibitory activity.\(^{339}\)

\[ \text{(148)} \]

Later in 2000 and 2001, Xie *et al.* described the synthesis of 6-azasteroids as potent phosphatidylinositol phosphalipase C (PI-PLC) inhibitors.\(^{340,341}\) Kasal *et al.* in 2005 reported an efficient synthesis of 6-aza-allopregnanolone as neurosteroid analogues but not evaluated them for 5α-reductase inhibitory activity.\(^{342}\)

\[ \text{(149)} \]
7-Azasteroids

Some 7-azasteroids were synthesized in early 1970’s. Morzycki and Sicinski reported the synthesis of 6,7-diazacholestane derivatives but they were not evaluated for the 5α-reductase inhibitory activity.

8-Azasteroids

Several 8-azasteroids have been synthesized and discussed as antifungal agents but none has been reported as 5α-reductase inhibitor.

9-Azasteroids

No work has been published on 9-aza steroids as 5α-reductase inhibitor although some fungicides have been known from this category.

19-Nor-10-azasteroids

On the basis of the molecular model of active site for type II isozyme and to increase the activity and selectivity of compounds towards both 5α-reductase type I and 5α-reductase type II. Guarna et al. synthesized a novel class of compounds 19-nor-10-azasteroids (Tables 25 and 26). Best results were obtained with 9:1 mixture of $\Delta^{9(11)}$ (154) and $\Delta^{8(9)}$ (155) 17 β-(N-tert -butyl carbamoyl)-19-nor-10-aza-4-androsten-3-one as it was found to be a good inhibitor of 5α-reductase type I and 5α-reductase type II.

The enamine structure of ring A of 10-aza-steroid (157) is analogous to that of the substrate like transition state.
Table 25: *In vitro* screening of compounds 150-156 against human steroid 5α-reductase type-II

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀(nM)</th>
<th>Finasteride IC₅₀(nM)</th>
<th>IC₅₀rel(nM) (IC₅₀rel=IC₅₀ Finasteride)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>4600±2990</td>
<td>3.4±2.2</td>
<td>1389±1295</td>
</tr>
<tr>
<td>150:151=5:1</td>
<td>4600±960</td>
<td>4.1±0.7</td>
<td>1123±318</td>
</tr>
<tr>
<td>152:153=22:1</td>
<td>2900±1190</td>
<td>3±1.4</td>
<td>981±609</td>
</tr>
<tr>
<td>154:155=9:1</td>
<td>37±6.7</td>
<td>2.2±0.4</td>
<td>16.9±0.4</td>
</tr>
<tr>
<td>154:155=3.5:1</td>
<td>150±33</td>
<td>4.3±1.5</td>
<td>34±12</td>
</tr>
<tr>
<td>156</td>
<td>460±229</td>
<td>5.5±2.1</td>
<td>83±52</td>
</tr>
</tbody>
</table>

Table 26: *In vitro* screening of compounds 150-156 against human steroid 5α-reductase type I in DU-145 Cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀(nM)</th>
<th>IC₅₀rel(nM)</th>
<th>Selectivity 5α-Reductase II: 5α-Reductase I</th>
</tr>
</thead>
<tbody>
<tr>
<td>150:151=5:1</td>
<td>263±63</td>
<td>6.7±2</td>
<td>1:17</td>
</tr>
<tr>
<td>152:153=9:1</td>
<td>127±12</td>
<td>2.8±0.4</td>
<td>1:1</td>
</tr>
<tr>
<td>156</td>
<td>1134±288</td>
<td>24.4±7</td>
<td>2.5:1</td>
</tr>
</tbody>
</table>

The presence of N atom at position 10 increases the nucleophilic character of the carbonyl group and stabilizes the carbocation intermediate (158) by delocalization at the positive charge.

The inhibitory potency of these compounds depends on the presence of bridgehead N-10 atom conjugated with 4-en-3-one moiety in A ring, unsaturation in C-ring and substituent at C-17 position. 19-nor-10-aza steroids have low transitional barrier energy values and more flexible as compared to 6-aza or 4-azasteroids.350
Some 10a-azasteroids were also synthesized from fusidic acid but were not evaluated for 5α-reductase inhibitors. Guarna et al. also synthesized 17β-[N-(phenyl) methyl/phenyl-amido] substituted 10-azasteroids. Unexpectedly, 5β-H compounds were found more active than their 5α-H counterparts, with (159) (IC50=279 and 2000 nM toward isoenzyme I and II, respectively) and (160) (IC50=913 and 247 nM toward isoenzymes I and II, respectively) being the most potent compounds of the series.

11-, 12a-, 13-Azasteroids

Though many 11-353-355, 12a-356 and 13-azasteroids357 have been prepared but 5α-reductase inhibitory activities have not been reported.

15- and 16-Azasteroids

Many 15-azasterols have been synthesized as antifungal agents358-360 but none of them have been evaluated for 5α-reductase inhibitory activity. 16-Azasteroids have been synthesized and evaluated but none of the compound has evolved as 5α-reductase inhibitor.361-362

17- and 17a-Aza-D-homosteroids

Regan and Hayes, in their exemplary work, have synthesized several 17- and 17a-aza-D-homosteroids from several 17-ketosteroid oximes.353 But 17a-azasteroids attracted more attention when chandonium diiodide was established as a potent neuromuscular blocker.364

17 and 17a-Azasteroids have been found to possess numerous biological activities like gamma amino butyric acid (GABA) receptor antagonistic365-367, antifungal368, antineoplastic, mutagenic369-370 and anti-inflammatory activity.371 The most interesting aspect concerning 17-D-homo-azasteroids is the possibility of “inverted action” or “backbinding” as proposed by McDonald et al.372 Their
proposition was based on the fact that the steroids have the potential to bind in two orientations in the active site of various metabolizing enzymes. Marcus and Talalay first reported that 3(17) β-hydroxysteroid dehydrogenase converts both T (23) and dehydroepiandrosterone (162) to androst-4-ene-3,17-dione (161) (Figure 17a). Other examples of enzyme inhibition by inverted steroids have also appeared. The oxiranes 164 and 165 were found to be active site-directed, irreversible inhibitors of 3-oxo-Δ5-steroid isomerase (Figure 17 b) and the bromoacetates 168 and 169 act as affinity labels for estradiol 17β-dehydrogenase (Figure 17c).

Research on 5α-reductase inhibitors has shown that steroids without side chains can bind to enzymes with the A-ring of the substance simulating the D-ring of the substrate, while the D-ring emulates the A-ring. This could lead to 17-D-homoazasteroids exhibiting same mechanism of action as 4-azasteroids. 17a-azasteroids remained unexplored avenue as far as their 5α-reductase inhibitory activity is concerned. Some 17a-azasteroids (170-173) evaluated for 5α-reductase inhibitory activity are summarized in the Table 27.

![Figure 17a: Action of 3(17) β-hydroxysteroid dehydrogenase](image-url)
Introduction

Figure 17b: Action of 3-oxo-Δ5-steroid isomerase

Figure 17c: Action of estradiol 17β-dehydrogenase

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Table 27: *In vitro* screening of compounds 170-173 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
<td>4</td>
</tr>
<tr>
<td>171</td>
<td>15</td>
</tr>
<tr>
<td>172</td>
<td>12</td>
</tr>
<tr>
<td>173</td>
<td>40</td>
</tr>
</tbody>
</table>

**Diazasteroids**

In 1996 Eberbach and co-workers reported a novel access to 4, 13-diazasteroid derivatives but they were not evaluated for 5α-reductase activity. In the same year Stuart *et al.* first reported 4, 17-diazasteroids as potential inhibitors of 5α-reductase. The Finasteride 17-aza-isomer (174) proved to be potent inhibitor of 5α-reductase II although less active than Finasteride (35) and its congeners. 4-Methylation (175) lowered the inhibition of the 5α-reductase II enzyme.

Removal of Δ¹(2) unsaturation led to the formation of compound (176) that is dual inhibitor of 5α-reductase type I and II and 4-methylation of 176 led to the increase in activity in 177. While compound with Δ⁵(6) (178) showed only a moderate inhibition of 5α-reductase II activity (*Table 28*).
Introduction

8, 13-Diaza steroids were also synthesized by Göndös et al. in 1998 but not evaluated for 5α-reductase inhibitory activity.  

11, 13, 15-Triazasteroids

Hirota et al. reported in 1995 the synthesis of 11, 13, 15-triazasteroid derivatives to investigate antidepressive activity. These analogues were also evaluated for anti-platelet aggregation activity and some derivatives exhibited positive action but no 5α-reductase activity has been investigated in these categories of steroids.  

Table 28: In vitro screening of compounds 174-178 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase</th>
<th>Type II 5α-reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀(nM)</td>
<td>IC₅₀(nM)</td>
</tr>
<tr>
<td>174</td>
<td>765(±70)</td>
<td>10.3(±1.1)</td>
</tr>
<tr>
<td>175</td>
<td>477(±29)</td>
<td>174(±42)</td>
</tr>
<tr>
<td>176</td>
<td>2200(±140)</td>
<td>40.1(±2.8)</td>
</tr>
<tr>
<td>177</td>
<td>28.0(±2.1)</td>
<td>3.6(±0.3)</td>
</tr>
<tr>
<td>178</td>
<td>~7000</td>
<td>52.0(±7.8)</td>
</tr>
<tr>
<td>4-MA³(34)</td>
<td>6.4(±0.2)</td>
<td>0.4(±0.04)</td>
</tr>
</tbody>
</table>

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B, D-Dihomo-azasteroids

Several steroidal B, D-dihomolactam have been synthesized and evaluated for antitumour activity but no 5α-reductase activity has been reported from this group till date.\textsuperscript{380-381}

Des-AB-azasteroids

Trehan \textit{et al.} synthesized des-AB-azasteroids but 5α-reductase activity studies were not done.\textsuperscript{382}

Steroidal 5α-reductase inhibitors which are extranuclear i.e. in which nitrogen is not the part of steroidal nucleus but forms part of the side chain or attached group have also been explored as 5α-reductase inhibitors, therefore are discussed next.

Steroidal 3-carboxylic / phosphonic/ phosphinic acids

A number of 3-androstene-3-carboxylic acids (179-187) (Table 29) were designed to mimic the putative enzyme-bound enolate intermediate by incorporating sp\(^2\)-hybridized centers at C-3 and C-4 and, most critically, an anionic carboxylic acid at C-3 as a charged replacement for the enolate oxyanion.

Because of presumably favorable electrostatic interaction between the carboxylate and the positively charged oxidized cofactor, the acrylate preferentially binds in a ternary complex with enzyme and NADP\(^+\), which leads to the uncompetitive kinetic mechanism.\textsuperscript{278,383,384}
Activity is enhanced in analogues possessing an additional unsaturation at C-5 (183-188) along with Δ^3- unsaturation. At C-17, diisopropyl (179) and pivalyl (184) amides were optimal. Epristeride (SK&F 105657) (184) entered the clinical trials for treatment of BPH and is a potent inhibitor of 5α-reductase II while a weak inhibitor of 5α-reductase I.

**Table 29: In vitro screening of compounds 179-187 against human steroid 5α-reductase**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>K_{iapp} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>179</td>
<td>30</td>
</tr>
<tr>
<td>180</td>
<td>7-18</td>
</tr>
<tr>
<td>181</td>
<td>26</td>
</tr>
<tr>
<td>182</td>
<td>7-12</td>
</tr>
<tr>
<td>183</td>
<td>7</td>
</tr>
<tr>
<td>184</td>
<td>30-36</td>
</tr>
<tr>
<td>185</td>
<td>32</td>
</tr>
<tr>
<td>186</td>
<td>35</td>
</tr>
<tr>
<td>187</td>
<td>50</td>
</tr>
</tbody>
</table>

A series of estratriene-3-carboxylic acids containing an aromatic A-ring had also been synthesized (188-192) (Table 30) with structural variations in the C-2 and C-4 substituents, in the degrees of unsaturation in the B and D rings, and in the C-17 carboxamide alkyl groups. Despite lacking C-19 methyl group these compounds were found to be potent inhibitors of 5α-reductase.385

![Chemical Structures](image)
Table 30: *In vitro* screening of compounds 188-192 against human steroid 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_{i\text{app}}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>188</td>
<td>20</td>
</tr>
<tr>
<td>189</td>
<td>30</td>
</tr>
<tr>
<td>190</td>
<td>10</td>
</tr>
<tr>
<td>191</td>
<td>35</td>
</tr>
<tr>
<td>192</td>
<td>36</td>
</tr>
</tbody>
</table>

Nitro derivatives also showed interesting structure activity relationship patterns compared to carboxylic acids, compound (193) was found to be potent competitive inhibitor by binding to E-NADPH complex. The sulphonic acid (194), phosphonic acid (195) and phosphinic acid (196) also proved to be the potent inhibitors of human 5α-reductase but not so of rat 5α-reductase. The affinities of phosphonic acid are relatively less than phosphinic acid derivatives because the increased bulk at the 3-substituent, leading to a steric intolerance for binding to enzyme. Overall 3-phosphosteroids were weaker inhibitors than their corresponding steroidal-3-carboxy steroids. The function of the C-3-moiety is presumably to act as an H-bond acceptor from a residue in the enzyme, which would normally donate a hydrogen bond to stabilize the enolate. Since the negatively charged groups (CO$_2^-$) or isosteres of the carboxylate (NO$_2$) best mimic this interaction it indicates that a pK$_a$-matched H-bond with a Lys or Arg donor may be operative.

