1. INTRODUCTION

1.1. Benign prostatic hyperplasia

Benign prostatic hypertrophy (BPH) also known as benign prostatic hyperplasia is non-malignant adenomatous overgrowth of the periurethral prostate gland. It involves hyperplasia of prostatic stromal and epithelial cells, resulting in the formation of large, fairly discrete nodules in the periurethral region of the prostate. When moderately large, the nodules compress the urethral canal to cause partial, or sometimes virtually complete, obstruction of the urethra, which interferes with the normal flow of urine (Figure 1). It is among the most common diseases of prostate gland and represent significant burden on male patients and health care system in many countries. The symptoms of BPH arise primarily due to an enlarged prostate and gradual loss of bladder function which results in incomplete emptying of bladder. BPH often presents as lower urinary tract symptoms (LUTS) due to difficulties in voiding and irritability of the bladder. It is a pathological condition that leads to but not the only cause of LUTS in ageing men.

![Figure 1. Enlargement of prostate in BPH](image)

The prevalence of BPH in elderly men is as high as 50% in their fifth decade and 90% in their ninth decade. Despite intense research efforts in the past five decades to elucidate the underlying cause of prostatic growth in older men, pathophysiology of BPH is far from complete understanding. The clinical symptoms associated with BPH are
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termed as 'prostatism' and range from mild to severe obstructive and irritative symptoms of weak stream, abdominal straining, hesitancy, incomplete bladder emptying, terminal dribbling, nocturia and dysuria. Other conditions associated with BPH are acute or chronic urinary tract infection, hematuria, acute urinary retention, and chronic renal failure.  

1.1.1. Etiology

Histopathologically, BPH is characterized by an increased number of epithelial and stromal cells in the periurethral area of the prostate. The observed increase in the prostatic mass is attributed due to epithelial and stromal proliferation or impaired programmed cell death leading to cellular accumulation. Androgens, estrogens, stromal-epithelial interactions, growth factors, neurotransmitters etc. may play role either singly or in combination in the etiology of the hyperplastic process.

The etiopathogenesis of BPH is still largely unresolved but multiple partially overlapping and complimentary hypotheses have been proposed, all of which seems to be operational at least to some extent.  

A relationship between BPH, LUTS, BPE (benign prostatic enlargement) & BOO (bladder outlet obstruction) is observed, where many of the men aging more than 40 years develop histological BPH, while some of them are associated with bothersome LUTS which was further overlapped with BPE and some with BOO, overall leading to a compromised quality of life (Figure 2).  

Figure 2. Relationship between BPH, LUTS, BPE, and BOO.
In the last few decades, lot of research has been carried out to investigate the various causes of BPH. The various possible causes are discussed below.

A. Tissue remodeling in the ageing prostate

Ageing is considered as the most significant factor which leads to development of BPH and associated disorders. Several studies have suggested a relationship between age and progression of BPH. Hyperplasia is initially seen in about the fifth decade, and increases with age. In ageing males, a significant tissue-remodeling process takes place within the prostate, especially in the transition zone. Interference in the balance of interacting growth factor signaling pathway occurs, and stromal-epithelial interactions generate an increase in prostate volume. The most significant modifications take place in the basal cells which change their intracellular metabolism and become enlarged and hypertrophic. 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 has also been implicated as a key cofactor in BPH development and progression. Earlier reported literature does not discriminate level of apoptosis in the epithelium of BPH relative to normal epithelium but some of the recent studies support this hypothesis.

B. Hormonal alterations

Although androgens do not cause BPH, but development of BPH requires presence of testicular androgens during prostate development, puberty, and ageing. Luminal secretory cells require androgens, particularly dihydrotestosterone (DHT) for terminal differentiation and secretory functions. It has been suggested that a higher DHT activity in BPH relative to normal prostate has been resulted as a permissive, rather than as a transformative, mediator in the development of BPH. This was further supported by studies in cadaver specimens where an increased accumulation of DHT was observed.

Testosterone (T) is secreted by Leydig cells of testes under the control luteinizing hormone (LH). Release of LH from pituitary is under the influence of LH-releasing hormone (LHRH) from the hypothalamus. Another source of T is the adrenal gland which is under the influence of prolactin and adrenocorticotropic hormone from the posterior pituitary. T reversibly binds to steroid hormone binding globulins in blood stream and
diffuses into the prostatic stromal and epithelial cells where it is converted into DHT by the action of membrane bound enzyme 5α-reductase (5AR) (figure 3).
the prostate. Normal circulating levels of androgens are required for the maintenance of structural function, growth and integrity of the prostate tissue. The formation of DHT–receptor complex in the prostate stimulates the expression of a range of growth factor promoters, such as epidermal growth factor (EGF), basic fibroblast growth factor, keratinocyte growth factor (KGF), and insulin-like growth factor (IGF), leading to cellular proliferation, and inhibitors, such as transforming growth factor-β (TGF-β), which increases the levels of apoptosis. Homeostasis is, therefore, achieved by the normally functioning prostate. Increased stimulation by promoters and hyperplastic growth, removal of the stimulus, through surgical or chemical means, leads to the opposite scenario, with a general move towards increased expression of cell growth inhibitors, such as TGF-β.

It has also been conclusively demonstrated that androgen signaling is significantly elevated in hyperplasic tissue relative to the adjacent normal prostate. Studies have also shown that circulating levels of free estradiol remain constant in the ageing man. In fact, the prevalence of fat tissues is responsible for the expression of high levels of aromatase, which induces estrogen conversion. The increased level of estrogens in the ageing man may lead to the reactivation of prostatic growth. In addition to epithelial effects, estrogens also promote stromal cell proliferation.

C. Metabolic syndrome

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 possible causes for the development of BPH. Both the patients of BPH and metabolic syndrome may share the same metabolic abnormalities of a defective insulin-mediated glucose uptake and secondary hyperinsulinaemia. These findings support the hypothesis of a causal relationship between high insulin levels and the development of BPH. Both BPH and diabetes are indeed associated with LUTS, including a reduced maximum flow rate and an increased post void residual volume. Reports have shown that vascular damage induced by Type 2 diabetes can promote BPH but the exact mechanism which correlates diabetes with BPH is unclear.

Hypertension has been suggested to be involved in the pathophysiology of BPH as supported by epidemiologic evidences and it has been also hypothesized that
noradrenergic nerves may contribute to the functional component of bladder outlet obstruction due to BPH.\textsuperscript{27, 28} Thus sympathetic nervous system is involved in the pathophysiology of LUTS because of its association with both arterial tone and voiding physiology. This is evident from the studies which suggest the use of adrenergic receptor blockers such as $\alpha$-adrenergic antagonists for the management of BPH.\textsuperscript{29-31}

D. Inflammation

Infective etiology studies have suggested the presence of heterogeneous bacterial and viral strains in BPH specimens. These strains produce inflammatory agents in prostatic stromal cells. The inflammatory infiltrates are mainly composed of activated T-cells and macrophages frequently associated with BPH nodules and causes increased production of cytokines and chemokines. These pro-inflammatory agents kill the surrounding cells leaving behind vacant space that are replaced by fibromuscular nodule and may lead to progression of BPH.\textsuperscript{32}

This is supported by the fact that healthy prostate do not express these cytokines whereas prostate with inflammation do express interleukins. Wang \textit{et al.} also demonstrated the presence of COX-2 in prostate with significant inflammation.\textsuperscript{33} These studies were consistent with differential role of inflammatory infiltrates in the etiology of BPH. Thus BPH may be considered as a form of symptomatic prostatitis. BPH has indeed been frequently associated with chronic prostatitis.\textsuperscript{34}

A pictorial relationship between various causing factors of BPH is shown in Figure 4.

![Figure 4. Relationship between various causing factors of BPH.](image-url)
1.1.2. Clinical symptoms associated with BPH

The clinical symptoms of BPH severely affect the quality of life. These may be classified as being obstructive (voiding) or irritative (storage) symptoms.\(^{35}\)

A. Voiding symptoms\(^{36,37}\)
- Hesitancy
- Poor urinary flow rate (>10ml/s) and straining.
- Sensation of incomplete bladder emptying
- Terminal or post-micturition dribbling
- Prolonged urination

B. Storage symptoms\(^{38}\)
- Voiding too frequently
- Nocturia
- Urgency
- Urge incontinence

1.1.3. Treatment approaches

The treatment approaches of BPH include non-pharmacological and pharmacological therapies.\(^{39,40}\) The non-pharmacological approach includes watchful waiting, lifestyle modification, urinary catheterization and surgery. This approach is generally used in the cases of mild and non-bothersome symptoms. The pharmacological approach includes clinically used drugs and emerging therapies. Currently \(\alpha\)-adrenergic antagonists and \(5\alpha\)-reductase inhibitors (5ARIs) are used for management of BPH either singly or in combination. Various other drugs which are discussed under emerging therapies includes phosphodiesterase inhibitors, progestogens, vitamin D3 analogues, botulinum toxin A, non steroidal anti-inflammatory agents (NSAIDs), luteinizing hormone releasing hormone (LHRH) antagonists, muscarinic receptor antagonists, carotenoids, cannabinoids, \(\beta\)-adrenoceptor antagonists, phytotherapy, and phytoestrogens.

The treatment option mainly depends upon age, size of the enlarged prostate, type and severity of the symptoms. The various approaches for management of BPH has been summarized in Figure 5.
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Management of BPH

Non-Pharmacological
- Watchful Waiting
- Lifestyle Modifications
- Urinary Catheterization
- Surgery
  - Transurethral Resection of Prostate (TURP)
  - Transurethral Incision of Prostate (TUIP)
  - Suprapubic or Retropubic Prostatectomy
  - Minimally Invasive Surgical Therapy (MIT)
  - Laser Surgery
  - Prostatic Stenting

Pharmacological

Current Therapies
- α-Adrenoceptor Antagonists
- 5α-Reductase Inhibitors
- Combination Therapy

Emerging Therapies
- Cannabinoids
- Progestogens
- Vitamin D3 Analogues
- Botulinum Toxin A
- Phytotherapy
- Carotenoids
- β-Adrenoceptor Agonists
- Phytoestrogens

Figure 5. Treatment approaches for management of BPH
A brief discussion on various pharmacological therapies including the emerging ones is as follows.

1.1.3.1. a-Adrenergic antagonists

The adrenergic receptor is a transmembrane glycoprotein receptor and has been sub-classified as $\alpha_1$, $\alpha_2$ and $\beta$ adrenoceptors. The prostate capsule, stroma, and the bladder neck are densely populated with $\alpha_1$-adrenergic receptor. These receptors are mainly responsible for contraction of smooth muscles of urinary bladder.41

Three subtypes of $\alpha_1$-adrenergic receptors localized in the prostate ($\alpha_{1a}$, $\alpha_{1b}$, and $\alpha_{1d}$) have been isolated and characterized. Immunohistochemical studies have revealed that $\alpha_{1a}$ adrenergic receptors are present only in the stroma.42 The $\alpha_{1b}$ receptors are located predominantly in epithelium with weak expression in stroma. The $\alpha_{1d}$ receptors are located in stromal elements including blood vessels and majority of them are found in detrusor muscle, neck of the bladder, and the sacral region of the spinal cord.43,44

Upon stimulation they increase the smooth muscle tone and closure of urethra exacerbating the bothersome symptoms of BPH including increased detrusor muscle pressure, frequency, urgency, straining, and a low urine flow. Antagonism of effect at $\alpha$-adrenoceptor reduces smooth muscle cell tone. Thus $\alpha_{1a}$ adrenergic antagonists have been used with better therapeutic index with regard to the cardiovascular side effects because of presence of these receptors exclusively in prostate.

Currently $\alpha_1$-adrenergic receptor antagonists are often used as first line medical treatment for BPH. The clinical demonstration of “uroselectivity” remains a primary consideration when choosing an $\alpha_1$-adrenergic receptor antagonist for LUTS associated with BPH.45 These agents have different side effects including dizziness, orthostatic hypertension, rhinitis, abnormal ejaculation and floppy iris syndrome. All $\alpha_1$-adrenergic receptor antagonists have been broadly classified as non-selective drugs and selective drugs. The non-selective drugs include phenoxybenzamine, thymoxamine etc. The selective drugs are further classified according to duration of action i.e. short acting like prazosin, alfuzosin, indoramin and long acting like terazosine, tamsulosin, doxazosine, naftopidil, and silodosin.

Phenoxybenzamine (1) was the first $\alpha$-adrenergic receptor antagonist introduced clinically for the treatment of BPH in 1976. It is a non selective haloalkylamine that binds
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covalently to the receptors causing irreversible blockade for long duration (14-48 hrs or longer).46

![Chemical Structure]

It is presumed that aryl alkylamine moiety of the molecule is responsible for its relative specificity. However its clinical use was limited because of side-effects such as tiredness, dizziness, impaired ejaculation, nasal stuffiness, and hypotension.47

Prazosin (2), a piperazinyl quinazoline, had emerged as the first $\alpha_1$ selective adrenergic receptor antagonist. It is 1000 fold more potent for $\alpha_1$ than $\alpha_2$-adrenergic receptor. It is metabolized extensively by the liver and only about 50% of the drug is available after oral administration. Prazosin leads to relaxation of both arterial and venous vascular smooth muscles and also those of prostate. Multiple daily dosing, and adverse hypotensive effects are clinical limitations.48,49

![Chemical Structure]

Alfuzosin (3), an uroselective $\alpha_1$-adrenergic antagonist, is a quinazoline derivative approved by the FDA for the treatment of symptomatic BPH.

![Chemical Structure]

It improved urinary voiding symptoms and increase urinary flow rates with fewer cardiovascular adverse effects.50
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Terazosin (4) was the first selective long-acting reversible \( \alpha_1 \)-adrenergic antagonist with half-life of 9-12 hrs and is used in the treatment of BPH and hypertension. It has high bioavailability but is extensively metabolized in the liver, with only a small fraction of unchanged drug being excreted in the urine.\(^{51}\)

Doxazosin (5), originally developed as an anti-hypertensive, was the second \( \alpha_1 \)-adrenergic antagonist approved by the FDA for symptomatic treatment of BPH.

It has moderate bioavailability and is extensively metabolized, with very little parent drug excreted in urine or faeces. This long-acting selective \( \alpha_1 \)-blockers (\( t_{1/2} = 22 \) hrs) is administered once daily with better tolerability compared with the shorter-acting drug like prazosin.\(^{52}\)

Tamsulosin (6) is an urospecific benzene sulphonamide containing \( \alpha_1 \)-adrenergic receptor antagonist with higher affinity for \( \alpha_{1a} \) and \( \alpha_{1d} \) receptors than for the \( \alpha_{1b} \) receptor subtype.

It is well absorbed orally and has \( t_{1/2} \) of 5 to 10 hrs.\(^{53}\) It is a chiral compound with (S) (+) tamsulosin enantiomer with greater levels of subtype selectivity than the (R) (-)
enantiomer. It is more effective in the treatment of BPH without reducing blood pressure in orthostatic hypotensive patients.

Silodosin (7) is an indoline derivative structurally related to tamsulosin. Like tamsulosin, its (R) enantiomer is more potent α1a adrenergic receptor antagonist. A trifluorothoxy group in silodosin has been inserted to block O-dealkylation which is considered as primary metabolic pathway of tamsulosin. Silodosin was approved by USFDA in 2008 for the treatment of BPH.

It acts as a superselective agent with a selectivity of 50-100 times for α1a receptor subtype than α1b or α1d which lead to its lower cardiovascular side effect profile. However its effect on ejaculation due to selective effect on seminal vesicle and vas deferens limit its scope to be a promising drug for BPH.

A large number of potential α1a subtype selective antagonists belonging to different chemical classes have been investigated. The potential molecules includes SNAP 5089 (8) and 5540 (9), but none of them have reached to clinic.
1.1.3.2. 5α-Reductase Inhibitors

1.1.3.2.1. 5α-Reductase (5AR)

Discovery of enzyme 5AR in rabbit liver homogenate in 1954 was a major milestone in understanding several androgen related disorders such as BPH, prostate cancer, acne, female hirsutism, and male pattern baldness. Steroid 5AR (3-oxo-5α-steroid:Δ4-oxidoreductase, EC 1.3.99.5) is a membrane bound, NADPH dependent enzyme which is responsible for conversion of major circulating androgen, T in male adult into 5α-dihydrotestosterone (DHT). Conversion of T into DHT amplifies the action of T by three to five times because of greater binding affinity of DHT as compared to T. The 5AR enzyme was characterized initially in rat liver slices based on its ability to convert deoxycorticosterone to 5α-reduced metabolites.57 In 1960 Tomkins and others showed that the enzyme utilized reduced pyridine nucleotide as a cofactor (NADPH) (Figure 6).

It has been shown that DHT can enlarge the undetectable prostate of male born with 5α-reductase deficiency,58 on the other hand levels of DHT in prostate of patients with BPH and prostate cancer are elevated which suggests that both enzyme and DHT play significant role in pathophysiology of the disease.59,60

1.1.3.2.2. 5AR Isozymes

Advancement in the molecular biology have helped the scientists in identifying five members of 5AR family which consists of three subfamilies. The five members has been named as 5α-reductase type 1 (5AR-1), 5α-reductase type 2 (5AR-2), 5α-reductase type 3 (5AR-3), glycoprotein synaptic 2 (GPSN2) and glycoprotein synaptic 2 like
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(GPSN2L) proteins. The cDNAs for 5AR-1 and 5AR-2 has been identified from rat liver and human prostate respectively. 5AR-1 enzyme present mainly in the hair follicles and peripheral skin whereas 5AR-2 is the major isozyme in genital tissues and a deletion in the gene leads to male pseudohermaphroditism.\(^61,62\)

5AR-1 is constitutively expressed in the brain, during adulthood it is mainly localized in the myelin membranes and has a catabolic inspite of an activating role in the brain. 5AR-2 which 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. These studies supports that 5AR-2 enzyme might participate in the perinatal differentiation of brain towards a male pattern.\(^63\)

5AR-1 and 5AR-2 isozymes differ in the constitution of amino acids as well as molecular weight. 5AR-1 is active at pH 6.0-8.5 while 5AR-2 is active at pH 5.0-5.5. The two isozymes also differ in the location of the gene structure where type 1 is located at 5p15 while type 2 is located at 2p22 although they had same gene structure.\(^64,65\)

Recently with the development of genome wide gene expression profile analysis a third type of isozyme 5AR-3 (SRD5A3) has been identified. GPSN2 and GPSNL2 proteins were identified using sequence searching and NCBI’s BLAST.\(^66\)

The 5AR-3 was recently identified in hormone-refractory prostate cancer cells (HRPC).\(^67,68\) This enzyme also converts T to DHT in HRPC cells in a similar way to type 1 enzyme and was found to be active at pH 6.9.\(^69\) This isozyme has been recognized as a ubiquitous enzyme in mammals.

This enzyme has been present in 4q12 location with a length of 25458. The isozyme is of 319 amino acid length with 6 transmembrane helixes. Northern blot and real time RT-PCR (real time polymerase chain reaction) analyses have shown the presence of this enzyme in androgen and non-androgen target human tissues such as pancreas, brain, prostate cancer cell lines, skin and adipose tissues.

This isozyme is specifically overexpressed in HRPC cells where it plays important role in HRPC growth and progression. Inhibition of this isozyme could be a better therapeutic target against HRPC and other androgen dependent cancers.