In addition, an interaction between the negatively charged C-3-moiety and the positively charged NADP$^+$ cofactor after the enzyme has turned over substrate is possible, especially if the cofactor lies directly under the A-ring of the steroidal...
skeleton in the transition state and mimics thereof. Compound 197 was not as active due to the presence of alcoholic group as it was not able to provide sufficient negative charge and hence was a weak inhibitor of rat and human 5α-reductase.

**Diazoketone Steroids**

The primary evidence of a dramatic increase in the affinity of 5α-reductase and an inhibitor with a 5-juncture of A/B ring and sp² hybridization at the C-3 and C-4 positions was obtained from the inhibition with a mechanism based inhibitor (5,20R)-4-diazo-21-hydroxy-20-methyl-pregn-6-en-3-one (198) (RMI-18,341). Diazoketone (198) had been reported to be a potent time-dependent inhibitor with a Ki of 35 nM (time-dependency is considered indicative of irreversibility).³⁸⁹

![Diazoketone Steroids](image)

A mechanism of inhibition was proposed that the protonation steps implicated in the normal enzymatic transformation activates the diazoketone functionality to a diazonium ion that could further alkylate some nucleophilic residue at the active site.³⁹⁰

**4-Substituted Steroids**

The observation that an excellent inhibitor possessed a conjugated system (sp²-sp²-sp²) at C-3,C-4,C-5 positions of A ring of steroids together with a lipophilic group at C-17, a range of 4-substituted-3-oxo-4-androstene-17β-carboxamides
(201-204) (Table 31) were prepared and compared with the Finasteride (35). Out of these 4-cyano compounds were found to be potent inhibitors of 5α-reductase type II enzyme and substitution with groups like thiol led to decreased activity. This series of compounds were also found to be potent androgen antagonists.\cite{391} Fei et al. carried out the synthesis of some novel 4-trifluoromethylsteroids and proposed them as novel 5α-reductase inhibitors. Out of the series 4-trifluoromethyl-N-(t-butyl)-4-androsten-17β-carboxamide (205) emerged as the most potent inhibitor being 4 times more active than Finasteride (35).\cite{392}

4-Cyanoprogesterone (206) was also found to be a potent inhibitor of both rat and human 5α-reductase enzymes (IC$_{50}$ values=0.045 and 0.050 μM, respectively). The mechanism of action of 4-cyano steroidal inhibitor was assumed to be the transition state inhibitor because on reduction by the enzyme compound would form a stable 5-3-enol that would remain tightly bound to the active site.\cite{393,394}

\begin{align*}
(201) \quad & R= \text{CN} \\
(202) \quad & R= \text{SH} \\
(203) \quad & R= \text{Cl} \\
(204) \quad & R= \text{Br} \\
(205) \quad & R= \text{CF}_3
\end{align*}

Table 31: \textit{In vitro} screening of compounds 201-204 against human 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Human 5α-reductase type I (transfected 293 cells) (IC$_{50}$ nM)</th>
<th>Human 5α reductase type II (transfected SW-13 cells) (IC$_{50}$ nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>201</td>
<td>2.9</td>
<td>218</td>
</tr>
<tr>
<td>202</td>
<td>709</td>
<td>437</td>
</tr>
<tr>
<td>203</td>
<td>&gt;1000</td>
<td>192</td>
</tr>
<tr>
<td>204</td>
<td>981</td>
<td>387</td>
</tr>
<tr>
<td>Finasteride(35)</td>
<td>218</td>
<td>8.47</td>
</tr>
</tbody>
</table>
Steroidal Oximes

A number of pregnenolone (207-211) and progesterone (212-215) based steroids were synthesized bearing a oxime group connected directly or via a spacer to the steroidal D ring, capable to form a coordinate bond with haeme iron of enzyme 5α-reductase. In contrast to the pregnenolone derivatives which showed no inhibition of 5α-reductase isozyme I and II, progesterone derivatives possessed marked inhibition towards type II.

Inhibitory potency of synthesized compounds against target enzyme using whole cell assay revealed that C-20 oxime (212) displayed strong inhibition against both isozymes (IC₅₀=1.63 μM against human type I and 0.58 μM against human type II).

Unsaturation in ring D (213,214) in conjugation with oxime group further enhances inhibitory activity. Almost complete loss of activity has been found toward type I in the derivative with keto group at C-6 (215).

Transferring the oxime group from position 20 to 21 caused an increased selectivity toward type II isozyme. Z-21-Hydroxyiminopregn-4-en-3-one (216) was
found to be a potential inhibitor of the type II (IC$_{50}$=1.95 µM against human type I and 0.30 µM against human type II).

However, none of the compounds showed activity near to that of the reference drug Finasteride (35) (IC$_{50}$ values being 45 and 3 nM for types I and II, respectively in the corresponding study).

Ma et al. in 2009 synthesized a series of 20-oxime pregnanes from pregnenolone. The 5α-reductase II inhibitory effects of compounds were investigated in a convenient screening model in which compounds 217, 218 and 219 were observed to be potential inhibitors of 5α-reductase. In particular 4-azasteroid 219 was active in the 5α-reductase II inhibitory test, and inhibited cell proliferation of androgen dependent cell and 218 was the most active in the 5α-reductase II inhibitory test, but interestingly, it inhibited PC-3 cells more potently than LNCaP cells.

Steroidal tetrahydrooxazin-2-ones

Wölfing and co-workers synthesized a novel series of steroidal tetrahydrooxazin-2-ones (220-225) containing heterocycles involving O and N heteroatoms at position 17β of androst-4-en-3-one, respectively as 5α-reductase inhibitors. The IC$_{50}$ values of compounds vary between 270 and 600 nM. The relative inhibitory effect of the unsubstituted N-phenyl compound 220 is 0.20.
Concerning the effects of substituents at position 4 of the phenyl ring in 220, the introduction of an ethyl (221) or ethoxy (223) group resulted in a weak enhancement of 5α-reductase inhibition. Substitution with halogens (224 and 225) or methoxy (222) caused lowering of inhibition ability. \[397\]

Steroid 5α-reductase inhibitors that don’t contain any nitrogen either as part of ring or extranuclear but yet found to inhibit 5α-reductase are discussed next.

16-Substituted steroids

A series of 16-methyl substituted derivatives of androst-4-ene and estr-4-ene originally prepared as antiandrogens, were tested for their inhibitory activity on rat and human prostatic 5α-reductase. The inhibitory activity data indicated that IC$_{50}$ increases in sequence in derivatives bearing 16α-methyl (227), 16β-methyl (228) and 16, 16-dimethyl substituents (229). Acylation of 17-hydroxy group significantly increases the inhibitory potency (IC$_{50}$ (231) = 4.8 nM, IC$_{50}$ (230) =23.5 nM in rat prostate and IC$_{50}$ (231) = 0.52 nM, IC$_{50}$ (230) = 0.62 nM in human prostate). Overall, in human prostate homogenates IC$_{50}$ varies between 0.6 and 120μM while in rat prostate it ranges from 1.6 to 1000 μM. This shows enzyme of human prostate is more sensitive than that of rat prostate to methyl substituted compounds. Overall 16-methyl steroids were found to be weak inhibitors both in rat and human enzymes compared to the existing ones. \[398\]
Certain 19-nor analogues have also been synthesized in order to improve the inhibitory activity in 16-methylated derivatives. **TSAA-291** (16-ethyl-17β-hydroxy-4-estren-3-one) (232) was found to be the first anti-androgen known to have dual action of competitive inhibition of 5α-reductase activity and androgen receptor complex formation. It showed a $K_i$ of 1400 nM to the purified nuclei from rat prostatic tissues.\(^{399}\)

6-Methylene steroidal derivatives

2',3'α-Tetrahydrofuran-2'-spiro-17-(6-methylene-4-androsten-3-one) (233; L612,710) is a potent time-dependent inhibitor which causes the highest percentage of inhibition (81%) of rat prostatic 5α-reductase enzyme. The structure activity relationship showed that 3-oxo-4-ene functionally was essential to the inhibitory activity and that substituents at C-17 influenced the inhibitory potency. The presence of the C-19 methyl group was not essential to the activity. The A ring appeared to interact with the entire active site of the enzyme. Furthermore, the affinity of an inhibitor to the enzyme was greatly enhanced by the introduction of a methylene group at C-6.
Activity was completely lost with a large radical such as iodomethylene group at C-7. Thus a series of 6-methylene steroids were prepared and examined as irreversible inhibitors of rat prostatic 5α-reductase. Another 6-methylene steroid that is potent inhibitor due to priming of its dienone group by electrophilic activation toward nucleophilic attack at the 6-methylene group is having structure (234).

Seco steroids

(4R)-5,10-Seco-estra-4,5-diene-3,10,17-trione (235) and (4R)-5,10-seco-19-nor-pregna-4,5-diene-3,10,20-trione (236) were first found to be noncompetitive and possibly irreversible inhibitors of epididymal 5α-reductase. Radiographic crystallography studies of both compounds showed that the conjugated allenic 3-oxo-5, 10-seco steroids (235) has a conformation similar to that of the normal tetracyclic steroid dione. Both compounds were non-competitive inhibitors of 5α-reductase and have an affinity label for the enzyme with Ki of 5470 and 980 nM, respectively.

Derivatives of natural substrate: Pregnane

As a consequence of the important observation that progesterone and deoxycortisone inhibits the synthesis of dihydrotestosterone by competing with 4-en-3-one function of the testosterone for the 5α-reductase enzyme it led Voigt and co-workers to synthesize number of progesterone derivatives.
The satisfactory result of 4-cyano-progesterone (206), which possessed marked inhibitory activity for 5α-reductase enzyme, stimulated great deal of interest to synthesize various 4- and 6-halo-progesterone analogs (237-241). These compounds were found to be potent antiandrogenic in nature when tested against gonadectomized hamster seminal vesicles and were also found to be inhibitors of 5α-reductase.404-406

Bratoeff et al. evaluated the antiandrogenic and 5α-reductase inhibitory activity of various 16-phenyl substituted-D-homo compounds (242-243), 16-methyl substituted steroids (244-246), 4-bromo compound (245) without a methyl group at C-16 and the epoxy compounds (248-249).

Compounds 243, 246 and 247 were found to possess both antiandrogenic and 5α-reductase inhibitory activity better than the Finasteride (35).406,407 The trienones having a more coplanar structure reacts faster with the nucleophilic portion of the enzyme in a Michael type addition reaction to form an irreversible adduct with a concomitant inhibition of the enzyme 5α-reductase than the dienones.

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A range of 4-bromo-17-substituted-4-pregnene-3, 20 diones were also synthesized and evaluated as 5α-reductase inhibitors on gonadectomized hamster seminal vesicle and flank organs. Small diameter of the pigmented flank organ and great reduction in the weight of seminal vesicle has been found with the compounds having p-fluorobenzoyloxy (250) and p-chlorobenzoyloxy (251) indicating that the presence of more electronegative substituent at C-17 position (p-halosubstituted phenyl) and halogen at C-4 enhances the antiandrogenic activity as well as 5α-reductase inhibitory activity.408,409

Several new pregnane derivatives were also synthesized and evaluated by the conversion of [3H] T to [3H] DHT in P.crustosum broths and the conversion of [1, 2-14C] sodium acetate into lipids. Compounds 252 and 253 were found out to be potent 5α-reductase inhibitors as they inhibit conversion of T (23) to DHT (24) and also decreased the incorporation of radiolabeled sodium acetate into lipids of the flank organs.410
Cabeza et al. also reported the 5α-reductase inhibitory activity and the antiandrogenic effect of novel 16-bromo substituted trienedione, 16β methyl substituted dienedione and the trienedione (254-256). Compounds 254 and 255 were found to exhibit 5α-reductase inhibitory activity higher than the commercially available Finasteride (35).

\[
\begin{align*}
(254) & \quad R_1 = \text{Br}, \Delta^1(2) \\
(255) & \quad R_1 = \text{CH}_3 \\
(256) & \quad R_1 = \text{CH}_3, \Delta^1(2)
\end{align*}
\]

The in vitro inhibitory activity of some novel progesterone derivatives was also determined and they were evaluated as 5α-reductase inhibitors as well as antagonists for the androgen receptor. The appropriate homologues extended by a methylene group at C-6 (257-260) showed better activity due to the presence of exocyclic double bond that can react faster with the enzyme in a Michael type addition reaction than the corresponding endocyclic diene. Compounds 257 and 258 showed IC$_{50}$ values of 19 nM and 100 nM, respectively.

\[
\begin{align*}
(257) & \quad R_1 = \text{OH} \\
(258) & \quad R_1 = \text{COC}_6\text{H}_4\text{Br} \\
(259) & \quad R_1 = \text{CH}_2\text{CH}_2\text{C}_3\text{H}_{10} \\
(260) & \quad R_1 = \text{CH}_2\text{CH}_2\text{CH}_2\text{C}_5\text{H}_{10}
\end{align*}
\]

In compound 261 which is an epoxy compound the nucleophilic enzyme attacked the electrophilic center at C-6 and in this process inhibits the enzyme. Several 3-substituted 4-pregnene-6,20-dione derivatives (262-270) have been synthesized and were evaluated for antiandrogenic as well as 5α-reductase inhibitory activity. The synthesized steroids have an α,β-unsaturated carbonyl moiety in common. It was found that accessible electrophilic β-carbon of an α,β-unsaturated carbonyl moiety reacted very readily with a variety of nucleophiles to form Michael

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adducts. The first step involved the formation of an enzyme-steroid activated complex and in a subsequent step the nucleophilic portion of the enzyme (amino group) attacked the conjugated double bond of the steroid in a Michael type addition reaction to form an irreversible adducts. \(^{414}\)

![Chemical Structures](image)