The characteristics of these isozymes has been presented in Table 1 including their length and location.\(^67\)
Table 1. Characteristics of genes and proteins of human 5AR isozymes

<table>
<thead>
<tr>
<th></th>
<th>5AR-1</th>
<th>5AR-2</th>
<th>5AR-3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene</strong></td>
<td>SRD5A1</td>
<td>SRD5A2</td>
<td>SRD5A3</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td>5p15</td>
<td>2p23</td>
<td>4q12</td>
</tr>
<tr>
<td><strong>Length (b)</strong></td>
<td>36,173</td>
<td>56,385</td>
<td>25,458</td>
</tr>
<tr>
<td><strong>Protein size (amino acids)</strong></td>
<td>259</td>
<td>254</td>
<td>319</td>
</tr>
<tr>
<td><strong>Transmembrane helices</strong></td>
<td>5</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td><strong>Protein weight (Da)</strong></td>
<td>29,459</td>
<td>28,393</td>
<td>36,521</td>
</tr>
<tr>
<td><strong>Optimal pH</strong></td>
<td>6-8.5</td>
<td>5-5.5</td>
<td>6.9</td>
</tr>
<tr>
<td><strong>Affinity for testosterone</strong></td>
<td>Km = 1.7 µM</td>
<td>Km = 0.2 µM</td>
<td>--</td>
</tr>
<tr>
<td><strong>In vitro inhibition by</strong></td>
<td>Kᵢ ≥ 300 nM</td>
<td>Kᵢ = 3–5 nM</td>
<td>--</td>
</tr>
<tr>
<td><strong>Localization (in tissues)</strong></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>
<td>Hormone refractory prostate cancer cells, pancrease, brain, skin, adipose tissue</td>
</tr>
<tr>
<td><strong>Selectivity to the inhibitors</strong></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>
<td>--</td>
</tr>
</tbody>
</table>

1.1.3.2.3. Mechanism of action of 5AR

The mechanism of action of 5AR can be studied broadly under two sub-heads i.e. chemical mechanism and kinetic mechanism.

A. Chemical mechanism

The reduction of T into DHT by 5AR based on the regio-reduction that involves formation of a binary complex between the enzyme and NADPH, followed by formation of a ternary complex with the substrate T. The interaction of electrophilic residue (E⁺) present in the active site with enone system generates a delocalized carbocation. Direct transfer of enantiotropic hydride from NADPH to 5α face of the delocalized carbocation leads to formation of enolate of DHT. This is a stereoselective reduction at C-5. This intermediate, which is coordinated with NADP⁺ on the α-face is
presumably attacked by the proton on β-face at C-4 giving a ternary complex of E-NADP⁺-DHT. Finally departure of DHT leaves binary NADP⁺-Enzyme complex and then the release of NADP⁺ regenerates the enzyme free for future catalytic cycles (Figure 7).[65]

![Chemical mechanism of 5AR catalysis](image)

Figure 7. Chemical mechanism of 5AR catalysis. Enz, Enzyme; E⁺, electrophile or electrophilic site; Nu⁻, nucleophile or nucleophilic site; R, adenine dinucleotide phosphate (ADP).

B. Kinetic mechanism

The enzymatic reduction of T into DHT is assisted by cofactor NADPH. This involves formation of enzyme and NADPH complex, which binds with the substrate to form ternary complex (E.NADPH.T) which intramolecularly through hydride shift reduces the T into DHT to form another complex (E.NADP⁺.DHT). Finally DHT departs and subsequently helps in proliferation of prostatic cells.[70, 71]
All these steps are reversible and the process is shown in Figure 8. The strategies to prevent the formation of DHT could be to inhibit this enzymatic process at A, B or C junctions through bisubstrate, competitive, uncompetitive inhibitors respectively.

![Figure 8. Kinetic mechanism of 5α-reductase action.](image)

Further the design of novel molecules that inhibit the enzymatic process at junction B is based upon the concept of transition state (TS) of the catalysis. Two such transition states have been postulated and shown in Figure 9.

![Figure 9. Transition states during 5AR catalysis](image)
Introduction

The hybridization of C-3, C-4 and C-5 in ‘substrate like’ TS are similar to those of intermediate, whereas hybridization of C-3, C-4 and C-5 in “product like” TS are similar to those of the enol form of DHT. 71, 72

It has been realized that the transition state analogs exhibit a great deal of binding to the enzyme and thus shows greater inhibition.

1.1.3.2.4. Development of 5α-Reductase inhibitors

The major limitation for development of 5ARIs is nonavailability of crystal structure of the enzyme because of its membrane bound nature thus posing difficulty in its isolation and purification. The only information available is their primary sequence estimated from cDNAs. Other homologous enzymes are present but there resemblance with 5AR is not significant enough to draw valuable information regarding suitable chemical architecture which can potentially inhibit the enzyme 5AR. A number of 5ARIs are reported till date. These can be classified into steroidal and non-steroidal inhibitors. The following section briefly discusses steroidal inhibitors reported with focus on their SAR and the key structural features responsible for inhibitory potency.

A. Steroidal 5ARIs

From the ongoing discussion it is evident that biosynthesis and metabolism of T constitute an attractive target for design and development of drugs for management of BPH. The phenotypic consequences of 5AR deficiency further reinforce to draw a strategy for development of drugs that may find application in BPH, acne, male pattern baldness, and prostatic cancer.

During 1970s steroidal hormones were first analyzed for their potential to inhibit the conversion of [4-14C]T to DHT by human skin 5AR and the K_m was found to be 1100 nm for T. 73 Progesterone which possess identical structure in ring A as in T has restrained transformation competitively upto 93.3% and was itself converted into 5α-pregnane-3,20-dione. The K_i was found to be slightly greater than T. It has been demonstrated that deoxycorticosterone acetate, deoxycorticosterone, and Δ4-dione, also inhibited the enzyme 5AR, to an extent of 85.8%, 84.7%, and 76.4%, respectively. But the other steroidal analogs like pregnenolone, DHT, androsterone, dehydroepiandrosterone, estradiol, and testolactone (an antiandrogen) has almost no significant effect on the inhibition of 5AR.
A close look at the structures of above mentioned steroids suggests that the structural characteristics required for an effective inhibition is 3-oxo-4-ene system in ring A and substitution at 17β position.

The first steroidal inhibitor had been designed by modifying the structure of natural steroidal substrate in which one of the carbon atom of the ring of the steroidal nucleus has been suitably replaced by a heteroatom such as nitrogen thereby forming azasteroids. Singh and co-workers74, 75 as well as other groups76-78 have published comprehensive reviews on biological activity of azasteroids. During the last two decades a large number of azasteroids have been reported for their potential application in the management of BPH. Based upon the location of N atom in the steroidal skeleton, the various classes of azasteroids are discussed below.

Among all the inhibitors discovered in recent years, three classes were based on testosterone skeleton, modified by the introduction of a nitrogen atom in ring A (4-azasteroids), B ring (6-azasteroids) and at bridgehead 10 (19-nor-10-azasteroids).

I. 2- and 3-Azasteroids

Although during early sixties Doorenbos and Wu79 and Mazur80 et al. synthesized some of the 3-azasteroids but, Anderson and Liao reported the 5AR inhibitory activity of steroidal N-oxido-3-aza-1,3,5(10)-triene in 1968.81 Based upon the understanding of chemical mechanism Haffner in 1994 reported synthesis of some novel 3-pyridyl-N-oxide steroids (10, 11) which mimic the enolate or enol like transition state of the enzyme-substrate complex.82

N-Oxide steroids 10 and 11 have shown potential inhibition against 5AR-2 with Kᵢ value of 0.031 and 0.104 μM, respectively. Other effective and stable transition state analogs were reported by Robinson et al. in 2003. These 2- and 3-azasteroidal derivatives (12-17) were evaluated for their inhibitory activity against both 5AR-1 and 5AR-2.83 Cyclic secondary amines (13 and 17) showed poor inhibitory activity against both 5AR-1
and 5AR-2 whereas ring A lactams (12 and 16) demonstrated marginal improvement against 5AR-2 isozyme. However both the nitrones (14 and 15) showed significant inhibition at 10 μM concentration.

II. 4-Azasteroids

One of the most extensively studied class of azasteroids as 5ARIs constitutes 4-azasteroids. Voigt et al. in 1970, screened a large number of steroids including natural substrates for their ability to inhibit 5AR by a crude cell free enzyme system isolated from rat ventral prostate. In continuation of these studies, they had synthesized a series of modified steroids and evaluated them for their 5AR inhibitory activity. It was evident from the study that 4-en-3-one function and 17β-side chain having one or more oxygen functionalities seems to be desired template for inhibition of 5AR. Molecules possessing these features act as competitive inhibitors of the enzyme. Among them 4-androsten-3-one-17-carboxylic acid (18) was identified as a potent inhibitor of 5AR with an inhibition of 87.7% for the microsomal enzyme of human skin.

However none of the compounds could interfere with in vivo conversion of the DHT, because of rapid conversion of the molecules into inactive 4,5-dihydro form by the
enzyme. Thus one of the major strategies could be design of molecules with steroidal skeleton in which the reducible portion in ring A is replaced by a nitrogen atom at 4th position.

In continuation for search of potent 5ARIs, Merck and Co. in 1980 reported a series of 4-aza steroids where C-4 of 3-oxo-5α-steroids had been replaced by nitrogen. This resulted not only an increase in 5AR inhibitory activity but also in vivo retention of activity.\(^{85,86}\)

Therefore, azasteroids were so designed that these mimic the enzyme bound intermediate formed by sp\(^2\)-hybridized center at C-3 and C-4 of natural substrate T. Presence of lactam in ring A (3-oxo-4aza) mimic the 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 4-aza inhibitors interact competitively at the active site as well as unlike the substrate (T), can not be further reduced to 5α metabolite and thus retain in vivo activity also.\(^{87}\) It is presumed that steroidal skeleton provides an anchor between ring A lactam and the 17β-substituent while the former helps in generating transition state mimic of intermediate enolate, whereas, the later significantly enhances potency by binding at a pocket considered largely lipophilic in nature. The structure activity relationship (SAR) of 4-aza-3-oxo-5α-androstane has been outlined below in Figure 10.\(^{88,89}\)

![Figure 10. Basic SAR of 4-aza-steroids](image-url)
The observation that substitution at 17β-position could dramatically affect the potency, a large number of modifications were carried out to identify potential inhibitors. Among them 4-MA (17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one) (19) was found to be a potent dual inhibitor having IC₅₀ value of 1.7 nM and 1.9 nM against human 5AR-1 and 5AR-2 respectively. However, it was withdrawn from the clinical developments due to hepatic toxicity and lack of selectivity over 3β-hydroxy steroid dehydrogenase enzyme. From the series a related A ring unsaturated analog 17β-(N-tert-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one (MK-906, finasteride) (20) emerged as drug. Finasteride is a potent inhibitor of 5AR-2 with IC₅₀ value of 9.4 and has weak affinity for 5AR-1 (IC₅₀ = 410 nM). The clinical dose is 5 mg/day and it causes lowering of plasma DHT levels by 65-80%. This drug was developed by Merck and was the first drug to be approved by USFDA for BPH. Long-term studies have further demonstrated that there is a sustained improvement in BPH symptoms and reduction in the prostate specific antigen (PSA) level.