The IC\(_{50}\) values of the compounds were found to increase progressively as the substituent on the phenyl group of the ester side chain at C-3 became more electropositive i.e. compound 265 has an IC\(_{50}\) value of 3.0 nM as compared to compound 267 which has an IC\(_{50}\) value of 4.0 nM. On the other hand the compounds 268-270 having saturated 4-pregnen-6, 20-dione skeleton for e.g. 269...
exhibited higher IC\textsubscript{50} value (3.7 nM) as compared to the unsaturated ones (264-267) for e.g. 265 with a value of 3.0 nM. It was also found that the presence of halogen substituents in ester moiety at C-3 as well as double bond at C-16 increased the binding affinity for the androgen receptor.\textsuperscript{415}

Several new progesterone derivatives were also synthesized by the same group having dienone moiety as reported earlier. Some of them (271-272) were found to have high 5α-reductase inhibitory activity like 272 showing an IC\textsubscript{50} value of 0.5 nM as compared to Finasteride (35) showing an IC\textsubscript{50} value of 8.5 nM in the same study.\textsuperscript{416, 417} 

![Picture of compounds](image1)

Bratoeff \textit{et al.} synthesized two novel steroidal carbamates (273-274) which are the esters of carbamic acid with substituents at the amino and esters ends (NHRCOOR\textsuperscript{1}) and proposed them as novel class of inhibitors for human and hamster steroid 5α-reductase. These compounds have a longer half life and are hydrolyzed slowly in the liver due to which they have a better pharmacological activity when compared to the conventional esters. Compound 273 has got a similar IC\textsubscript{50} value (10 nM) as that of Finasteride (8.5 nM) (35) while compound 274 has a higher IC\textsubscript{50} value of about 50 nM apparently due to the presence of large bromine atom.\textsuperscript{418}

![Picture of compounds](image2)

Working on similar lines some more novel 4, 16-pregnadiene-6, 20-dione derivatives were synthesized and evaluated as 5α-reductase inhibitors. In this work,
it has been demonstrated that compounds containing chlorine (275), bromine (276), iodine (277) atoms, and (278); without any substituent in the ester moiety) at C-3 produce a significant decrease of the prostate weight in castrated animals treated with testosterone (23). Therefore, it was proposed that the ester moiety at C-3 is functioning as a pharmacophore, enriched by the presence of halogens in these steroidal derivatives leading to the increase in the inhibition of 5α-reductase enzyme as determined by the IC₅₀ values. Compound 276 was found to be most potent with an IC₅₀ value of 1.8 nM while Compounds 275 and 277 showed values 14 and 10 nM, respectively in comparison to Finasteride (35) having value 8.5 nM. Compound 278 was not that active when compared to halogenated compounds thus further demonstrating the need of a halogen atom in the ester side chain.\textsuperscript{419}

Recently, Cabeza \textit{et al.} synthesized several C-6 substituted and unsubstituted pregnane derivatives as potential 5α-reductase inhibitors. It has been found that steroids that lack a chlorine atom in C-6 (279-281) exhibited a higher capacity for inhibition of the activity of 5α-reductase (IC₅₀ in the range of 25-63 nM) than the compounds (282-284) having this atom (IC₅₀ in the range of 920-990 nM in comparison to Finasteride (35) having an IC₅₀ 8.5 nM).
The presence of bromine atom in C-6 of compound 285 however doesn’t affect the inhibitory activity of enzyme (IC$_{50}$ being 33 nM). Also, the presence of an ester moiety in C-17 α on the steroidal skeleton tends to increase the inhibition of the activity of the enzyme.$^{420}$

Cabeza and co-workers in 2010 reported the synthesis and biological evaluation of 6- and 17-substituted progesterone derivatives (286-289). Compounds 286-288 significantly decreased the weight of the prostate as compared to testosterone-treated animals and this reduction of prostate weight was comparable to that produced by Finasteride (35). Steroid 8 was the most effective of the tested compounds. On the other hand, 286-289 exhibited a high inhibitory activity for the human 5α-reductase enzyme with IC$_{50}$ values of 10, 70, 22, and 19 nM, respectively. It was proposed that compounds that contained the acetate ester moiety in the molecule inhibited the activity of 5α-reductase and decreased the weight of the prostate. Nevertheless, the double bond in ring B seems to diminish the inhibitory potency (287 and 289), since 286, which does not possess a double bond at C-6, had the highest inhibitory activity (the lowest IC$_{50}$ value).$^{421}$

Steroidal oxazolines

Szécsi and co-workers recently reported the 5α-reductase type I inhibitory activity against rat isozyme of novel (5’S)-17β-(4,5-dihydrooxazol-5-yl)androst-5-en-
3-one compounds containing various derivatized phenyl substituents coupled to the exo-heterocyclic moiety. The 2-chlorophenyl substituent (290) of the oxazolines significantly increases the inhibitory potential while presence of fluorine (291) in the position 4 of the phenyl ring decreases inhibition. Although the compounds were not as active (showing µM inhibitions) as compared to standard drug Finasteride (35) having 8nM inhibition.422

Non-steroidal 5α-reductase inhibitors

A number of classes of non-steroloidal inhibitors of 5α-reductase have now been identified. It was anticipated that the use of non-steroloidal template can decrease the potential interaction with other enzyme or receptor of the steroidal endocrine system and can limit the complexity of target compound synthesis.423 They have in fact emerged either from the design of compounds mimic of azasteroidal inhibitors, generally by the formal removing of one or more rings from the azasteroidal structure or by early non-steroidal lead (ONO-3805) (292) which was prepared as leukotriene synthesis inhibitor 424 or by high throughput screening. These compounds are generally thought to act all as competitive inhibitors vs. testosterone with exception of the epristeride analogues which are uncompetitive inhibitors. Non-steroidal inhibitors include benzo[f]quinolinones, pyridones and quinolinones which were mimics of 4-azasteroid inhibitors. Benzo[c]quinolinones were synthesized as mimics of 6-azasteroids while benzo[c]quinolizinones were designed as mimics of 10-azasteroids. The most potent and selective inhibitors of human type I 5α-reductase are found among these classes of compounds. Almost all the other non-steroidal inhibitors can be grouped as carboxylic acid (generally butanoic acid) derivatives which are thought to act as non-competitive inhibitors versus testosterone in analogy to ONO 3805 (292).
ONO-3805 (292)

Mimics of 4-azasteroids: Benzo[f]quinolinones

Benzo[f]quinolinones were the first non-steroidal inhibitors prepared by the Lilly's researchers. They were derived by the removal of the D ring from 4-azasteroids and replacing the C ring with an aromatic one. Most of these compounds are type I selective, although dual inhibitors can be obtained if an appropriate substitution is present at the position 8 on the aromatic ring.

Two main classes of benzo[f]quinolinones have been described, the hexahydro derivatives (293-296), which have an unsaturation at position 4a–10a, and the octahydro derivatives (297-302).

(293) R= H, X=H
(294) R= H, X= Cl
(295) R= CH₃, X=CI
(296) R=CH₃, X= Br

(297) R₁= H, R₂= H, X=Cl
(298) R₁= CH₃, R₂= CH₃, X=Cl
(299) R₁= CH₃, R₂= H, X=Cl
(300) R₁= CH₃, R₂= H, X=CH₃
(301) R₁= CH₃, R₂= H, X=F
(302) R₁= CH₃, R₂= H, X=Br
In general octahydro derivatives are more potent inhibitors than the corresponding 4α-10α unsaturated compounds (Tables 32 and 33) and in both series the potency toward type I 5α-reductase increases if an halogen atom is present at position 8 (in particular a Cl atom) and a methyl group at position 4; in fact the most potent inhibitor of the series is LY191704 (299) with IC50 = 8 nM (Table 33). This molecule has progressed into human clinical trials.

Table 32: *In vitro* screening of compounds 293-296 against human 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase</th>
<th>Type II 5α-reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC50 (nM)</td>
<td>IC50 (nM)</td>
</tr>
<tr>
<td>293</td>
<td>6500</td>
<td>-</td>
</tr>
<tr>
<td>294</td>
<td>460</td>
<td>-</td>
</tr>
<tr>
<td>295</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>296</td>
<td>60</td>
<td>-</td>
</tr>
</tbody>
</table>

The quantitative structure activity relationship study of these compounds has focused on the effect of 8 substituent on the aromatic ring, which can be accounted for by its lipophilic character.426 They found that the optimum activity may reside in the property space around the chlorine substituent. The substitution of the 8-Cl atom by a F (301) or a Br atom (302) decreased very slightly the potency. Finally, several kinds of substituents, including complex aromatic groups, were introduced at the position 8 427, and some potent type I selective inhibitors such as compounds 303, 304 and 305 were prepared.
Table 33: *In vitro* screening of compounds 297-305 against human 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase IC₅₀(nM)</th>
<th>Type II 5α-reductase IC₅₀(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>297</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>298</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>299</td>
<td>8</td>
<td>10000</td>
</tr>
<tr>
<td>300</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>301</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>302</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>303</td>
<td>59</td>
<td>&gt;10</td>
</tr>
<tr>
<td>304</td>
<td>6</td>
<td>1400</td>
</tr>
<tr>
<td>305</td>
<td>6</td>
<td>1340</td>
</tr>
</tbody>
</table>

Pyridones, quinolinones and piperidines

Abell *et al.* synthesized a number of tricyclic thiolactams (306-307), aryl acid (308), bicyclic lactams (309-312) and bicyclic thiolactam (313) and evaluated them *in vitro* as inhibitors of types I and II steroid 5α-reductase (Table 34). Removal of two or more rings from 4-azasteroids resulted in a strong decrease of potency. The tricyclic thiolactams were found to be selective type I 5α-reductase inhibitors and in general were less active than the corresponding lactams. The aryl acid 308 showed good dual isozyme inhibitory properties with significantly enhanced type II activity.

Bicyclic lactams, in general, were found to be less active against type I 5α-reductase than the tricycles. For example, compounds (309-310), lacking the B and D steroidal rings, were poor type I 5α-reductase inhibitors, with the highest potency associated to the presence of the Cl atom on the aromatic ring of 310. 428

![Chemical structures](image-url)
A styryl (or azo) substituent dramatically enhances type II activity (and indeed type 1 activity with the bicycles) (311-312) and provided dual inhibitors of types I and II.

Table 34: *In vitro* screening of compounds 306-313 against human 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase IC₅₀(nM)</th>
<th>Type II 5α-reductase IC₅₀(nM)</th>
<th>or percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>306</td>
<td>377</td>
<td>13.2%@ 40µM</td>
<td></td>
</tr>
<tr>
<td>307</td>
<td>183</td>
<td>21.6%@40µM</td>
<td></td>
</tr>
<tr>
<td>308</td>
<td>152</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>309</td>
<td>2477</td>
<td>13.5%@ 40µM</td>
<td></td>
</tr>
<tr>
<td>310</td>
<td>1690</td>
<td>12350</td>
<td></td>
</tr>
<tr>
<td>311</td>
<td>302</td>
<td>579</td>
<td></td>
</tr>
<tr>
<td>312</td>
<td>107</td>
<td>617</td>
<td></td>
</tr>
<tr>
<td>313</td>
<td>3360</td>
<td>14%@40µM</td>
<td></td>
</tr>
</tbody>
</table>

Hartmann and co workers synthesized pyridones of the general formula 314 and 315 where the B and C rings of the steroid system have been replaced by an
Introduction

acyclic linker but these compounds display relatively weak activity versus both the rat and human 5α-reductase isozymes (Ki>20μM).^429-431

\[
\text{Scheme 1}
\]

(314) \( n = 1, 2 \); \( X = \text{CON} \ (i-\text{Pr})_2 \)

(315) \( R_1, R_2 = \text{H, Me}; X = \text{CON} \ (i-\text{Pr})_2 \)

Their poor potency however illustrates the need for both A- and B-rings to be present with the correct fusion pattern for good recognition at the enzyme active site. A series of 5-phenyl substituted 1-methyl-2-pyridones have also been prepared and tested against human and rat 5α-reductase type I and type II.

Compound 316 bearing bulky carboxamide substituents exhibited excellent 5α-reductase type II inhibitory activity with IC\(_{50}\) value of 10 μM.^432

\[
\text{Scheme 2}
\]

(316)

McCarthy and co-workers have recently synthesized a series of 4’- substituted 5-aryl pyridones along with corresponding 1-aryl-pyridone derivatives and tested them against 5α-reductase types I and II expressed in transfected human embryonic kidney cells to examine structure activity relationship for the 4’ position in pyridones.

\[
\text{Scheme 3}
\]

(317) \( R = i-\text{Pr} \) (318) \( R = \text{allyl} \)

(319)
Weak inhibition was observed against the type I isozyme for 4'-N-substituted acetamide compounds (317-320) while potent inhibition of type I isozyme was observed for compound 321 having 4'-benzoyl substituent and also for compounds (322-223) having long carbon chain tethers attached to the 4'-acetamide (Table 35). Thus further proving that large hydrophobic groups are tolerated in a region of the active site not involved in the enzymatic reaction.433

**Table 35: In vitro screening of compounds 317-323 against human 5α-reductase**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase (% inhibition at 10μM)</th>
<th>Type II 5α-reductase (% inhibition at 10μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>317</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>318</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>319</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>320</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>321</td>
<td>61</td>
<td>-</td>
</tr>
<tr>
<td>322</td>
<td>43</td>
<td>-</td>
</tr>
<tr>
<td>323</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>Finasteride (35)</td>
<td>453 (IC₅₀(Nm))</td>
<td>25 (IC₅₀(Nm))</td>
</tr>
</tbody>
</table>
Few quinolinone derivatives such as 6-substituted 1H-quinolin-2-ones (324-325) and 2-methoxy quinolines (326-327) have also been synthesized. The most active inhibitor for the human type II isozyme was 6-[4-(N, N-diisopropylcarbamoyl) phenyl]-1H-quinolin-2-one 324 having $K_i$ 800 ± 85 nM, showing mostly competitive inhibitory patterns. A type I selective inhibitor could be identified with 6-[4-(N, N-diisopropylcarbamoyl) phenyl]-N-methyl-quinolin-2-one 325 ($IC_{50} =$510 nM) but 2-methoxy quinolines were not found to be active.434

![Chemical structure of 324, 325, 326, 327](image)

Hartmann and co-workers synthesized and evaluated a series of 2'-Substituted 4-(4'-carboxy- or 4'-carboxymethylbenzylidene)-N-acylpiperidines as active steroid 5α-reductase type II inhibitors. They synthesized several compounds from N-acyl-4-benzylidene-piperidine-4'-carboxylic acids. In the dicyclohexylacetyl series, fluorination in the 2-position of the benzene nucleus (328), exchange of the carboxy group by a carboxymethyl moiety (329) and combination of both structural modifications (330) led to highly active inhibitors of the human 5α-reductase type II isozyme with $IC_{50}$ values of 328, 329 and 330 being 11, 6 and 7 nM respectively in comparison to Finasteride (35) having value of 5nM.435

![Chemical structures of 328, 329, 330](image)

Earlier Hartmann et al. have reported a series of N-substituted piperidine-4-(benzylidene-4-carboxylic acids) (331-334) as potent non-steroidal dual inhibitors of...
5α-reductase. In rat, compounds 331 (IC50=3.44 and 0.37 μM for type I and II, respectively) and 333 (IC50=0.54 and 0.69 μM for type I and II, respectively) displayed the best inhibition toward both isozymes. Compound 332 showed a strong inhibition toward type II human and rat enzyme (IC50=60 and 80 nM) but only a moderate activity versus type I enzyme (IC50 approximately 10 μM for rat and human enzyme).

\[
\text{HOOC} \quad \text{R} \quad \text{O} \\
\text{(331) R =Methyl diphenyl} \quad \text{(332) R =Methyl dicyclohexyl} \\
\text{(333) R =Diphenylamino} \quad \text{(334) R =4-Heptyl}
\]

Compound 334 (IC50 in human type II enzyme being 0.26μM and in rat type II enzyme being 0.29 μM) was found to be a moderate dual inhibitor probably due to higher flexibility of the open ring substituent. 436 Methyl esters of N-(dicyclohexyl)acetyl-piperidine-4-(benzylidene-4-carboxylic acid) (335) were designed and monitored for dual inhibition toward type II isozyme in BPH cell free preparation and for type I isozyme in DU 145 cells.

Methyl esters, applied as hydrolytically stable precursor drugs to facilitate cell permeation, will yield the corresponding carboxylic acids as type II inhibitors after hydrolysis in the target organ. The esters themselves stable in human plasma and Caco-2 cells act as potent drug toward 5α-reductase type I. Thus, dual inhibition of 5α-reductase type I and type II can be achieved by applying a single parent compound 437

\[
\text{(335)}
\]

**Mimics of 6-azasteroids: Benzo[c] quinolinones**

On the basis of 6-aza-androst-4-en-3-one derivatives (Figure 16) in which a vinylogous amide was inserted into a steroid nucleus as a transition state mimic for
conversion of T (23) to DHT (24) and Lilly’s Benzoquinoline derivatives (298-299), novel phenanthridin-3-one derivatives (336-338) were synthesized having vinylogous amide pharmacophore.

![Chemical Structure](attachment:image.jpg)

(336) \(R_1=CH_3, R_2=H\)  
(337) \(R_1=CH_3, R_2=CH_3\)  
(338) \(R_1=H, R_2=CH_3\)

Although compounds were found to be 5α-reductase type I selective and poor inhibitors of 5α-reductase type II but overall these compounds didn’t showed promising inhibitory activity. The potency of compounds was found to increased from 336 (\(K_i>10\mu M\)) to 337 (\(K_i=1.1\mu M\)) and 338 (\(K_i=0.92\mu M\)) this was due to the presence of methyl group on the A ring corresponding to the 4-Me of Eli Lilly inhibitors. This effect is due to the presence of hydrophobic pocket in the active site of enzyme which is able to accommodate a small alkyl group located at the position 4 of the A ring of these tricyclic inhibitors.  

**Mimics of 10-Azasteroids: Benzo [c] quinolinones**

Guarna et al. had synthesized two series of benzo[c]quinolinin-3-ones as novel inhibitors of human 5α-reductase type I: \(4aH\)-series with a double bond between the positions 1 and 2 (339-342) and \(1H\)-series with a double bond between the positions 4 and 4a (343-348). The efficacy and selectivity of these compounds have been demonstrated on recombinant human 5α-reductase type I expressed in CHO cells but they displayed very poor or no inhibition towards 5α-reductase type II (Table 36). Increased activity of the compounds of \(1H\)-series than those of corresponding inhibitors of \(4aH\)-series has been attributed to the presence of double bond enabling conjugation between carbonyl and nitrogen atom.  

The presence of a methyl group at position 4 (342,344,345,347) associated with a substituent at position 8, gave potent compounds in comparison with Finasteride (35) and the known 5α-reductase I selective inhibitor LY191704 (299).
All compounds inhibited the enzyme through a reversible competitive mechanism. The structure activity relationship carried out on this class especially methyl group at positions 1, 4, 5, 6 and 8 can be summarized as follows (Figure 18):

Figure 18: SAR for Δ⁴-Benz[c] quinolizin-3-ones

It was found that presence of substituent at position 8, either a chlorine or methyl group, generally increased the potency of the inhibitors. Thus compound 340 and 341 were potent were more active than unsubstituted compound 339 in 4aH-series. Similarly in 1H-series compound 343 and 345 were more potent than unsubstituted compounds due to presence of 8-chlorine group.

The introduction of a methyl group at position 4 was found to increase the potency in both series with effect being higher in 1H-series. A very strong increase of potency is observed in 8-chloro-4-methyl derivative 345 when compared to 4-methyl unsubstituted compound 343. The substitution with a methyl group at position 6 also
Introduction

affects potency in both series with effect being more prominent in 1H-series with compound 346 being more potent than unsubstituted 343.

This effect of methyl substitution at position 6 seems consistent with the observation that the introduction of the same group on the corresponding position 7 in 4-azasteroids increased their 5α-reductase I selectivity. Presence of methyl group at position 5 found to decrease the potency of the compounds while introduction of methyl at position 1 as in compound 348 caused only a slight decrease in activity when compared to unsubstituted compound 343.

In 2001, Guarna et al. studied the effect of C-ring modifications in benzo[c]quinolizin-3-ones. They synthesized several octahydro- and decahydrobenzo[c]quinolizin-3-one derivatives containing partially or fully saturated C-ring. These compounds were found to be selective 5α-reductase I inhibitors.

Table 36: In vitro screening of compounds 339-348 against recombinant 5α-reductase I expressed in CHO cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>339</td>
<td>5130±130</td>
</tr>
<tr>
<td>340</td>
<td>176±17</td>
</tr>
<tr>
<td>341</td>
<td>459±118</td>
</tr>
<tr>
<td>342</td>
<td>137±58</td>
</tr>
<tr>
<td>343</td>
<td>49±19</td>
</tr>
<tr>
<td>344</td>
<td>20±8</td>
</tr>
<tr>
<td>345</td>
<td>7.6±0.9</td>
</tr>
<tr>
<td>346</td>
<td>14.3±5.9</td>
</tr>
<tr>
<td>347</td>
<td>8.5±2.1</td>
</tr>
<tr>
<td>348</td>
<td>204±49</td>
</tr>
</tbody>
</table>

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh
Benzo[c]quinolizin-3-one inhibitor lacking the aromatic C ring but with a double bond at 6a–10a 349 displayed an inhibitory potency 345-fold higher than that of the corresponding 6a–10a saturated, trans-fused compound 350 (Table 37).\textsuperscript{442}

Table 37: In vitro screening of compounds 349-350 against recombinant 5α-reductase I and II expressed in CHO cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase IC\textsubscript{50}(nM)</th>
<th>Type II 5α-reductase IC\textsubscript{50}(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>349\textsuperscript{a}</td>
<td>58±2.1</td>
<td>No inhibition</td>
</tr>
<tr>
<td>350</td>
<td>20000±400</td>
<td>No inhibition</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Mixture of Δ\textsuperscript{6a(10a)}/Δ\textsuperscript{6(10a)} isomers in 10:1 ratio.

A 3D-QSAR model correlating the potency of the inhibitors with their physicochemical features using density functional theory (DFT) was also developed for a series of benzo[c]quinolizin-3-ones derivatives by adding two “non standard” variables (dipole moment and log P) to the classical electrostatic and steric comparative molecular field analysis (CoMFA) fields.\textsuperscript{443}

With the aim to discover new dual non steroidal inhibitors of 5α-reductase I and II a series of benzo[c]quinolizin-3-ones derivatives (351-356) bearing diverse substituents at position 8 were synthesized in 2005.\textsuperscript{444} They were tested towards 5α-reductase I and II expressed by Chinese Hamster Ovary cells (CHO 1827 and CHO 1829), respectively. It was found out that most potent dual inhibitors were obtained when F atom was introduced on the phenol moiety of these esters. All compounds displayed inhibition towards 5α-reductase I in the range of 93-165 nM (Table 38). Compound 353 was found to be the most potent dual inhibitor of the series with IC\textsubscript{50} values about 100 nM for both enzymes.

\[\text{IC}_{50}\]

![Chemical structures of compounds](image_url)
Table 38: In vitro screening of compounds 351-356 against recombinant 5α-reductase I and II expressed in CHO cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase (% inhibition at 10μM)</th>
<th>Type II 5α-reductase (% inhibition at 10μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>351</td>
<td>102</td>
<td>553</td>
</tr>
<tr>
<td>352</td>
<td>129</td>
<td>584</td>
</tr>
<tr>
<td>353</td>
<td>93</td>
<td>119</td>
</tr>
<tr>
<td>354</td>
<td>160</td>
<td>134</td>
</tr>
<tr>
<td>355</td>
<td>138</td>
<td>166</td>
</tr>
<tr>
<td>356</td>
<td>42</td>
<td>368</td>
</tr>
</tbody>
</table>

Non steroidal aryl acids

Some novel 9, 10-dihydrophenanthrene-2-carboxylic acids (357-359) were prepared by formally removing D ring from parent androstene carboxylic acid inhibitors and contrary to them, were found to be selective 5α-reductase I inhibitors. Introduction of a bromine atom at position 7 in compound 359 gave the most potent compound of the series. Substitution by chlorine at position 7 in compound 358 doesn’t result in increase in potency as compared to unsubstituted compound 357. These compounds are supposed to interact with the positively charged Enzyme-NADP+ complex in an uncompetitive manner versus testosterone.445 Moreover when double bond was introduced in the B ring as in compound 360 (formally obtained by removing the D ring from Epristeride 184) the selectivity toward 5α-reductase I was found to be lost in favor of an increased potency towards 5α-reductase II (Table 39).446
Introduction

On removal of two or more rings from the parent steroidal compounds several aryl acids mimics of steroidal carboxylic acids have been synthesized (362-367). Hartmann et al. synthesized several N-substituted 4-(5-indolyl) benzoic acids but potent and selective human 5α-reductase I inhibitors were not found from the series. Only compound 362 with IC$_{50}$ value of 67 nM against human 5α-reductase I was the most potent inhibitor.447

Table 39: In vitro screening of compounds 357-361 against recombinant human 5α-reductase I and II

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Type I 5α-reductase (Ki,app) (nM)</th>
<th>Type II 5α-reductase (Ki,app) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>357</td>
<td>315</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>358</td>
<td>320</td>
<td>~2500</td>
</tr>
<tr>
<td>359</td>
<td>26</td>
<td>10000</td>
</tr>
<tr>
<td>360</td>
<td>1200</td>
<td>260</td>
</tr>
<tr>
<td>361</td>
<td>1900</td>
<td>1600</td>
</tr>
</tbody>
</table>

Similarly, compound 363 was found to possess IC$_{50}$ value of 680 nM.448

Igarashi et al. synthesized a new series of indole derivatives as potent human prostatic 5α-reductase inhibitors. Compounds (364-367) were found to be most potent among the series with 4-[(1-benzyl-1H-indol-5-yl) oxy]-3-chlorobenzoic acid 365 having an IC$_{50}$ value of 0.44 nM while 3-chloro-4-{1-(4-phenoxybenzoyl)-1H-indol-5-yl}oxy)benzoic acid 366 showed inhibitory activities for both human and rat prostatic 5α-reductase with IC$_{50}$ values of 2.1 and 73 nM, respectively.449
Screening of aryl carboxylate versus the human 5α-reductase isozymes by SmithKline-Beecham company led to the discovery of two series of selective and potent 5α-reductase II nonsteroidal inhibitors based on benzophenone and indolecarboxylic acids skeleton. In benzophenone series of inhibitors the linker between the A-ring and B-ring proved to be crucial whereas linker between B and C-rings was more tolerant of variation. Both the A-ring carboxylic acid and C-ring are critical for activity. Compounds 368 and 369 were found to be most potent among the series with Ki, app being 10 and 5 nM respectively. In indole series there was a strong preference for substitution at the 5- or 6-position of the indole ring. Compounds 370 and 371 were most active among the series with Ki, app being 10 and 40 nM, respectively. The structure activity relationships of both the series are summarized as below (Figures 19 and 20).
Takami and co-workers synthesized various indole derivatives with varied substituents on the $\alpha,\beta$-unsaturated double bond and evaluated them for activity to inhibit rat prostatic 5$\alpha$-reductase. Among the various derivatives they found that (Z)-4-{2-[[3-[1-(4,4'-difluorobenzhydryl)indol-5-yl]-2-pentenoyl]-amino]phenox-y} butyric acid (372, KF 20405) was the most potent compound having activity about 20 times greater than Finasteride (35) and an IC$_{50}$ value of $0.48 \pm 0.086$ nM.  