Merck and Glaxo had reported in 1996 that finasteride and its close analogs are mechanism based inactivators of 5AR-2 and acts as an alternate substrate to T and finally reduced to dihydrofinasteride. This reduction of Δ¹,₂ proceeds through a very slow dissociating enzyme bound NADP-dihydrofinasteride adduct. Earlier it was believed that finasteride acts 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 has been established that the most likely cause of the slow offset inhibition is rate-limiting hydride transfer from NADPH to the Δ¹-double bond of finasteride. Reduction of Δ¹ by hydride transfer from NADPH enables the nucleophilic attack of C-2 of finasteride on oxidized nicotinamide C-4. This reduction results in the formation of lactam enolate which is not suitably positioned for efficient protonation by the enzyme. Instead the
enolate is trapped by the pyridinium cation of the NADP yielding a covalent adduct to the co-factor and to the protein (Figure 11).

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 and has a dissociation constant $K_i \leq 1 \times 10^{13}$ M. Finasteride is also a mechanism-based inhibitor of the human skin (5AR-1) isozyme, but it is processed with a much smaller second-order rate constant, $k_i/K_i = 3 \times 10^3$ M$^{-1}$ s$^{-1}$, which attenuates its activity against this isozyme in vivo.$^{98-100}$

It was reported in 1981 that 17ß-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (21) strongly inhibited the 5AR both in vivo and in vitro.$^{101}$ Also it was reported that diazoketone (22) act as potent time dependent inhibitor as well as bis-norcholane side chain at C-17 position is compatible with high enzyme affinity. Several 2
oxa-steroids have also been reported with diverse biological activity and are considered as 2-carba analogs. This replacement of carbon by an oxygen will not cause steric perturbations and may convert 4-aza-3-one steroid into a urethane derivative (OCONH) thus enhancing the polarity of the 3-oxo.

This enhanced polarity of the carbonyl may help in binding the enzyme at the active site with great affinity. Based upon these observations Weintraub et al. had reported the synthesis of 20-(hydroxymethyl)-4-methyl-4-aza-2-oxa-5α-pregnan-3-ones and their corresponding 3-thiones (23-26), but all the synthesized compounds had shown weak in vitro inhibitory activity against 5AR with Kᵢ’s around $10^{-7}$ M.\(^\text{102}\)

Taking lead from A ring of finasteride, Bakshi and co-workers reported synthesis and in vitro 5AR activities of carboxamides.\(^\text{103}\) These molecules comprise of a double bond between C-1 and C-2 and an aryl cabamoyl moiety at C-17 similar to finasteride. It was assumed that appending an aryl moiety helps in interacting this part of the molecule with enzyme with greater affinity. However some of the azasteroids were found to be potent dual inhibitors having activity against 5AR-1 and 5AR-2. Among the series compound (27) was found to be most potent with IC₅₀ of 5.6 and <0.1 nM against 5AR-1 and 5AR-2 respectively.

Further rigorous investigations in search of 4-azasteroids as potential 5ARI's led to development of dutasteride (GG745, 28).
It is $17\beta$-N-(2,5-bis(trifluoromethyl)phenyl))-3-oxo-4-aza-5α-androst-1-ene-17-carboxamide (28) and is most potent dual inhibitor. It has been approved by USFDA in 2002, for the symptomatic treatment of BPH.\textsuperscript{104}

Being a time dependent competitive inhibitor of both 5AR-1 and 5AR-2 it reduces DHT levels >90% following one year oral administration.\textsuperscript{105} It forms a stable complex with a slow rate of dissociation constant and does not bind to the androgen receptor.\textsuperscript{106} By reducing DHT level, it helps in reducing the size of enlarged prostate thus improving the urinary flow rate. It is about 60 times more potent than finasteride and has been shown to decrease the risk of acute urinary retention and BPH related surgery.\textsuperscript{107,108} Dual inhibition of 5AR is more beneficial over selective 5AR-2 inhibition as this does not allow synthesis of DHT mediated through 5AR-1 present in peripheral system, thus increases the efficacy to a greater extent.

Di Salle \textit{et al.} in early 1990s reported the synthesis and 5AR inhibitory activity of a series of 4-azasteroids with more polar substituents at C-17 position. It was observed that derivatives containing N-methyl group and saturated A ring exhibited greater potency.\textsuperscript{109} Among the series turosteride (29) was found to be most active compound. It contained lactam in A ring and substituted acylurea appended at C-17 position. Structurally it was a close analog of 4-MA (19) but devoid of binding to the rat androgen receptor and a weak inhibitor of $3β$-hydroxy steroid dehydrogenase unlike 4-MA with an \( IC_{50} \) value of 55 and 53 nM against human and rat 5AR respectively.\textsuperscript{110}
Other azasteroids which retained 5α-reductase inhibitory activity are 2-substituted (30), A-homo- (31) and 19-nor- (32) analogues. Li et al. (1995) reported a number of 17β-(N-alkyl/aryl formamido) and 17β-[(N-alkyl/aryl)alkyl/arylamido]-3-oxo-4-aza-5α-steroids as 5ARIs and androgen receptor antagonist and compared them to 4-MA (19). It was observed that 5AR-1 has preference for 17β N-substituted linear alkyl side chain of up to five carbon atoms. Compound (33) and (34) showed dual inhibition of both isozymes of 5AR (IC\textsubscript{50} = 9.57 and 16.9 nM for 5AR-1 and 14 and 18.4 nM for 5AR-2 respectively). 17β-[(N-alkyl/aryl)alkyl/arylamides] were found to be more active toward 5AR-1 as compared to 5AR-2 isozyme. The compound 35 was found to be dual inhibitor with IC\textsubscript{50} = 2.93 and 3.75 nM, respectively for 5AR-1 and 5AR-2.\textsuperscript{111}
To evaluate the contribution of substituents at C-7 position, a series of 7β-substituted derivatives (36-42) were synthesized and evaluated as 5ARIs. It was found that substitution of B ring at 7β position resulted in potent selective inhibitor of 5AR-1, provided substituted lactam in ring A is preserved.112 Among the series 4,7β-dimethyl-4-aza-5α-cholestan-3-one (42) emerged as one of the most potent inhibitor with IC\textsubscript{50} of 0.9 nM against 5AR-1.113

Salle and co-workers reported 5AR inhibitory activities of various 4-azasteroids. The structures of the reported compounds were similar to finasteride with fluoro substituted 17β-amidic side chains.114 Among the series FCE 27837 (N-[1,1,1-trifluoro-2-oxo-but-3-yl]-3-oxo-4-aza-5α-androstan-17β-carboxamide, 43), was found to be active against both human and rat 5AR with IC\textsubscript{50} values of 51 and 60 nM respectively.115

Labrie and associates patented several steroidal compounds having lactam in ring A which is devoid of any unsaturation at Δ\textsuperscript{1(2)} position, as well as derivatives of urea appended at the 17β position. Among these compounds, 44 was found to be most active against human 5AR with K\textsubscript{i} value of 0.5 nM.116
Panzeri and co-workers reported the synthesis of several $\Delta^{1(2)}$ unsaturated $17\beta$-substituted 4-aza-5α-androstan-3-one carboxamides. These were found to be highly potent against human 5AR. Compound 45 was very active with IC$_{50}$ value of 8 nM against human 5AR but most of the compounds were devoid of selectivity.\textsuperscript{117}

Another potent carboxamide FCE 28260 [(22R,S)-N-(1,1,1-trifluoro-2-phenylprop-2-yl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide, 46] was reported by Giudici et al. in 1996. It was a dual inhibitor of both 5AR isozymes and caused 74% reduction in the DHT levels with IC$_{50}$ values of 15 and 16 nM for rat and human prostatic 5AR respectively.\textsuperscript{118}

Ciba-Geigy Ltd. reported the synthesis of CGP53153 (N-(2-cyano-2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide, 47). On evaluation it was found to be a non selective moderately active compound with IC$_{50}$ value of 36 and 262 nM against rat and human prostatic 5AR respectively.\textsuperscript{119}
Attempts were also made by Ishibashi and co-workers for synthesis of more polar steroidal compounds by incorporating oxygen functionality at C-11 position with diaryl carbamoyl moiety at C-17 (48-52). The 4-methyl 11β-hydroxy-4-aza-5α-androstane derivative (50) was found to be most potent with 74 % inhibition of rat 5AR at $10^{-8}$ M concentration. The relative inhibitory potency of this compound was 2.9 times more as compared to finasteride.$^{120}$

Finasteride was first steroidal drug approved by USFDA for BPH. In search for potent 5AR inhibitors Merck in 1997 synthesized several other 4-aza 5α-androstan-3-one 17β-(N-substituted carboxamides) (53, 54) in which one of the substituents at carboxamide nitrogen was aromatic nucleus. Most of the synthesized compounds showed potent dual inhibition with 54 as the most potent inhibitor with an IC$_{50}$ of 5 and 11 mM for 5AR-1 and 5AR-2 respectively.$^{121}$
Taking guidance from the structure of dutasteride Salle et al. in 1998 reported synthesis of another series of carboxamide in which both the trifluoromethyl group were not part of aromatic nucleus. The compound PNU 157706 [N-(1,1,1,3,3,3-hexafluorophenylpropyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carbox-amide, 55] as a potent dual 5AR inhibitor. The IC₅₀ value of the compound was found to be 3.9 and 1.8 nM against 5AR-1 and 5AR-2 respectively.¹²²

Another patented molecule was reported by Menzenbach et al. It was not a carboxamide, but a 17 methylene 4-azasteroid. The compound 56 was found to be inhibitor of 5AR with IC₅₀ = 3.4 nM for prostate and 2.5 nM for seminal vesicle associated 5AR.¹²³

### III. 6-Azasteroids

The stimulation for synthesis of 6-aza androsten-4-ene-3-one as potent 5ARIs lies in the assumption of transition state paradigm of substrate T. The design of this class of inhibitors was based on 3-keto-4-ene-6-amino functionalities to mimic the structural and charge polarization features of transition state of 5AR catalysed transfer of enantiotropic hydride from NADPH to T. These 6-azasteroids does not act as substrate for 5AR but has higher reduction potential than α-β unsaturated ketone and thus show slow offset inhibition instead of irreversible as demonstrated by 4-azasteroids analogs.⁹⁹ Frye and associates have reported basic SAR of 6-azasteroids based upon the synthesis and biological evaluation of a large number of substituted 6-azasteroids (Figure 12).¹²⁴,¹²⁵
Introduction

C4-methyl improves 5AR-1 potency
C-17/?-anilides preferred
NHR
Alkylation tolerated but acetylation reduces potency versus 5AR-1 & 5AR-2

Figure 12. Basic SAR of 6-azasteroids

In order to understand the SAR of A and B ring vs. 5AR-1 and 5AR-2 activity, a set of various derivatives (57-64) has been synthesized in which substitution at N-6, C-1, C-2, C-4 were explored.