KF20405 (372)

A novel series of indole and benzimidazole derivatives were also synthesized and evaluated for their inhibitory activity of rat prostatic 5$\alpha$-reductase.
Among the series, 4-{2-[1-(4,4'-dipropylbenzhydryl)indole-5-carbox-amido]phenoxy}butyric acid (373) and its benzimidazole analogue (374) showed potent inhibitory activities with IC$_{50}$ values of 9.6±1.0 and 13±1.5 nM, respectively.$^{452}$

![Chemical Structure](image1)

![Chemical Structure](image2)

Sawada et al. synthesized a novel series of indolizinebutyric acids with various benzoyl substituents. FK687 (375) (S)-4-[1-[4-[[1-(4-isobutylphenyl)butyl]oxy]benzoyl]indolizin-3-yl]butyric acid displayed strongest *in vitro* inhibitory activity (IC$_{50}$=4.6 nM) against the human enzyme and *in vivo* inhibitory activity (IC$_{50}$=1.7 nM) against the castrated young rat model among the series.$^{453}$

FK 687 (375)

In 2002, various N-substituted 4'-biphenyl-4-carboxylic acids were synthesized by increasing the conformational flexibility using an ether linker between the steroidal A–C ring mimetics and were tested against human and rat 5α-reductase type I and II. Two compounds were found to be most potent with compound 376 showing an IC$_{50}$ value of 60nM while 377 showed an IC$_{50}$ value improved by a factor of 5 from 1.9μM to 0.38 μM in comparison with the parent biphenyl compound 378.$^{454}$

![Chemical Structure](image3)

![Chemical Structure](image4)
Baston et al. synthesized several 3,4-dihydro-naphthalene-2-carboxylic acids and evaluated them for 5α-reductase inhibitory activity. The most active inhibitors were 6-[3-\((N, N\)-dicyclohexyl aminocarbonyl) phenyl]-3, 4-dihydro-naphthalene-2-carboxylic acid (379) (IC\(_{50}\) = 0.75\(\mu\)M, human type II; IC\(_{50}\) = 0.81\(\mu\)M, human type I) and 6-[4-\((N, N\)-diisopropylamino-carbonyl) phenyl] naphthalene-2-carboxylic acid (380) (IC\(_{50}\) = 0.2\(\mu\)M, human type II). The latter compound was shown to deactivate the enzyme in an uncompetitive manner (K\(_i\) = 90 nM; K\(_m\), T = 0.8–1.0 \(\mu\)M) similar to the steroidal inhibitor Epristeride (184).

Novel substituted benzoyl benzoic acids and phenylacetic acids were synthesized by Salem et al. based on the template structure 369 and were evaluated for the inhibition of rat and human steroid 5α-reductase isozymes I and II. The phenylacetic acid derivatives were more potent than the analogous benzoic acids.

Bromination in the 4-position of the phenoxy moiety led to the strongest inhibitor of the series against human 5α-reductase II (383; IC\(_{50}\) = 5 nM), which was...
equivpotent to Finasteride (35) while compounds 381 (IC_{50} = 23 nM) and 382 (IC_{50} = 27 nM) were also found to potent inhibitors against 5α-reductase type II.456

Kato and co-workers synthesized a series of pyrrole butyric acid derivatives and evaluated them for inhibitory activity on human and rat steroid 5α-reductase. In case of para-aminobenzoyl pyrrole derivatives (384-387), the introduction of ethyl (385) or isopropyl (386) at C-4 increased the activity [IC_{50} being 32 and 9.2 respectively], whereas the replacement with carboxyl (387) resulted in compounds with decreased inhibitory activity against human 5α-reductase enzyme, indicating steric restriction in the binding site of the enzyme. Compound with m-amino benzyl pyrrole (388) moiety was found to be more active than the corresponding para isomer (386) [IC_{50} = 3.2 nM].

![Chemical structure of compounds](image)

(384) R=H, n=3, side chain at p position
(385) R= C_2H_5, n=3, side chain at p position
(386) R= CH(CH_3)_2, n=3, side chain at p position
(387) R=COOH, n=3, side chain at p position
(388) R= CH(CH_3)_2, n=3, side chain at m position

(389) R= Hexyl, M=1/2 Ca

Compound 389, having 2-hexyloctylamino group, was found to be most potent inhibitor among the compounds with IC_{50} being 0.60 nM against human and 5.8 nM against rat 5α-reductase.457
A novel series of indole-3-alkanoic acids with varied N-benzyl substituents were also synthesized. Amongst these 4-[1-(6, 6-dimethyl-6H-dibenzo[b,d]pyran-3-y1) methyl indol-3-yl]-butyric acid (390; FR119680) displayed very high inhibitory activity against rat prostatic 5α-reductase (IC₅₀=5.0nM). ⁴⁵⁸

![FR119680 (390)](image)

Igarashi et al. found a novel series of phenoxybenzoic acids derivatives as potent inhibitors of human prostatic 5α-reductase. It was found that introduction of a chloro (391), fluoro (392) or methoxy (393) group at 3-position of benzene ring (R₁) leads to formation of compounds with high inhibitory activity with IC₅₀ being 0.87, 0.67 and 0.56 nM, respectively. ⁴⁵⁹

![Structures](image)

A series of indoline and aniline derivatives have also been synthesized so as to inhibit both human and rat prostatic 5α-reductase. Among the indoline series, 3-chloro-4-[(1-(4-phenoxybenzyl)indolin-5-yl]oxy]benzoic acid (394; YM-36117) was found to be the most potent inhibitor against human enzyme having an IC₅₀ value of 5.3nM and 46 nM against the rat enzyme while in aniline series, 3-chloro-4-{4-[N-(4-phenoxybenzyl)amino] phenoxy}benzoic acid (395) turned out to be most potent inhibitor with an IC₅₀ against human and rat enzyme as 10 and 5.5 nM, respectively. ⁴⁶⁰
Bisubstrate inhibitors

Ishibashi et al. synthesized a series of novel benzofuran derivatives with both carboxy and 5- or 6-diphenylmethylcarbamoyl groups and their inhibitory activities against rat and human testosterone 5α-reductase were tested in vitro. The derivatives were more active against human type I enzyme than against type II enzyme. The 6-carbamoyl derivative such as 396 tended to be more potent than the 5-carbamoyl ones such as 397 with 396 being the most potent compound having IC$_{50}$ value of 37.9, 50 and 340 nM against rat, human type I and human type II isozymes.\(^{461}\)

Later, they also synthesized a series of 2-phenylbenzofuran derivatives with a carbamoyl, alkylamino, or alkyloxy group at the 5 or 6 position of the benzofuran ring. It was found that carbamoyl derivatives had more potent inhibitory activities than the alkylamino or alkyloxy derivatives against the rat enzyme and the 6-carbamoyl derivatives tended to be more potent than the 5-carbamoyl ones. The 6-carbamoyl and 6-alkylamino derivatives were found to be more potent inhibitors against human type I enzyme than type II but on whole compounds were found not to be selective.\(^{462}\) The nonsteroidal o-hydroxyaniline (292; ONO-3805) was a weak compound in vitro versus human 5α-reductase II. It is a bi-substrate inhibitor in which the butanoic acid moiety is thought to be localized in the region of the phosphate group of NADPH and the lipophilic part could be orientated in the region of the steroidal C and D ring, thus occupying the hydrophobic pocket of the enzyme.
The fact that this compound acts as a non-competitive inhibitor (versus T) and not as uncompetitive one, supports this hypothesis. This prompted Pfizer to prepare the derivatives of 292 and subsequently C-3 acylindole (398) was prepared which had improved potency versus both human 5α-reductase enzymes. The benzodioxolane (399) adopts a similar minimum conformation to the ether (398) and proved to be a potent dual inhibitor of both 5α-reductase enzymes. In common with the steroidal carboxylic acid inhibitors, these compounds require the carboxylic acid moiety for potency and the 3-acylindole motif was found to be crucial for dual activity presumably by allowing access to both the conformations 400 and 401. The corresponding 2-methyl analog 402 which adopts conformation 401 due to the presence of methyl group on the indole ring was found to be a selective inhibitor of 5α-reductase I (Table 40).  

FK-143 (403) 4- [3- [3- [bis (4- isobutylphenyl) methyl amino] benzoyl]-l H-indol-l-yl] butyric acid was disclosed by Sawada et al. as a potent dual inhibitor of both human 5α-reductase isozymes. It inhibited in vitro human and rat prostatic 5α-reductase in a dose-dependent manner with an IC50 of 1.9 and 4.2 nM respectively in a noncompetitive fashion while in vivo showed potent inhibitory activity against castrated young rat model. This compound can be a potential drug for the treatment of benign prostatic hyperplasia.

Table 40: In vitro screening of compounds 398-402 against human 5α-reductase I and II and rat 5α-reductase

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Rat 5α-reductase IC50(nM)</th>
<th>Human Type I 5α-reductase IC50(nM)</th>
<th>Human Type II 5α-reductase IC50(nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>398</td>
<td>1</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td>399</td>
<td>9</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>402</td>
<td>588</td>
<td>10</td>
<td>6,300</td>
</tr>
<tr>
<td>ONO-3805(292)</td>
<td>1.7</td>
<td>-</td>
<td>256</td>
</tr>
</tbody>
</table>

Sawada et al. University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh

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Introduction

Miscellaneous non-steroidal inhibitors

In search of novel nonsteroidal mimics of steroidal inhibitors of 5α-reductase, 4-(2-phenylethyl)cyclohex-1-ene carboxylic acids were synthesized with different substituents in para position of the phenyl ring such as N, N-diisopropylcarbamoyl (404), phenyl, phenoxy etc. They turned out to be good inhibitors of the human prostatic 5α-reductase isozyme II with 404 being the most potent one (IC$_{50}$ = 760 nM).

Fan et al. evaluated a series of umbelliferone (7-hydroxycoumarin) derivatives as inhibitors of 5α-reductase I on LNCaP cells. The coumarin skeleton was considered as a mimic of the proposed transition state of the natural substrate and as well as bioisostere of quinolin-2-one. 1',1'-dimethylallyloxycoumarin (405) showed potent inhibitory activity (IC$_{50}$=1.3 μM) for the 5α-reductase I.

This was possibly a result of conformational effects of geminal dimethyl group. 8-allyl-7-hydroxycoumarin (406) also exhibited potent inhibitory activity (IC$_{50}$=0.99
Introduction

μM) against 5α-reductase I enzyme. Introduction of a carbonyl group at 7-position (407) resulted in only a slight increase in 5α-reductase I inhibitory activity (IC50=0.49 μM). 470

Due to the excellent estrogen receptor binding affinity of a series of 2’, 6’-disubstituted 4-hydroxy-4’-hydroxymethyl biphenyl derivatives Lesuisse et al. designed various biphenyls as surrogates of the steroidal backbone. They hypothesized that by introducing appropriate substituents non steroidal estrogens could be tailored into 5α-reductase inhibitors. Two compounds (408 and 409) emerged as potent type II 5α-reductase inhibitors with IC50 being 71 and 9.8 nM, respectively. 471

Chen et al. evaluated isoflavonoids as potential nonsteroidal inhibitors of rat 5α-reductase by using the hypothetical pharmacophore of 5α-reductase. They proposed that although these compounds (410-413) were inhibitors of rat 5α-reductase in the range of 27-49 μM they could be evaluated as human 5α-reductase inhibitors. 472
Hosoda et al. in 2007 designed and synthesized novel type I 5α-reductase inhibitors by using 3, 3-diphenylpentane skeleton as a substitute for the usual steroid skeleton. 4-(3-(4-(N-Methylacetamido) phenyl) pentan-3-yl) phenyldibenzylcarbamate (414) was found to be a competitive 5α-reductase type I inhibitor with the IC$_{50}$ value of 0.84 µM among the series.$^{473}$

5. Androgen Deprivation Therapy

Antiandrogen therapy can be directed toward any of the regulatory steps in androgen production or action (Figure 21). Therefore, an understanding of antiandrogen effects must include some insight into the regulation of the pituitary-gonadal axis, and the action of androgen in the prostate. Hypothalamic-pituitary peptide hormones mediate the control of androgen production in the testis.