(57) R₁, R₂= H  (58) R₁= H, R₂= CH₃
(59) R₁= Cl, R₂=H  (60) R₁= Br, R₂= H
(61) R₁= CH₃, R₂= H  (62) R₁= H, R₂= CH₂, Δ₁
(63) R₁= H, R₂=H,1, 2α-methano
(64) R₁= H, R₂= H, 2α, β-CH₃

It was found that methylation at N-6 (58) and substitution by lipophilic groups such as Cl (59), Br (60) and CH₃ (61) at C-4 increases 5AR-1 selectivity four-fold whereas incorporation of unsaturation (62), 1,2 cyclopropanation (63) and C-2-methylation (64) decreases the 5AR-1 activity. Compound 61 was identified as most potent with IC₅₀ of 40 and 3.9 nM against 5AR-1 and 5AR-2 respectively.

Further optimization was carried out by making suitable substitutions at C-17 along with A and B ring which resulted in development of dual inhibitors (65-77). ¹²⁴
Introduction

Generation of both ester (69, 71) and carboxamide (72, 73) resulted in compounds having 16-200 fold more selectivity towards 5AR-1. However a large lipophilic group at C-17 resulted in compound 76 as the most potent dual 5ARI with an IC\textsubscript{50} of 1.0 and <0.1 nM against 5AR-1 and 5AR-2 respectively.

B-Homologated analogue, 17\(\beta\)-N, \(\text{N'}\)-diethylcarboxy-6-aza-androst-4-en-3-one (78) has also been found to be potent 5ARI with IC\textsubscript{50} = 318 nM.\textsuperscript{126}

Similar to 4-azasteroids with cholesterol side chain, the 6-azacholenest-3-ones (79-81) were found to possess little selectivity between 5AR-1 and 5AR-2.\textsuperscript{127} All three compounds were found to be potent dual inhibitors with IC\textsubscript{50} value in the range of 0.8-1.0 and 1.2-7.9 nM respectively for 5AR-1 and 5AR-2 isozymes. Introduction of C-7 methyl group might segregate 5AR selectivity. Again the \(\alpha\)-C-7 methyl diastereomer (79) was found to be 7-fold more potent 5AR-1 inhibitor than \(\beta\)-diastereomer (80).

Fang and Sharp in 1996 reported large number of 6-azaandrostenones of the general structure 82 as 5ARIs.\textsuperscript{128} But none of them had shown promising results.
Another series of 6-azaandrosterone was reported by Aster and co-workers where aryloxy, alkoxy and heteroaryloxy groups were incorporated at 16β position. Compound 83 was found to be most potent 5AR-1 inhibitor among the series.

None of the molecule from 6-azasteroid class reported so far in the last three decades, could reach the clinical development stage.

**IV. 19-Nor-10-azasteroids**

SAR studies have suggested the link between inhibition potency of azasteroids with type of substitution at 17 position and the presence as well as position of unsaturation in ring A and C. In this context Guama et al. synthesized a novel class of compounds 19-nor-10-azasteroids (84-90). In this study, $\Delta^{9(11)}$.19-nor-10-azaandrost-4-ene-3,17-dione (84) and 19-nor-10-azaandrost-4-ene-3,17-dione (86) were reported as weak inhibitors of 5AR-2 but more potent inhibitors of 5AR-1 ($IC_{50} = 263$ and 299 nM, respectively), whereas 19-nor-10-aza-5α-androstane-3,17-dione (87) was inactive against both of the isoenzymes. Best results were obtained with 9:1 mixture of $\Delta^{9(11)}$ (88) and
Δ(9) 17β-(N-tert-butyl carbamoyl)-19-nor-10-aza-4-androsten-3-one as it was found to be potent dual 5ARI. The results of ab initio calculations suggested that inhibitory potency of 19-nor-10-azasteroids is directly related to nucleophilicity of carbonyl group at 3rd position.\textsuperscript{131}

It has now been established fact that polar group on C-17 modulate the biological profile of testosterone derived inhibitors. Taking this into consideration Guarna et al. introduced 17β-[N-(aryl)alkyl/aryl-amido] group which is analogous to 17β carbamoyl group in 10-azasteroids. Three pairs of 5α-H/5β-H epimers were synthesized and evaluated as 5ARIs. However, 5β-H compounds were found to be more active than their 5α-H counterparts, with \textsuperscript{91} (IC\textsubscript{50} = 279 and 2000 nM for 5AR-1 and 5AR-2 respectively) and \textsuperscript{92} (IC\textsubscript{50} = 913 and 247 nM for 5AR-1 and 5AR-2 respectively) being the most potent agents from the series.\textsuperscript{131}

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\section*{V. 17- and 17a-Aza-D-homosteroids}

The pioneering work regarding synthesis of 17 & 17a-aza-D-homosteroids was started by Regan and Hayes in 1955.\textsuperscript{132} Introduction of nitrogen at 17a position was accomplished by Beckmann rearrangement of 17-keto steroid oxime whereas rearrangement of 16-oximino-17-keto steroid gave 17-aza homosteroids. 17 and 17a-aza...
steroids were chosen particularly since their isosters oxa-D-homo steroids were shown to possess interesting physiological properties. Later research on 17a-azasteroids attracted more attention when Chandonium diiodide was established as a potent neuromuscular blocker.133

These azasteroids have been found to possess numerous biological activities such as γ-amino butyric acid (GABA) receptor antagonists,134-136 antifungal,137 antineoplastic, mutagenic,138 139 and anti-inflammatory.140 We have seen that clinically available drugs as well as other potential 5AR inhibitors possess lactam in ring A. Taking this into consideration McDonald et al. proposed 17-D-homo-azasteroids as possible scaffold which might be acting by inverted action or backbinding.141

Various 17-oximino-5-androsten-3-β-yl esters, 17-oxo-17a-aza-D-homo-androsten-3-β-yl esters were reported from the laboratory of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh and these compounds were evaluated for their antiproliferative, androgenic activities and acute toxicity against finasteride as positive control. Some of the compounds (93-96) exhibited better antiandrogenic and cytotoxic activities compared to finasteride. Indirectly antiandrogenic activity could be related to 5AR inhibition.142 143

It is hypothesized that steroids without side chains at C-17 position can bind to enzymes with the A-ring of the substance simulating the D-ring, and the D-ring emulates the A-ring. Thus 17-D-homo-azasteroids may exhibit the same mechanism of action as that of 4-azasteroids. Till date 17a-Azasteroids remained largely unexplored avenue as far as their 5AR inhibitory activity is concerned. On the basis of this fact some 17a-azasteroids (97-100) were synthesized and found to be active against 5AR with IC50 value in the range of 4-40 μM.141
VL Hybrids

Yao et al. in 2011 reported a new series of compounds containing features of both finasteride and epristeride (Figure 13). It was hypothesised that keeping the A and B ring of epristeride, and replacing the A ring of finasteride with the D ring of epristeride would increase the inhibition activity of 5AR. The compounds were tested for 5AR inhibition and were found to be equipotent to finasteride. These results indicated, although the hybrid compound possesses the main bulk of epristeride, but its inhibitory mechanism is same as that of finasteride.144

Figure 13. Hybrid of finasteride and epristeride
Parallely, our laboratory also reported the synthesis of 17a-substituted 3-cyano-17a-aza-D-homo-3,5-androstadien-17-one, 17a-substituted 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid, and 17-oxo-19-nor-3,5-androstadien-3-oic acid derivatives. In vitro biological evaluation using human embryonic kidney cells line (HEK 293), demonstrated most of the synthesised compounds as potent 5ARIs when compared to standard drug finasteride.

VII. Diazasteroids

It is an established fact that incorporation of nitrogen in both ring A and D produces molecules that show 5AR inhibitory activity. The lactam in ring A helps in inhibition of enzyme catalysis whereas D ring nitrogen might be interacting with the binding pocket. Eberbach et al. in 1996, reported a series of 4,13-diazasteroids but the compounds were not evaluated as 5ARIs. In the same year Stuart et al. reported synthesis and biological activity of 4,17-diazasteroids. Compound 101, a 17-aza isomer of finasteride exhibited potent inhibition of 5AR-2 although less active than finasteride and its congeners. Methylation at nitrogen (102) led to decrease in activity whereas removal of unsaturation in ring A produced a dual inhibitor 103 which when methylated at nitrogen led to a more active compound 104. Incorporation of unsaturation in ring B 105 showed only a moderate inhibition.

Attempts were made to synthesize 8, 13-diazasteroids by Göndös et al. in 1998, but none of the compounds had shown any promising results.

VIII. Steroidal 3-carboxylic / phosphonic/ phosphinic acids

Development of large number of 3-androstene-3-carboxylic acids were based on the structure of intermediate formed when T is converted into DHT. These carboxylic
Introduction

Acid derivatives contain sp²-hybridized centers at C-3 and C-4 and an anionic carboxylic acid moiety at C-3 which acts as mimic to the putative enzyme-bound enolate intermediate and as a charged replacement for the enolate oxyanion respectively. These acrylates preferentially bind in a ternary complex with enzyme and cofactor (NADP⁺), and depict uncompetitive kinetic mechanism because of favorable electrostatic interaction between the carboxylate and the positively charged oxidized cofactor.⁷⁰, ¹⁵⁰, ¹⁵¹

It was further observed that unsaturation in ring B in conjugation with ring A enhances activity. Intensive research in this class of molecules led to development of epristeride having Kᵣ of 30-36 nM. It has even reached to phase III clinical trials in US but could not qualify as clinical drug for BPH.¹⁵¹

A series of (19-nor) estratriene-3-carboxylic acids has also been reported. SAR studies were carried out by varying substituents at C-2 and C-4 position, by introducing unsaturation in the B and D rings, and appending carboxamide group at C-17 position. Although the compounds were not like natural substrate T, these 19-nor compounds showed potent inhibition of 5AR.¹⁵² Among the series, compound ¹⁰⁶ was most active with Kᵣ of 10 nM.