Neurons in the preoptic area of the hypothalamus secrete gonadotropin-releasing hormone (LHRH), a decapeptide that subsequently interacts with high-affinity cell surface receptor sites on the plasma membrane of pituitary cells. LHRH stimulates the release of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by a calcium-dependent mechanism. LH secretion is controlled by the action of androgens and estrogens on the hypothalamus and pituitary.$^{474}$

The control of LH in men occurs primarily by negative feedback since testicular steroids inhibit LH secretion. Both testosterone and estradiol can inhibit LH secretion. Although testosterone can be converted to estradiol in the brain and pituitary, the two hormones probably act independently. Testosterone acts on the
central nervous system (CNS) to slow the hypothalamic pulse-generator and consequently decreases the frequency of LH pulsatile secretion. In addition, testosterone appears to have negative feedback action on LH secretion in the pituitary. LH reaches the testis via the peripheral circulation, where it interacts with specific high-affinity cell surface receptors on the plasma membranes of the Leydig cells. The binding of LH to its receptor stimulates biosynthesis of testosterone.°

**Figure 21: Antiandrogen approaches in BPH**

In peripheral tissues, testosterone may act directly (e.g., in the CNS, skeletal muscle, and seminiferous epithelium), or serve as a circulating prohormone for the formation of DHT (e.g., prostate) and estrogen.°° In the prostate, testosterone (23) diffuses into the cell, where it is reduced by 5α-reductase to DHT (24). Ninety percent of total prostatic androgen is in the form of DHT, principally derived from testicular androgens. The remaining 10% of the prostatic androgens are produced in the adrenal glands. Inside the cells of the prostate, both testosterone and DHT bind to the same high-affinity androgen-receptor protein. The hormone-receptor complex then binds to specific DNA binding sites in the nucleus of prostatic cells. This results in increased transcription of androgen-dependent genes and ultimately stimulation of protein synthesis. Conversely, androgen withdrawal from androgen-sensitive tissues results in a decrease in protein synthesis, tissue involution, and in some cases, cell
The biological basis of androgen ablation lies in the observation that the vast majority of natural and malignant prostate cells undergo programmed cell death when deprived of androgen.\textsuperscript{477}

Androgen deprivation decreases the size of prostate and resistance to outflow through the prostate urethra and the ability of many patients to urinate improves (Figure 22).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{androgen_receptor_signalling_pathway.png}
\caption{Androgen receptor signalling pathway \textsuperscript{478}}
\end{figure}

The androgen receptor (AR), a member of steroidal hormone receptor subfamily, is responsible for signal transduction of 5α-DHT.\textsuperscript{479} Androgen receptor (AR) belongs to steroid hormone receptor transcription factor superfamily.\textsuperscript{480}

In the absence of ligand, heatshock proteins (HSP-70 and HSP-90) bind to AR and protect it from degradation.\textsuperscript{481} Androgens bind to the ligand-binding domain (LBD) of inactive AR in cytoplasm and induce a conformational change in the receptor structure that leads to dissociation of heat shock proteins and receptor dimerization. Ligand-binding also causes AR phosphorylation, which stabilizes ligand/receptor complex. The dimerized AR then moves into the nucleus, binds to

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androgen-response element in the promoter region of target genes, and regulates their transcription. DHT is the most potent ligand for AR and is obtained from testosterone by the enzyme 5α-reductase.\textsuperscript{482, 483}

### Anti-Androgens

Androgen receptor functions as ligand dependent transcription factor. The receptor contains three functional domains: an amino terminal trans activating domain, a DNA binding domain and a carboxy terminal ligand binding domain. A hinge region between DNA and ligand binding domain centre is important for nuclear trafficking.\textsuperscript{484} Since an essential step in the action of androgens in target cells is binding to the androgen receptor (Figure 23), a logical approach for neutralizing the androgens is the use of antiandrogens or compounds which prevent the interaction of natural ligands T and DHT with the androgen receptor. 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. DHT has a more potent binding affinity to the androgen receptor. Prostatic epithelial cells are also able to convert the weaker adrenal androgens to DHT.\textsuperscript{485}

![Diagram of Androgen Receptor](image)

**Figure 23: Androgen Receptor**

Anti-androgens are competitive inhibitors that prevent the natural ligand of AR from binding to the receptor. The anti-androgens are divided into steroidal and nonsteroidal anti androgens.\textsuperscript{486}

#### a. Steroidal Anti-Androgens

They are weak partial agonists and competitive inhibitors of the AR in the target tissue. In addition they have progestational agonistic property that causes negative feedback at the level of pituitary which lowers LH secretion. Consequently, LH stimulated testosterone production decreases. The loss of libido and decreased sexual potency are the common effects.
Cyproterone acetate (415) an analogue of hydroxyprogesterone acts as a competitive androgen receptor inhibitor and it inhibits 5α-reductase activity. In addition, it suppresses serum gonadotropin and androgen levels and increases hepatic clearance of testosterone.\(^487\)

![Chemical structure of Cyproterone acetate (415)](attachment)

Chlormadinone acetate (416) is a derivative of naturally secreted progesterone that shows high affinity and activity for the progesterone receptor. It has an anti-estrogenic effect and, in contrast to natural progesterone, shows moderate anti-androgenic properties. Chlormadinone acetate acts by blocking androgen receptors in target organs and by reducing the activity of skin 5α-reductase. It suppresses gonadotropin secretion and thereby reduces ovarian and adrenal androgen production.\(^488,489\)

![Chemical structure of Chlormadinone acetate (416)](attachment)

b. Non-Steroidal Anti-Androgens

They are competitive inhibitors of androgen binding without clinically significant agonistic activity at the AR in vivo. They block testosterone binding to AR in CNS and this interrupt the negative feedback of testosterone on gonadotrophin secretion. They cause a rise in plasma estradiol levels due to the increased aromatization of testosterone and they maintain libido and potency due to the elevated or normal level of plasma testosterone.

Toluidide derivatives are considered as pureantiandrogens since they posses little or no intrinsic androgenic activity when bound to AR. They do not have cross reactivity with any other steroidal receptors. The non-steroidal antiandrogens that are used clinically for BPH are Flutamide (417), Nilutamide (418) and Bicalutamide (419).
The in vivo antiandrogenic activity of bicalutamide arises almost entirely from its R-isomer that has approximately 30-fold greater binding affinity than the S-isomer. Bicalutamide in a dose of 25 mg/day proved to be effective in women with severe hirsutism. Anti-androgens in combination with surgical or medical castration has been widely used for the BPH treatment.490

COMBINATION THERAPY

Combination of α-adrenergic blocker with 5α-reductase inhibitors

5α-reductase inhibitors or α-adrenergic blockers alone cannot provide rapid symptom relief in those patients who have bothersome symptoms. A hypothesis was proposed that a short-term combination therapy with α1-blocker and a 5α-reductase inhibitor for BPH could provide a rapid onset of symptomatic relief (α1-blocker) and patient comfort while initiating modification of the underlying disease process (5α-reductase inhibitor). Treatment alone with α1-blockers provides rapid symptom relief but does not have any impact on the underlying disease. Combination therapy of both would help optimizing treatment for patients with large prostates and bothersome LUTS thereby providing early relief of symptoms and long-term lasting relief with a reduction in prostate volume.491

Medical Therapy of Prostatic Symptoms (MTOPS) study recruited 3047 men with BPH and investigated combination therapy with the 5α-reductase inhibitor
Finasteride and the α1-blocker doxazosin over 4 years and compared its efficacy with that of placebo and the two drugs used in monotherapy. The reduction in risk of BPH progression was found to be 67% with combination treatment, 39% with doxazosin treatment and 34% with finasteride treatment. Doxazosin monotherapy delayed the time to progression of AUR and the need for invasive therapy, but did not reduce the overall risk of either event. In addition, in selected patients, there appeared to be additive symptomatic benefits of receiving long-term combination treatment with a 5α-reductase inhibitor and an α1-blocker, as symptom deterioration resulting from underlying BPH progression was less in patients receiving the combination than in those receiving either monotherapy.492

The Symptom Management After Reducing Therapy (SMART-1) study was designed to examine short-term combination therapy with an α1-blocker tamsulosin and dutasteride, followed by removal of the α1-blocker and continuation of dutasteride monotherapy. It aimed to provide physicians with information on how to manage patients with symptomatic BPH using combination therapy. Results suggest that patients with more severe symptoms may benefit from an initial period of combination therapy longer than 24 weeks before withdrawal of tamsulosin.493

The 2003 AUA management guideline for BPH recommends that combination therapy is appropriate and effective treatment for patients with LUTS who have enlarged (>40 g) prostate glands. Also those patients who would most likely to be benefited from this combination therapy are those with larger prostate glands (>40 g) and higher PSA values (>4 ng/mL).494

**Combination of antiandrogens with 5α-reductase inhibitors**

Antiandrogens have also been evaluated in combination with finasteride (Proscar) as androgen ablative therapy for patients with androgen dependent advanced disease. It is hoped that this combination of drugs may provide effective AR blockade without the undesirable side effects of castrate testosterone levels on muscle and bone mass, energy level and libido. The effects of long-term castrate testosterone levels are of particular concern with regards to skeletal complications. The combination of flutamide 150 mg TID and finasteride 5 mg/day has been evaluated in several efficacy trials in hormone therapy naïve patients.495,496
Emerging Treatments for BPH

Storage lower urinary tract symptoms are often associated with bladder outlet obstruction due to benign prostate hyperplasia (BPH) in men. The pathophysiology of male LUTS that is suggestive of BPH is multifactorial. Recent investigations have shown that multiple pathways are involved in bladder dysfunction.

Therefore, several novel strategies to treat BPH and LUTS have been proposed and are under clinical trials.\(^{497, 498}\)

1. LHRH agonists

Testosterone production is controlled by the higher centers of the brain in which hypothalamus and pituitary are of paramount importance. Pulsatile release of luteinizing hormone releasing hormone also known as LHRH stimulate the release of LH and follicle stimulating hormone (FSH), which subsequently control the hormonal and reproductive function of the gonads. So, targeting the upper brain centers was sought as an option to suppress the androgen stimulated growth of the prostate. The therapeutic methods involve the use of LHRH agonists like busel, leuprolide, goserelin, histrelin, deslorelin, triptorelin and nafarelin that inhibit the secretion of lutenizing hormone from the anterior pituitary gland that results in the decreased testosterone production from the leydig cells of the testis. Although they are indicated for prostate cancer, endometriosis and breast tumor but none of them is still approved for BPH although goserelin, busel, leuprolide have undergone clinical trials with limited success but various side effects like erectile dysfunction, decreased libido, hot flushes marred their success.\(^{27}\)

2. LHRH (GnRH) antagonists

These are the compounds that are the competitive ant-agonists of the GnRH receptor, which causes an immediate and rapid, reversible suppression of gonadotropin secretion. There are several LHRH antagonists available, namely degarelix, tevarelix, cetrorelix and ozarelix. Cetrorelix and Teverelix are indicated for BPH and are in phase II clinical trials. Another molecule D-63153 is a decapeptide and is currently undergoing phase II clinical trials for BPH. Good efficacy along with tolerability and no flare up response made them ideal candidates for treatment of BPH but they are marred by poor oral bioavailability.\(^{499}\) There are LHRH receptors in human prostate tissue also and it is thought that LHRH antagonists work by reducing
prostate volume and hence work on the static component of obstructive symptoms of LUTS.\textsuperscript{500, 501}

3. COX-2 / 5-LOX inhibitors

Cyclooxygenase (COX-2) and Lipooxygenase (5-LOX) are the arachidonic acid metabolizing enzymes that have been implicated in variety of cancer, including BPH. This approach has led to the identification of new molecular targets in cancer prevention. Number of diarylpyrazole based derivative have been designed as COX-2/5-LOX inhibitors and have been found to mediate aptoptic death in cancer cells.\textsuperscript{502}

4. Hexokinase inhibitors

The secretory epithelial cells of human prostate possess a unique citrate related metabolic pathway regulated by testosterone and prolactin which is distinguishable from the rest of cells in the body. Normal and BPH prostates accumulates high levels of zinc in addition to citrate which inhibits the m-aconitase activity, which thereby inhibits the citrate oxidation into isocitrate which essentially truncates the Kreb’s cycle which is a major ATP producing pathway. Prostate cells depend highly on the glycolysis for energy production and hexokinase inhibitor by inhibiting hexokinase enzyme of glycolysis impairs the energy production of prostate cells. Lonidamine (420) an oral hexokinase inhibitor inactivates glycolysis resulting in cell death. Lonidamine is in phase III studies. Lonidamine (420), an orally administered small molecule that inhibits glycolysis by the inactivation of hexokinase, may represent a unique and novel approach to the treatment of BPH.\textsuperscript{503}

![Lonidamine](image)

5. Oxytocin antagonists

Stromal and epithelial cells of the prostate contain oxytocin receptors and hence oxytocin has been implicated in the regulation of prostate growth. It may acts as autocrine or paracrine mediator to regulate the prostate growth by either binding
Introduction

to Gq-coupled receptor leading to cell proliferation or due to its regulatory activity secondary to its effect on the enzyme 5α-reductase converting testosterone into dihydrotestosterone. SSR-126768 is an oxytocin antagonist implicated in BPH. Although it has high potency and good bioavailability but it may cause side effects like problem in erection and ejaculation.27, 504

6. Vitamin D₃ (calcitriol) analogues

Human prostate cells express Vitamin D receptor (VDR) and respond to VDR agonists by decreasing their proliferation. Therefore, VDR agonists could represent a novel option for the treatment of BPH. However VDR agonist induces hypercalcemia and hyperphosphatemia. Hence, non-hypercalcemic 1,25-dihydroxyvitamin D₃ (421) analogues could represent good candidates to become novel and attractive therapeutic agents for BPH. BXL-628 (elocalcitol, 1-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalficiferol;422) is an analogue that decrease testosterone-stimulated human BPH cell proliferation and promoted BPH cell apoptosis even in the presence of growth factors.505

![1,25-dihydroxyvitamin D₃ (421) and BXL-628 (422)](image)

7. NX-1207

NX-1207 involves a new targeted approach to the treatment of BPO. It was originally identified from research into Alzheimer’s disease treatments. NX-1207 is injected directly into the zone of the prostate where the enlargement occurs.506

8. PRX302

PRX302 is a pore-forming pro-drug that is activated by specific proteases produced at elevated levels on the surface of target cells. PRX302 has been generated by engineering the naturally occurring toxin pro-aerolysin so that it is
activated by PSA. Once activated, the drug punches holes in the cells causing the contents to leak out and ultimately cell death.507