Instead of carboxylic acid, nitro derivatives ¹⁰⁷⁻¹¹¹ were also prepared and compound ¹⁰⁷ was found to be potent competitive inhibitor by binding to E-NADPH complex.¹⁵³ Although sulphonic acid (¹⁰⁸),¹⁵⁴ phosphonic acid (¹⁰⁹) and phosphinic acid (¹¹⁰) derivatives were also prepared to mimic carboxylic acid but these were weak inhibitors of 5AR compared to steroidal 3-carboxylic acid derivatives.¹⁵⁵
IX. Diazoketone Steroids

It was found that, carboxysteroids having sp\(^2\) hybridization at C-3 and C-4 position induces 5\(\alpha\)R affinity to a considerable extent. This was demonstrated with diazoketone i.e. \((5,20\beta)-4\text{-diazoo-21-hydroxy-20-methyl-pregn-6-en-3-one}\) (112), which is a mechanism based inhibitor with \(K_i\) value of 35 nM.\(^{156}\)

![Image of compound 112]

X. 4-Substituted Steroids

Conjugated enone system consisting of three sp\(^2\) hybridized carbons at C-3, C-4 and C-5 position of steroid A ring with a lipophilic group at C-17 exhibited excellent 5\(\alpha\)R inhibition. A series of 4-substituted-3-oxo-4-androstene-17\(\beta\)-carboxamides were reported and among them 4-cyano compound 113 was found to be potent inhibitor of 5\(\alpha\)R-2 with IC\(_{50}\) value of 2.9 nM.\(^{157}\) Replacement of 4-cyano with trifluoromethyl as in compound 114 enhanced the inhibitory property four times than that of the finasteride.\(^{158}\)

![Image of compounds 113 and 114]

Progesterone with 4-cyano (115) group also acted as inhibitor of both rat and human 5\(\alpha\)R enzyme with IC\(_{50}\) values of 0.045 and 0.050 \(\mu M\) respectively. It was assumed that the cyano steroidal inhibitor acted as a transition state inhibitor because on reduction these compounds would form stable 5,3-enol that would remain tightly bound to the active site of the enzyme.\(^{159, 160}\)
XI. Steroidal Oximes

Heme iron of 5AR has important role in metabolism of T. It was observed that oxime group connected directly or indirectly on steroidal D-ring coordinates with heme iron of 5AR. Various pregnenolone and progesterone oximes (116, 117) were synthesized and evaluated for 5AR inhibitory activity, progesterone derivatives exhibited marked inhibition towards 5AR enzyme in comparison to pregnenolone derivatives. Incorporation of a spacer between oxime and steroidal nucleus increased selectivity towards 5AR-2. One such isomer Z-21-hydroxyiminopregn-4-en-3-one (117) was found to be a potential inhibitor of the 5AR-2 (IC50=1.95 μM for 5AR-1 and 0.30 μM for 5AR-2). However, none of the compound showed better activity than that of finasteride.161

Kim et al. in 2009 has reported synthesis of several epoxy and/or 20-oxime derivatives of pregnane. The compounds were evaluated for their cytotoxic activity towards LNCaP (androgen dependent) and PC-3 (androgen independent) prostate cancer cell lines. The most active compound was 118 among the series.162
XII. Steroidal tetrahydrooxazin-2-ones

Efforts were also made to attach heterocyclic molecules at C-17 position of androst-4-ene-3-one. Wolfing et al. has reported steroidal tetrahydrooxazin-2-ones (119-124) having IC$_{50}$ value ranging between 27-600 nM. Any further substitution in the phenyl ring attached at nitrogen of tetrahydrooxazin-2-one led to decreased activity.$^{163}$

![Chemical Structure](image)

(119) $R =$ Phenyl  
(120) $R = p-$C$_2$H$_5$-Phenyl  
(121) $R = p-$CH$_2$O-Phenyl  
(122) $R = p-$C$_2$H$_4$O-Phenyl  
(123) $R = p-$Br-Phenyl  
(124) $R = p-$Cl-Phenyl

The class of steroidal molecules discussed below do not contain nitrogen either as part of the ring or extranuclear but have shown to possess 5AR inhibitory activities.

XIII. 16-Substituted steroids

A series of 16-methyl substituted derivatives (125-130) both of androst-4-ene and estr-4-ene were originally prepared as antiandrogens but these also exhibited inhibitory activity on rat and human prostatic 5AR enzyme. Moreover compounds 126-128 having alkyl group at C-16 position has shown to increase the inhibition with respect to rat and human prostatic 5AR.$^{164}$

![Chemical Structure](image)

(125) $R_1, R_3 = (\text{-O}), R_2 = R_4 = \text{H}$  
(126) $R_1, R_3 = (\text{-O}), R_2 = \text{CH}_3, R_4 = \text{H}$  
(127) $R_1, R_3 = (\text{-O}), R_2 = \text{H}, R_4 = \text{CH}_3$  
(128) $R_1, R_3 = (\text{-O}), R_2 = R_4 = \text{CH}_3$  
(129) $R_1 = \text{H}, R_2 = \text{OH}, R_3 = \text{CH}_3, R_4 = \text{H}$  
(130) $R_1 = \text{H}, R_2 = \text{OAc}, R_3 = \text{CH}_3, R_4 = \text{H}$
Certain C-16 alkylated estran-4-ene/9-nor analogues were prepared and tested as antiandrogens. From this study TSAA-291 (16-ethyl-17β-hydroxy-4-estren-3-one, 131) was identified as potent antiandrogen with dual action of competitive inhibition of 5AR and androgen receptor.165

XIV. 6-Methylene steroidal derivatives

Affinity for the enzyme is enhanced by addition of a methylene group at C-6 in B ring of the steroid nucleus. Based upon these observations, compound 132 and 133 were synthesized and have shown time dependent rat prostatic 5AR inhibition.166 It was believed that inhibition is due to priming of its dienone group by electrophilic activation toward nucleophilic attack at the 6-methylene group.167

XV. Seco steroids

Two seco-steroids namely (4R)-5,10-seco-estra-4,5-diene-3,10,17-trione (134) and (4R)-5,10-seco-19-nor-pregna-4,5-diene-3,10,20-trione (135) were found to be noncompetitive and possibly irreversible inhibitors of epididymal 5AR with Kᵣ of 5470 and 980 nM, respectively.168,169
XVI. Derivatives of natural substrate

On the basis of important observation that natural substrate progesterone and deoxycorticosterone inhibits the synthesis of DHT by competing with 4-en-3-one function of the T during catalysis, Voigt et al. reported synthesis of a number of progesterone derivatives.84

4-Cyano progesterone (115),159 which exhibited marked 5AR inhibition stimulated great deal of interest to synthesize various 6- and 4- halo progesterone analogs (136-140). Some of the compounds among the series were found to be potent antiandrogens as well as 5ARIs.170-172

\[
\begin{align*}
(136) & \text{ } R=\text{COCH}_3, X=\text{Cl} \\
(137) & \text{ } R=\text{COCH}_3, X=\text{Br} \\
(138) & \text{ } R=\text{CO(CH}_2)_3\text{CH}_3, X=\text{Br} \\
(139) & \text{ } X=\text{Cl} \\
(140) & \text{ } X=\text{Br}
\end{align*}
\]

Bratoeff et al. evaluated various 6-phenyl-D-homo (141, 142), 16-methyl (143-145), 4-bromo (146) derivatives without a methyl group at C-16 and the epoxy compounds (147, 148) for their antiandrogenic and 5AR inhibitory potential. Compounds 142, 145 and 146 were found to possess both antiandrogenic as well as 5AR inhibitory activity compared to finasteride.173

\[
\begin{align*}
(141) & \text{ } R=\text{OH} \\
(142) & \text{ } R=\text{OCOCH}_3 \\
(143) & \text{ } R=\text{OH} \\
(144) & \text{ } R=\text{OCOCH}_3 \\
(145) & \text{ } R=\text{OCO(CH}_2)_3\text{CH}_3
\end{align*}
\]
Various other 4-bromo-17-substituted-4-pregnene-3,20-diones were also synthesized and evaluated as 5ARIs on gonadectomized hamster seminal vesicle and flank organs. Compounds having p-fluorobenzoyloxy (149) and p-chlorobenzoyloxy (150) moiety were effective in reducing the diameter of the pigmented flank organ and weight of seminal vesicle indicating that the presence of more electronegative substituent at C-17 position (p-halosubstituted phenyl) and halogen at C-4 were favorable for 5AR inhibition as well as antiandrogenic activity.\(^{174,175}\)

Two other analogs 151 and 152 having additional unsaturation at \(\Delta^1,2\) were identified as potent inhibitors of 5AR.\(^{174}\)

Based upon the observation that 17\(\alpha\)-acyloyloxy-16-\(\beta\)-methyl substituted pregnadiene-3,20-dione derivatives have high affinity for 5AR as well as androgen receptors, several new 17\(\alpha\)-acyloyloxy-16-\(\beta\)-methyl substituted pregnadiene-3,20-diones
and pregnatriene 3,20 triones were synthesized. Among which compound 153 was found to be moderately active as 5ARI.\textsuperscript{176}

\begin{center}
\includegraphics[width=0.3\textwidth]{153.png}
\end{center}

It was observed that when length and size of side chain at C-17 position increases, 5AR inhibitory potency decreases. Thus, in an effort to optimise the length and size of side chain at C-17 position several compounds were synthesised and it was observed that compounds having hydroxy (154) and \( p \)-bromo benzyloxy (155) groups were most active with an \( IC_{50} \) value of 19 and 100 nM, respectively.\textsuperscript{177, 178}

\begin{center}
\includegraphics[width=0.3\textwidth]{154.png}
\end{center}

\begin{center}
(154) \( R=\text{OH} \)  
(155) \( R=\text{COC}_6\text{H}_5\text{Br} \)
\end{center}

Various 3-substituted-4-pregnene-6,20-diones were also synthesized and on evaluation as 5ARIs, compound 156 was found to be potent inhibitor with \( IC_{50} \) value of 3.0 nM. It was argued that the presence of halogen substituents in ester part at C-3 and \( \Delta^{16, 17} \) increased the binding affinity for the androgen receptor.\textsuperscript{179}

\begin{center}
\includegraphics[width=0.3\textwidth]{156.png}
\end{center}

\begin{center}
(156)
\end{center}
Synthesis has also been carried out where the ester moiety present at C-3 has been appended at position C-17 of D-homo steroid. Compound 157 has shown promising 5AR inhibitory activity with an IC$_{50}$ value of 0.5 nM.$^{180,181}$

In continuation to this, two new steroids (158 and 159) based on the progesterone skeleton and having carbamate groups were identified as 5ARIs with an IC$_{50}$ value of 10, and 50 nM, respectively.$^{182}$

Further, attempts were made to synthesize novel steroids having ester moiety at C-3 and conjugated double bonds at C-4 and C-16 in pregnane series as 5ARIs and compound 160 was found to be most potent with an IC$_{50}$ value of 1.8 nM.$^{183}$
Several progesterone derivatives (161-164) having identical groups attached at C-17 position with minor modifications at B ring were investigated and these have shown IC$_{50}$ value ranging between 10-70 nM.$^{184}$

Although lot of efforts were made to exploit the natural substrate progesterone having identical structure with testosterone i.e. 4-ene-3-one, but none of the compound among the series could reach the clinical phase.

XVII. Steroidal oxazolines

Szécsi et al. have studied the 5AR inhibitory activity of novel compounds (165, 166) containing various derivatised phenyl substituents coupled to oxazoline moiety appended at C-17 of steroidal nucleus. However the compounds were not much active and the activity was in micromolar range.$^{185}$
B. Nonsteroidal inhibitors

Because of ease in synthesis and undesirable hormonal side effects pertaining to steroidal drugs, a large number of non-steroidal 5ARIs have been investigated by academic as well as industrial researchers.186 These inhibitors has been designed as mimics of azasteroidal inhibitors by removing one or more ring from the azasteroidal skeleton or by early non-steroidal lead (ONO-3805, 167) which was prepared as leukotriene synthesis inhibitor187 or by high throughput screening.

These compounds are generally thought to act as competitive inhibitors verses T with exception of the epristeride analogues which are uncompetitive inhibitors. Non-steroidal inhibitors include benzo[f]quinolinones, pyridines, quinolinones and piperidines which were mimics of 4-azasteroids. Benzo[c]quinolinones were synthesized as mimics of 6-azasteroids while benzo[c]quinolizinones were designed as mimics of 10-azasteroids. All the other reported non-steroidal inhibitors has been collectively considered as carboxylic acid derivatives and are thought to act as non-competitively in analogy to ONO 3805 (167).187

I. Mimics of 4-azasteroids: Benzo[f]quinolinones

Benzof[f]quinolinones obtained by removing D ring from 4-azasteroids and replacing C ring with an aromatic one has been first reported by researchers at Lilly.188 Most of these compounds exhibited 5AR-1 inhibition although dual inhibitors can be obtained with an appropriate substitution at position 8 of the aromatic ring.

Two main classes of benzof[f]quinolinones have been discussed, the hexahydro derivatives 168-171, which have an unsaturation at position 4a-10a, and the octahydro derivatives 172-177 with aromatic C ring in both the series. In general, octahydro derivatives were found to be more potent as compared to their corresponding 4a-10a...
unsaturated derivatives. The presence of halogen at C-8, methyl at C-4, lead to enhanced potency toward 5AR-1 in both of the series. The most potent compound from the series was \(174\) with \(IC_{50}\) of 8 nM.

[Chemical structures and images]

The effect of substituents on 8\(^{th}\) position has been studied using QSAR and the lipophilic group at this position was found to be favorable for activity.\(^{189}\) On the basis of observation that optimum activity reside in the property space around the chlorine substituent, a number of 8-substituted 5AR-1 inhibitors were synthesized (\(178-180\)).\(^{200}\)

II. Pyridones, quinolinones and piperidines

Abell et al. reported tricyclic thiolactams (\(181, 182\)), bicyclic lactams (\(183-186\)) and bicyclic thiolactam (\(187\)) as 5ARIs. Removal of two or more rings from 4-azasteroids resulted in a strongly diminished potency. The tricyclic thiolactams were found to be selective 5AR-1 inhibitors and in general were less active than the corresponding lactams.\(^{191}\)
Hartmann et al. reported testosterone mimics comprising of a pyridone or piperidine moiety mimicking ring A and diisopropyl or t-butyl benzamide mimicking ring D of steroid nucleus. Most active compound was N, N-diisopropyl-4-[2-(1-methyl-2-oxo-piperidine-5-yl)-ethylene] benzamide (188) with an IC$_{50}$ of 13 µM.\textsuperscript{192-194}

A series of 5-phenyl substituted 1-methyl-2-pyridones have also been reported as 5ARIs. Compound 189 having a bulky carboxamide substituents exhibited good 5AR-2 inhibitory activity with IC$_{50}$ value of 10 µM.\textsuperscript{195}

McCarthy et al. reported a series of 4' substituted 5-aryl pyridones along with corresponding 1-aryl-pyridone derivatives as 5ARIs (190-196). Bicyclic pyridines containing long chain or 4' benzoyl showed more potent inhibition against 5AR-1. Compound 193 was emerged as most potent inhibitor with IC$_{50}$ of 3 nM against 5AR-1. It was concluded that large hydrophobic groups are tolerated in a region of the active site which is not taking part in the catalysis of conversion of T into DHT.\textsuperscript{196}
A series of compounds belonging to 1\textit{H}-quinolin-2-ones (197, 198) and 2-methoxy quinolines (199, 200) have also been reported as dual 5ARIs. While compound 6-[4-(\(N, N\)-diisopropylcarbamoyl) phenyl]-1\textit{H}-quinolin-2-one (197) was active against 5AR-2 with \(K_i\) value of 800 nM, compound 6-[4-(\(N, N\)-diisopropylcarbamoyl) phenyl]-\(N\)-methyl-quinolin-2-one (198) was a potent 5AR-1 inhibitor with an \(IC_{50}\) value of 510 nM.\(^{197}\)

Hartmann \textit{et al.} had designed an attractive new series of compounds i.e. \(N\)-substituted benzylidine piperidine 4' carboxylic acids as 5ARIs showing promising \textit{in vivo} results. To further optimize the lead, various modifications were carried out and a series
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of 2'-substituted 4-(4'-carboxy- or 4'-carboxymethylbenzylidene)-N-acylpiperidines were synthesized and evaluated for inhibition of 5AR-2 isozyme.

In the dicyclohexylacetyl series, fluorination at C-2 of benzene nucleus (201), exchange of the carboxy group by a carboxymethyl moiety (202) and combination of both structural modifications (203) led to development of potent 5AR-2 inhibitors with IC\textsubscript{50} values of 11, 6 and 7 nM respectively.\textsuperscript{198}

\begin{align*}
(201) & \\
(202) & R = \text{H} \quad (203) & R = \text{F}
\end{align*}

N-Substituted 4/5-(indolyl) benzoic acid had been reported to exhibit dual inhibition of 5AR with moderate potency and it mimic steroidal substrate in contrast to steroidal inhibitors which has a lipophilic substituent at 17\beta-position, the substituent in indole compounds is located in the plane of the molecule. On the basis of this observation Hartmann et al.\textsuperscript{199} designed and synthesized N-substituted piperidine-4-(benzylidine-4-carboxylic acids), (204-208) as potent non-steroidal dual 5ARIs which are similar to steroid skeleton. Compound 204 (IC\textsubscript{50}=3.44 and 0.37 \mu M for 5AR-1 and 5AR-2, respectively) and 206 (IC\textsubscript{50}=0.54 and 0.69 \mu M for 5AR-1 and 5AR-2, respectively) were found to be inhibitors of rat isozymes. While compound 205 showed a strong inhibition toward human and rat 5AR-2 (IC\textsubscript{50}=60 and 80 nM respectively) but moderately active towards 5AR-1. Compound 207 (IC\textsubscript{50} = 0.26 \mu M human 5AR-2 and 0.29 \mu M for rat 5AR-2) was found to be a moderate dual inhibitor possibly due to higher flexibility of the open ring substituent.\textsuperscript{200}

\begin{align*}
(204) & R = \text{Methyl diphenyl} \quad (205) & R = \text{Methyl dicyclohexyl} \\
(206) & R = \text{Diphenyl amino} \quad (207) & R = \text{4-Heptyl} \\
(208) &
\end{align*}
III. Mimics of 6-azasteroids: Benzo[c] quinolinones

Taking lead from Lilly’s benzoquinoline derivatives and skeleton of 6-aza-androsten-3-one, various phenanthridin-3-one derivatives (209-211) containing vinylogous amide functionality were reported. These inhibitors were thought to act as transition state mimic of conversion of T to DHT. Most of the compounds were found to be poor inhibitors of 5AR-2 as compared to 5AR-1.\(^{201}\)

![Image of 6-azasteroid structure]

\[(209) \text{R=CH}_3, \text{R}_1=\text{H} \]
\[(210) \text{R, R,=CH}_3 \]
\[(211) \text{R=H, R}_1=\text{CH}_3 \]

IV. Mimics of 10-Azasteroids: Benzo[c] quinolizinones

A series of 19-nor-10-azasteroids was reported by Guarna et al. as possible transition state mimic of the conversion of T to DHT. The compounds were dual inhibitors with potency comprehensively dependent upon the presence, position and number of unsaturation on A, B & C rings as well type of substituents at C-17. The observation that potency increases with unsaturation on C-ring is regarded on the fact that some flatness is involved in azasteroidal skeleton.