9. Botulinum toxin A

At the neuromuscular junction, Botulinum toxin A (BoNT-A), blocks the acetylcholine. Several mechanisms have been proposed for the method of action in the prostate and improvement in LUTS/BPO. It could induce relaxation of the prostate, atrophy of striated and smooth muscle fibers, and reduction in prostate size through inhibition of the trophic effect of the ganglionic and post-ganglionic fibers of the autonomic system on the prostate gland or through inhibition of sensory afferents from the prostate to the spinal cord.508, 509

10. Vanilloid Receptors

The role of vanilloid receptors such as transient receptor potential vanilloid (TRPV-1) in the pathophysiology of neurogenic or idiopathic detrusor over activity has been recognized. C-fiber input plays an important role in the generation of the urgency sensation. Resiniferatoxin (RTX) is the sole compound with a desensitizing effect suitable for human use. Furthermore, bladder instillation of vanilloid agents, such as capsaicin and RTX, resulted in increased bladder capacity and decreased urge incontinence.510

11. Endothelin Antagonists

Endothelin-1 (ET-1) is an endogenous vasoactive peptide that binds two receptor subtypes, the endothelin-A (ETA) and endothelin-B (ETB) receptors. ET-1 can directly activate C-fiber afferent nerves in the bladder, and suppression of ETA receptors might be effective for the treatment of detrusor over activity by reducing activation of C-fiber afferent pathways. Bladder hypertrophy is common in various pathologies of the bladder or its outlets, such as BPH or neuropathic bladder.511

12. NSAIDs

It has been suggested that NSAIDS may prevent or delay development of BPH and its consequences. Celocoxib512 and loxoprofen513 have been successful in the treatment of nocturia secondary to BPO. They can also play a role in the inflammatory process involved in the development of BPH/BPE/BPO.
13. Antimuscarinic agents

Anticholinergic drugs are competitive antagonists of bladder muscarinic receptors. During bladder filling, the drugs suppress involuntary detrusor contractions caused by basal release of neuronal and urothelial acetylcholine. During the voiding phase, massive parasympathetic outflow and acetylcholine release might overcome the effects of antimuscarinic medication. Recent studies have demonstrated both the effectiveness and safety of antimuscarinic drug and α-antagonist combinations, improving urodynamic and patient-reported outcomes in men with LUTS and BPH.497,498,514

14. β3-Adrenoreceptor Agonists

Real-time reverse transcription–polymerase chain reaction analysis revealed the predominant expression of β3-adrenoreceptor (AR) mRNA in the human detrusor muscle, which was 97% of total β-ARs. Functional studies have demonstrated that AR agonist-evoked relaxation is mediated primarily by β3-ARs in human bladder tissue.515,516 In vivo studies indicate that β3-AR agonists increase bladder capacity with no change in micturition pressure and residual volume, indicating that β3-AR agonists are useful in storage symptoms secondary to obstructed bladder.517

15. Progestogens

There are two progestogens known to be used (mainly in Japan) for the treatment of LUTS/BPO, allylestrenol and chlormadinone acetate (CMA). These are thought to act by anti-androgenic mechanisms that cause prostatic atrophy.518,519

16. PDE5 inhibitors

Erectile dysfunction, ejaculatory disorders and pain during intercourse are correlated with the severity of LUTS. Several pathophysiology common to LUTS and ED, such as age, atherosclerosis-induced vascular insufficiency in the bladder and corpora, and autonomic nervous system hyperactivity, have been reported and are potentially mediated by nitric oxide pathways. In addition, there are significant amounts of phosphodiesterase types 4 and 5 in the transition zone of the prostate. Given the potential common etiology of LUTS and ED, phosphodiesterase-5 inhibitors and α-blockers are advantageous to both diseases.520,521 There are three
PDE5 inhibitors (PDE5I), namely tadalafil (Cialis), vardenafil (Levitra) and sildenafil (Viagra), used in the treatment of ED. Both BPO and ED increase with age and PDE5 inhibition mediates smooth muscle relaxation in the lower urinary tract, making the use of PDE5I a potential for the treatment of LUTS/BPO. The exact mechanism by which these drugs affect LUTS is poorly understood but they may reduce the smooth muscle tone of the prostate, inhibit human stromal cell proliferation of the prostate mediated by cGMP accumulation, as well as produce vascular changes.\(^{522}\)

17. MISCELLANEOUS

(i) CH5036249, a novel non secoestroidal VDR agonist, exhibits favorable characteristics with potential as a new drug candidate for the treatment of BPH. At the very low dosage of 0.03µg/kg, the compound showed efficacy in a spontaneous BPH beagle model 2. A Phase I randomized, double-blind, placebo-controlled clinical trial of pomegranate tablets versus placebo is underway to look at the effects on symptoms suggestive of BPO (NCT00381108).\(^{523}\)

(ii) Photodynamic therapy

It involves creating localized tissue necrosis with light, most conveniently from a low power red laser after prior administration of photosensitizing agent, thereby initiating a non-thermal cytotoxic effect and tissue necrosis. Porfimer sodium is in discovery phase for BPH although it is used for lung and bladder tumour. Another molecule belonging to the third generation photosensitizers, lemuteporfin is undergoing phase II clinical trials for BPH but this therapy is associated with poor patient compliance and may cause local burning, itching or blistering at the site of the skin.\(^{523}\)

(iii) Gene therapy

The discovery of novel proprietary gene containing a prostate tissue specific enhancer (PSE) and creation of organ specific attenuated adenoviruses prompted Calydon to develop CN-73X series which is an attenuated adenovirus series which may have a potential role in BPH. But high cost, risk of insertional mutagenesis, inefficient DNA transfer to human cells locally and at distant sites along with the lack of viral specificity marred this technique.\(^{27,524}\)
Current status of 5α-reductase inhibitors in the management of lower urinary tract symptoms and BPH

Safety-adverse events

5α-Reductase inhibitors are well tolerated and have only minimal side effects. The most common adverse events (AE) are sexual dysfunction including, reduced libido, erectile dysfunction and, less frequently, ejaculation disorders which occur more frequently in recipients with 5α-reductase inhibitors than with placebo. Gynecomastia appears in approximately 1-2% of patients. The new onset of most drug-related adverse effects usually arises within the first year of treatment, and new-onset generates after the first year are often similar to those among patients receiving placebo.525, 526

Finasteride (35) and Dutasteride (49) are the only two steroidal clinically used drugs that have evolved from research on steroids as 5α-reductase inhibitors but many compounds have shown promising results such as Epristeride (184). Although both are 5α-reductase inhibitors, their pharmacologic and clinical profiles are different. Key differences of both the drugs are listed in Table 41

Table 41: Key Pharmacokinetic and pharmacodynamic characteristics of 5α-reductase inhibitors

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dutasteride</th>
<th>Finasteride</th>
</tr>
</thead>
<tbody>
<tr>
<td>5α-reductase inhibition target</td>
<td>Type 1 and 2</td>
<td>Type 2</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Liver</td>
<td>Liver</td>
</tr>
<tr>
<td>Daily dose(mg)</td>
<td>1×0.5</td>
<td>1×5.0</td>
</tr>
<tr>
<td>Oral Bioavailability (%)</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>Time for peak serum concentration(h)</td>
<td>1-3</td>
<td>2</td>
</tr>
<tr>
<td>Elimination half life</td>
<td>5 weeks</td>
<td>6-8 h</td>
</tr>
<tr>
<td>Plasma protein binding (%)</td>
<td>99.5</td>
<td>90</td>
</tr>
</tbody>
</table>

METHODS OF BIOLOGICAL EVALUATION

Till date a number of in-vitro and in-vivo models are available for biological evaluation of 5α-reductase inhibitory activity in both humans and animals. A brief summary of these methods has been described below.
I. In Vitro ASSAY METHODS

(i) Inhibition of 5α-Reductase

5α-Reductase can be prepared from prostates of various species, such as human, dog or rats. Prostatic particulates are prepared from either of the species which is under consideration by homogenizing, mincing and treating in a given set of conditions. For the 5α-reductase assay, reaction solutions are prepared in duplicate tubes containing 1 μM \([14C] \) testosterone. Further the test compounds or standard as inhibitors are added in 5 μl ethanol at concentrations between \(10^{-9}-10^{-5}\) M. The control tubes receive the same volume of ethanol. This is followed by the addition of the above mentioned prostate particulates. The reactions are linear for at least 1.0 hr at 37 °C. The reactions are carried out for 10-30 min. and are stopped with 2.0 ml of ethyl acetate containing testosterone, 5α-dihydrotestosterone, and androstanedione (10 μg each). The supernatant is then removed, centrifuged and analysed using chromatography. The radioactivity profiles are determined by scanning the plates or by scraping the silica in sections and counting in a scintillation counter. 5α-Dihydrotestosterone is the only radioactive product for the rat and human enzymes, however, a set of three products are observed in the case of dog enzymes. The radioactivities of the first 3 products are combined for the calculation of the 5α-reductase activity. IC\(_{50}\) values are calculated based on at least 5 dilutions of test preparations or standard. 527

(ii) DU-145 Assay Method

In vitro inhibitory activity of the compounds can be evaluated using DU-145, PC-3 and SW-13 as a source of 5α-reductase type I and type II. It is carried out by incubating different concentrations of the compounds along with \([H^3]\) androstenedione.190,528 After 24 hr incubation, media is extracted twice with solvent ether and different steroids can be separated by TLC. Radioactivity is counted and results expressed as the amount of the androstenedione, androsterone and epiandrosterone formed as a percentage of the control value.

(iii) Penicillium crustosum Broth Method

The in vitro biological activity of compounds can be determined by measuring the transformation of testosterone (T) to dihydrotestosterone (DHT) produced by 5α-
reductase enzyme in *P. crustosum* broths. Conversion of T to DHT has been demonstrated in *P. decumbens* and *P. crustosum* broths obtained from fermented pistachios, lemons and corn tortillas. For the determination of the biological activity of the new steroids, as 5α-reductase inhibitors, the conversion of T to DHT in *P. crustosum* broths is recorded. The conversion of radiolabeled T to DHT in the incubated medium increases significantly as compared to T plus Finasteride (35) or a combination of test steroids with testosterone (T). The conversion of T to its metabolite DHT is determined by the reverse isotope dilution technique. Thus, this model indicates the efficacy of the newly synthesized compounds in inhibiting the conversion of T to DHT.

(iv) HEK 293 Cells Method

In this method human embryonic kidney cell line HEK293, which lacks endogenous 5α-reductase activity was transfected with the cDNA for either of the isoforms of 5α-reductase i.e. type I or II. Stable clones were selected, tested on enzyme activity and established as permanent cell lines. The cell lines were used to test selected compounds as well as the steroidal inhibitors such as Finasteride (35) used as control. Using this strategy dual and selective inhibitors of both 5α-reductase isozymes could be identified. HEK293 cells (300,000/ well) transfected either with pRcCMV-I or with pRcCMV-II were seeded in a 24-well tissue culture plate and incubated overnight to allow attachment of the cells. Lysates are obtained after harvesting and resuspending 80% confluent cells in homogenate buffer (containing 300mM saccharose, 5 mM Tris-HCl and 0.1 mM EDTA) followed by homogenization using ultrasonication. Suspensions of both cell lines were used for all following assays. In the inhibitor assays, the compounds dissolved in DMSO were mixed with androstenedione (test concentration containing 3H androstenedione: 500 nM), which served as a substrate, NADPH regenerating system (containing NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase) and tris buffer. To start the incubation one volume of the cell suspension was added to a total volume of 500 μl. After an incubation of 30 min at 37 °C, the reaction was stopped by the addition of ether. The steroids were extracted, dried and resuspended in methanol followed by radioactivity HPLC based detection. The amount of converted tritiated...
androstenedione was measured for each sample which served to determine the inhibitory activity of the compounds.530-531

II. In Vivo ASSAY METHODS

(i) Chicken Comb Method

This classical bioassay based on growth of the capon comb has been used by many authors for androgenic activity and found to be extremely useful for the isolation and structural elucidation of natural androgens. Newly hatched chicks of either sex have been used to study the growth of combs after systemic or local administration. White Leghorn chicks are used at an age of 2-3 days. They are kept in a brooder with a thermostatic control. An oily solution (0.05 ml) of the test compound or the standard is applied on the comb daily for a period of 7 days. Twenty-four hours after the last application, the animals are autopsied. Body weights are determined. The combs are removed by two longitudinal incisions along the base of the comb at its juncture with the scalp. The comb is freed from the scalp, touched lightly on a towel to remove blood and weighed. Dose-response curves are established.532-533

(ii) Hamster Flank Organ Test Method

Flank organs of the gonadectomized Syrian Golden male hamsters (150-200 g) are also used for the screening of new antiandrogenic drugs. Hamster flank organs are dorsal spots on the skin that are composed of pilosebaceous tissue. 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 testosterone or progesterone stimuli. In the 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 testosterone restored the original diameter of the spot. The flank organs can convert testosterone (T) to dihydrotestosterone (DHT) in both intact and gonadectomized animal since the 5α-reductase enzyme is present in this tissue. Varying doses of the test compound and the control i.e. testosterone is administered via subcutaneous route. After these treatments, the animals were sacrificed by ether anesthesia. Both flank organs of the animals were shaven and the diameter of the pigmented spot was measured.
test compounds such as Finasteride (35) significantly reduces the diameter of the pigmented spot on the flank organs as compared to that of the testosterone one.

Testosterone increases the rate of incorporation of labelled glucose or sodium acetate into lipids, principally in glycerides and fatty acids and decreases the polar lipids synthesis in flank organs. Hence the effect of the hormone treatments on radioactive sodium acetate incorporation into lipids under culture condition can also be evaluated to establish the efficacy of the test compound relative to known compounds.404,416,534

(ii) Scrotal Incision Method

In this method, male rats are castrated by scrotal incision under ether anesthesia. Varying doses of the test compounds viz., of 9.2-11.4 mg/kg (in oil or vehicle), Finasteride (1 mg/kg) and testosterone propionate (1 mg/kg) are applied to these animals by separate subcutaneous injections once daily for 4 days. Twenty four hours after the last application, the rats are sacrificed by CO2 inhalation, and the ventral prostates of all the animals are removed. The mean percentage of inhibition of the T-induced hypertrophic response in these organs is calculated according to the following equation:

\[
\% \text{ Inhibition} = 100 \times \frac{(C_t - D)}{(C_t - C_c)}
\]

where \( C_t \), \( C_c \), and \( D \) are the mean prostate weights of T-treated control, castrated control and drug treated groups, respectively. Furthermore, the suitable statistical studies are performed to establish the statistical significant difference between the known molecules (testosterone and Finasteride) and the test compounds.326,436,468

(iii) Seminal Vesicles Test Method

In this test the effect of steroids on seminal vesicles from castrated male hamsters is determined. The animals are administered with subcutaneous (s.c.) injections of the steroids at different doses dissolved in 0.5 ml castor oil every day for 3 days. After treatments, the animals are sacrificed by ether anesthesia, and the seminal vesicles were dissected out and weighed on a balance. The results obtained
Introduction

are analyzed for significant difference between the known and test compounds using one-way analysis of variance with the aid of suitable statistical software.\textsuperscript{404, 408}

(iv) Effect on Steroid (Androgen) Level

5α-Dihydrotestosterone (DHT) is a specific androgenic hormone present in prostate formed due to conversion of testosterone by 5α-reductase located in the prostate. Circulating levels of testosterone and dihydrotestosterone hormone level or tissue concentration can be measured after administering 5α-reductase inhibitor by radioimmunoassay or ELISA giving an indication of level of inhibition by the compound and can be compared with that of standard.\textsuperscript{527, 535}

(v) Change in rat prostate weight method (In Mature Male Rats)

For each inhibitor to be evaluated, mature male rats were domesticated and oral administration of 5 ml/kg suspension of drug was started on 9 weeks old rats for 14 days by oral gavage. Drugs were suspended in 0.5% methylcellulose solution. On day 15, each rat was killed by asphyxiation. After determination of total body weight, the adrenal glands, liver, ventral prostate, seminal vesicles and right testicle were removed, cleared of adherent tissue and weighed. Values are reported as mean ± S.E.M. for actual weights and percentage of vehicle control and were obtained by using the analysis of variance feature.\textsuperscript{468, 536}

III. Cytotoxicity Assays

Testing antineoplastic agents on cell lines is a critical component of the drug development process. \textit{In vitro} cytotoxicity studies prior, to animal testing, is cheap alternative for the screening of drugs. The rapid and accurate assessment of viable cell number and cell proliferation is useful for determination of cytostatic potential of anticancer compounds and to evaluate the cytotoxic effect in toxicology studies. In general one of two parameters is used to measure the health of cells: cell viability or cell proliferation. In almost all cases, these parameters are measured by assaying for “vital functions” that are characteristic of healthy cells.\textsuperscript{537}

(i) Cell Viability

Cell viability can be defined as the number of healthy cells in a sample. Whether the cells are actively dividing or are quiescent is not distinguished. Cell
viability assays are often useful when non-dividing cells (such as primary cells) are isolated and maintained in culture to determine optimal culture conditions for cell populations. The most straightforward method for determining viable cell number is the direct counting of the cells in a haemocytometer. Sometimes viable cells are scored based on morphology alone; however, it is more helpful to stain the cells with a dye such as trypan blue. In this case, viability is measured by the ability of cells with uncompromised membrane integrity to exclude the dye.

(II) Cell Proliferation

Cell proliferation is the measurement of the number of cells that are dividing in a culture. One way of measuring this parameter is by performing clonogenic assays. In these assays, a defined number of cells are plated into the appropriate matrix, and the number of colonies that are formed after a period of growth are enumerated. Drawbacks to this type of technique are that it is tedious and not practical for a large number of samples. In addition, if cells divide only a few times and then become quiescent, colonies may be too small to be counted and the number of dividing cells may be underestimated. Alternatively, growth curves could be established, which is also time-consuming and laborious. Another way to analyze cell proliferation is the measurement of DNA synthesis as a marker for proliferation. In these assays, labeled DNA precursors ($^3$H-thymidine or bromodeoxyuridine) are added to cells, and their incorporation into DNA is quantified after incubation. The amount of labeled precursor incorporated into DNA is quantified either by measuring the total amount of labeled DNA in a population, or by microscopically detecting the labelled nuclei. Incorporation of the labeled precursor into DNA is directly proportional to the amount of cell division occurring in the culture. Cell proliferation can also be measured using more indirect parameters. In these techniques, molecules that regulate the cell cycle are measured either by their activity (e.g., CDK assays) or by their amounts (e.g., Western blots, ELISA, or immunohistochemistry). Assay using 96-well microplate offer several advantages over colony forming or dye exclusion assay including automation, rapid sample handling, the ability to change the medium and condition similar to the standard cell culture. These assays can employ colorimetrically measured dyes, radioactive isotopes, fluorochromes to detect viable cells.
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(iii) MTT Assay Method

In vitro cytotoxicity using DU-145 or PC-3 cells in the preliminary evaluation of anticancer drugs enables us to select most potent compound, but cytotoxic agents, however, frequently exhibit unspecific toxicity. Nevertheless the ability to selectively kill the target cell remains a highly desirable property of potential new therapeutic cytotoxic agents. The MTT assay, which is based on the colorimetric measurement of formazan derivative of (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) that is formed by live cells has been the most frequently used assay for in vitro cytotoxicity testing. Formazan crystals then can be dissolved and quantized by measuring the absorbance of the solution at 550 nm and resulted value is related to the number of living cells.540,541

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP

Quantitative structure-activity relationship (QSAR) and three-dimensional quantitative structure-activity relationship (3D-QSAR) form a powerful tool for the optimization of the lead compound. It is a rapidly expanding field. The QSAR studies mainly focus on the development of quantitative correlations between three-dimensional properties of molecules and their biological activity, by means of a statistical or mathematical tool. The derived model is then used to analyze the results and to predict the activity of untested compounds.542,543 The major goal of this study is to contribute to the rational design of new and more active compounds starting from the known ones, as property prediction is of paramount importance in medicinal chemistry. A great number of investigators contributed to the development of the QSAR approaches; among these investigators, a fundamental contribution was made by Prof. C. Hansch in the 1960's. The key elements of his theory which aided in the development of the quantitative approaches are enumerated as follows: (a) recognition of the fundamental role of the hydrophobic interaction, and definition of methods for their parameterization; (b) adoption of quantitative models for the formalization and treatment of the data, and the use of statistics; (c) use of multiparametric models and of multivariate statistics in order to take into account the presence of different molecular determinants of the action.544 The QSAR area is basically founded on the systematic use of mathematical models and of the
multivariate point of view. A drug molecule exerts its biological activities by binding specifically to a target macromolecule, or receptor, in the body. Receptors mainly consist of proteins and the main functions of these receptors depend on the protein constituents. The molecular recognition between a receptor and drug is supposed to be transduced into signals. Evaluation of these interactions between the drug and receptor site leads to the "receptor-based" models of drug design and account for the rationale approaches of drug design. With the advent of more modern techniques for structural evaluation of proteins viz. X-ray crystallography, NMR, and homology modeling, the number of such applications is also rapidly increasing.

The QSAR method has been developed mainly for drug design in cases where the structure of the receptor is not known. The basic hypothesis which forms the basis of QSAR studies is that the structure of a molecule contains features (geometric and/or electronic) responsible for its physical, chemical, or biological properties. Thus, for a given biological process from a set of active molecules are assumed to have the same (or very similar) mode of action (MOA), it becomes possible to define a model relating structure and activity provided that the molecular structure can be represented by a set of structural descriptors (numerical values, fragments, etc). This corresponds to "ligand-based" models. The parameters characterizing the molecular structure may be as follows: Descriptors calculated from the 2D molecular formula or the actual 3D geometry or Physicochemical quantities (measured or calculated) such as partition coefficients, vapour pressures, ionization constants, and orbital energies.

However, the method has a limitation that the design of new molecules as well as the interpretation of the structure activity relationships must usually remain within the framework of derivatives with the same skeletal structure. It is necessary to establish approaches with three-dimensional structures of molecules, in order to compare the structures and properties of known drugs with different skeletons.

The newly introduced 3D-QSAR models take into account the spatial characteristics of molecules (geometry, shape, and electron distribution), and even evaluate the fields they create in their surrounding or their interactions with neighboring structures (solvents, or receptors). This results in definite improvement
in the field. A more detailed and accurate picture of the molecular behaviors is thus accessible.\textsuperscript{550,551} Currently, these models are also used as an invaluable tool not only for predicting the biological activity as well as for predicting the toxicity potential of various compounds. These methods offer a rapid and cost-effective first-pass screening capability to assess toxicity when conventional toxicology data are limited or lacking, with the potential to identify compounds that would be appropriate for further testing. Thus the results obtained from these studies can be used as a guide to chemical synthesis when new chemical entities are developed. QSAR’s are thus regarded as valuable, scientifically credible tools in drug discovery and environmental toxicology programs.\textsuperscript{552,553}

\textbf{SOMFA (SELF ORGANIZING MOLECULAR FIELD ANALYSIS)}

The SOMFA method was proposed by Robinson and co-workers as an intuitive 3D-QSAR method avoiding complex statistical tools and variable selection procedure. SOMFA is a grid-based, alignment-dependent method. Molecules are embedded in a lattice of nodes as in comparative molecular field analysis (CoMFA) but the main difference is that SOMFA does not need calculation of fields on these nodes and rather considers intrinsic molecular properties (such as shape and electrostatic potential) and so shows some resemblance with similarity-based methods. To each grid node, values of a shape indicator and of electrostatic potential calculated from partial atomic charges are given.\textsuperscript{554} At every node, these values for a given molecule are multiplied by the mean centered activity for that molecule (so as to give less interest to molecules close to the mean activity). Mean-centered activity is represented on a logarithmic scale. The QSAR model relating activity to a property (such as shape and potential) is then derived by linear regression. A series of grid points corresponding to larger minimal and maximal values for shape and electrostatic potential are then visualized, in order to screen the regions more likely to control the biological activity under consideration. Thus, it has an inherent simplicity relative to CoMFA which leads to a greater potential for development, particularly in regard to the alignment and conformational problems inherent in 3D-QSAR.\textsuperscript{555}
As with all QSAR techniques a model is built from a set of molecules of known activity; these molecules constitute the training set. Crucial to SOMFA is the notion of the “mean centered activity”. By subtracting the mean activity of the molecular training set from each molecule’s activity, we obtain a scale where the most active molecules have positive values and the least active molecules have negative values. Three-dimensional grids are created as in other QSAR techniques with values at the grid points representing the shape or electrostatic potential. Shape values are given a value of 1 inside the van der Waals envelope, 0 outside. Electrostatic potential values at grid points are calculated in the normal manner from the partial charges distributed across the atom centers. The most important step is that the value of the shape or electrostatic potential at every grid point for a given molecule is multiplied by the mean centered activity for that molecule. These weighs the grid points so that the most active and least active molecules have higher values than the less interesting molecules close to the mean activity. It thus acts as a form of descriptor filtering. In general, a SOMFA grid can be trained on any calculable molecular property. The grids for each molecule in the training set are combined to give master grids for each property. The value of a SOMFA master grid point at a given x,y,z is defined by manner from the partial charges distributed across the atom centers.

\[ \text{SOMFA}_{x,y,z} = \sum_{i} \text{Property}_{i}(x,y,z) \text{ Mean\_Centred\_Activity}_{i} \quad (1) \]

The values at each point of a property master grid can be displayed to highlight features favorable or unfavorable to activity. For example, the shape master grid is a template of the areas of steric bulk which enhance or detract from activity. A QSAR relating a property, such as shape, to molecular activity can be derived from the property master grid in the following way. For every molecule (i) in the training and prediction set, an estimate of the activity of the molecule as defined by a certain property can be obtained by using equation 2:

\[ \text{SOMFA}_{\text{property}i} = \sum_{x,y,z} \text{Property}_{i}(x,y,z) \text{SOMFA}_{x,y,z} \quad (2) \]

Linear regressions between the SOMFA\text{property} values and the logarithms of the experimental activities for the training set are then derived. Calculating the correlation coefficient indicates the potential importance of a given property. The linear equations produced can be used to predict the activity of compounds in the
prediction set from their SOMFA_{property,i} values. A better method is to combine the predictive power of the different SOMFA_{property,i}. On combination of the individual property predictions using a weighted average of the shape and electrostatic potential based QSAR, using a mixing coefficient (c\_i) as in equation 3:

\[ \text{Activity} = c_i \text{Activity}_{\text{Shape}} + (1 - c_i) \text{Activity}_{\text{ESP}} \quad (3) \]

Using equation 3 instead gives greater insight into the resultant model by allowing the study of the variation in predictive power with different values of c\_i. 554

Till date many elaborative studies have been successfully performed employing SOMFA viz., steroids, sulfonamides, endothelin inhibitors, dihydropyridines derivatives, 1,5-diarylimidazoles selective inhibitors of COX-2 and aryl-piperazines. 556-559

The work carried out is discussed under the head: “RESUMÉ AND DISCUSSION”