This prompted the development of benzo[c]quinolizin-3-one derivatives as 5ARIs. These compounds while preserving the A ring enamine-one moiety as essential feature of 19-nor-10-azasteroids lacked D ring and instead further incorporated a more planer benzene ring in place of C ring.\(^{202}\)

Guarna et al. described synthesis of two series of benzo[c]quinolizin-3-ones as 5AR-1 inhibitors. 4aH-Series with a double bond between the positions 1 and 2 (212-215) and 1H-series with a double bond between the positions 4 and 4a (216-221). Increased potency of both of the series was attributed to the presence of double bond enabling more conjugation between carbon and nitrogen atom.\(^{203,204}\)
In continuation of earlier studies, Guama et al. in 2001 reported several octahydro- and decahydrobenzo[c]quinolizin-3-one derivatives having partially or fully saturated C-ring to study the effect of unsaturation. These compounds were found to be selective 5AR-1 inhibitors. Compound 222 lacking the aromatic C ring but with a double bond at 6a–10a was 345-fold potent than that of the corresponding 6a–10a saturated, trans-fused compound 223.\(^{205}\)

Another series of benzo[c]quinolizin-3-ones derivatives (224-229) having ester functionality attached at position 8 and aromatic C ring were synthesized.\(^{206}\) On evaluation against 5AR-1 and 5AR-2, compound 226 bearing fluorine at para position of phenyl ring was identified as most potent dual inhibitor with IC\(_{50}\) values of about 100 nM for both of the isozymes.
V. Aryl carboxylic acids

It was observed that lactam of 6-substituted 1H-quinolin-2-ones mimic the steroidal A ring of finasteride and biphenyl-4-carboxylic acids are steroidimimetic of A-C ring and were more appropriate for 5AR inhibition than ring A-C mimetics, 5-phenyl-1-methyl-2-pyridones. On the basis of these facts various non-steroidal carboxylic acids were designed and synthesized as the structural mimetic of epristeride. Most of the non-steroidal carboxylic acid derivatives reported in literature as 5ARIs has been designed on the basis of above facts. 9, 10-dihydropyrenanthrene-2-carboxylic acids (230-234) were prepared and found to be selective 5AR-1 inhibitors. Incorporation of a bromine atom at position 7 as in compound 232 further potentiate its action. It is believed that these compounds interact with enzyme-NADPH complex in an uncompetitive manner vs. T ($K_i = 26$ nM). Another series of 4a-methyl-3,4,4a-9-tetrahydrophenanthrene-2-carboxylic acids has been prepared and evaluated as 5AR out of which compound 5(7-chloro 4a-methyl-3,4,4a-tetrahydrophenanthrene-2-carboxylic acid (233) was marginally selective inhibitor of 5AR ($K_i = 260$ nM). The related phosphonic acid derivatives (234) were weak inhibitors of 5AR-1 and 5AR-2.207,208

On removal of ring from the parent steroid skeleton several steroidal carboxylic acid mimics (235, 236) had been synthesized. Hartmann et al. reported synthesis and 5AR inhibitory activity of several N-substituted 4-(5-indolyl) benzoic acids. Two of the compounds 235 and 236 were active against human 5AR-1 having IC$_{50}$ of 67 nM and 680 nM, respectively.209,210
Another new series of indole derivatives (237-240) were synthesized by Igarashi et al. as potent human 5ARIs. The compound 4-[(1-benzyl-1H-indol-5-yl)oxy]-3-chlorobenzoic acid (238) and 3-chloro-4-(1-(4-phenoxybenzoyl)-1H-indol-5-yl)oxy)benzoic acid (239) were found to be active with an IC\textsubscript{50} value of 0.44 and 2.1 nM, respectively.\textsuperscript{210}

The observation that aryl carboxylate act as 5ARIs by SmithKline-Beecham led to discovery of two series of non-steroidal compounds based on benzophenone and indole carboxylic acid skeletons, respectively. In benzophenone series, compounds 241 and 242 were found to be most potent with $K_c$ of 10 and 5 nM respectively. In indole series compounds 243 and 244 were most active with $K_c$ of 10 and 40 nM, respectively.\textsuperscript{88, 210}

Takami et al. reported many indole based 5ARIs where carboxylic group was not directly attached to the indole nucleus but present as butyric acid. Compound (Z)-4-(2-
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[[3-[1-(4,4'-Difluorobenzhydryl)indol-5-yl]-2-pentenoyl]-amino] phenoxy) butyric acid (245) was identified as the most potent 5ARI with an IC$_{50}$ value of 0.48 nM.$^{211}$

Another related benzimidazole derivatives are compound 4-(2-[1-(4,4'-dipropylbenzhydryl)indole-5-carboxamido]phenoxy)butyric acid (246) and its methyl ester (247) with an IC$_{50}$ values of 9.6 and 13 nM, respectively.$^{212}$

Sawada et al. reported a series of indolizine butyric acid derivatives. Compound (S)-4-[1-[4-[1-(4-isobutylphenyl)butyl]oxy]benzoyl]indolizin-3-yl] butyric acid (248) displayed highest in vitro inhibitory activity (IC$_{50} = 4.6$ nM) against the human 5AR enzyme and in vivo inhibitory activity (IC$_{50}=1.7$ nM) against the castrated rat model of BPH.$^{213}$

It was suggested that ether linkage between steroidal A-C ring mimetic lead to increased conformational flexibility and thus might enable an inhibitor to better...
accommodate into active site of the enzyme. On the basis of this hypothesis several carboxamides, biphenyl and phenyl acetic acid derivatives were synthesized and evaluated as dual 5ARIs. Compound 249 was found to be a potent inhibitor with an IC₅₀ value of 60 nM while 250 had an IC₅₀ value of 0.38 pM.²¹⁴

Baston et al. reported several 5ARIs comprised of 3,4-dihydro-naphthalene and naphthalene 2-carboxylic acid moiety mimicking steroidal A-B rings for generation of potent transition state analogue of substrate T and androstandione. The most potent inhibitors were 6-[3-(A,A-dicyclohexylaminocarbonyl) phenyl]-3, 4-dihydro-naphthalene-2-carboxylic acid (251) (IC₅₀ = 0.81μM, human 5AR-1; IC₅₀ = 0.75μM, human 5AR-2) and 6-[4-(N,N-diisopropylamino-carbonyl) phenyl]naphthalene-2-carboxylic acid (252) (IC₅₀ = 0.2μM, human 5AR-2).²¹⁵

Recently Hartmann at el. reported a series of benzylidine esters and claim their action as hybrid inhibition. These esters acted as inhibitors of 5AR-1 in the periphery and are cleaved in the prostate to corresponding acids which are potent inhibitors of 5AR-2. In this regard synthesis, biological evaluation and molecular modeling of novel benzoyl benzoic acid and phenylacetic acid derivatives were reported.

The phenylacetic acid derivatives were more potent than the analogous benzoic acids. Various potent inhibitors from this series were 253 (IC₅₀ = 23 nM), 254 (IC₅₀ = 5 nM), and 255 (IC₅₀ = 27 nM).²¹⁶
Igarashi et al. synthesized and evaluated a series of indoline and aniline derivatives as human and rat 5ARls. Among the indoline series, 3-chloro-4-([1-(4-phenoxybenzyl)indolin-5-y]oxy)benzoic acid (256) was found to be the most potent inhibitor against human enzyme with an IC\textsubscript{50} value of 5.3 nM, whereas 3-chloro-4-(4-[N-(4-phenoxybenzyl)amino] phenoxy)benzoic acid (257), from the aniline series, emerged as potent inhibitors with an IC\textsubscript{50} value of 10 and 5.5 nM against human and rat enzymes, respectively.\textsuperscript{217}

4-(2-phenylethyl)cyclohex-1-ene carboxylic acids were synthesized with phenyl, phenoxy and isopropyl substituents at para position of the phenyl ring. Compound N, N-diisopropylcarbamoyl (258) (IC\textsubscript{50} = 760 nM) was found to be potent inhibitor among the series.\textsuperscript{218}

On the basis of estrogen receptor binding affinity of a series of 2', 6'-disubstituted 4-hydroxy-4'-hydroxymethyl biphenyl derivatives, Lesuisse et al. designed various such derivatives as surrogates of the steroidal backbone. It was thought that by introducing appropriate substituents, non steroidal estrogens could be tailored into 5ARls. Compounds 259 and 260 emerged as potent 5AR-2 inhibitors with IC\textsubscript{50} being 71 and 9.8 nM, respectively.\textsuperscript{219}
Several other carboxylic acid derivatives have been explored as 5ARIs but till date none could reach the clinic.\textsuperscript{220, 221}

\textbf{VI. Bisubstrate inhibitors}

The nonsteroidal \textit{o}-hydroxyaniline (167, ONO-3805) was a weak, bisubstrate inhibitor of 5AR-2, in which the butanoic acid moiety is supposed to be lying 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. This is further supported by the fact that it acts as non-competitive inhibitor instead as an uncompetitive inhibitor.\textsuperscript{186, 222, 223} This prompted Pfizer to prepare the derivatives of 167 and subsequently C-3 acylindole (261) was found to be a potent dual 5ARI. The benzodioxolane (262) was found to be a potent dual 5ARI. Introduction of a methyl group on indole ring (263) increased its selectivity towards 5AR-1.\textsuperscript{224}

Further Ishibashi \textit{et al.} reported a series of novel benzofuran derivatives with both carboxy and 5- or 6-diphenylmethylcarbamoyl groups as rat and human 5ARIs. The compounds were more active against human 5AR-1 than 5AR-2. The 6-carbamoyl derivative (264) was found to be most potent compound having IC\textsubscript{50} value of 37.9, 50 and 340 nM against rat, human 5AR-1 and 5AR-2 isozymes respectively.\textsuperscript{225}
Later a series of 2-phenylbenzofuran derivatives with a carbamoyl, alkylamino, or alkyloxy group at the 5 or 6 position of the benzofuran ring was also synthesized. It was found that carbamoyl derivatives were more potent than the alkylamino or alkyloxy derivatives against the rat enzyme. The 6-carbamoyl and 6-alkylamino derivatives were found to be more potent inhibitors against human 5AR-1 than 5AR-2.\textsuperscript{226}

Sawada \textit{et al.} reported 4-[3-[3-[bis(4-isobutylphenyl) methyl amino] benzoyl]-1H-indol-1-yl] butyric acid (265) as a potent dual inhibitor of both human 5AR isozymes with an IC\textsubscript{50} of 1.9 and 4.2 nM for human and rat isozymes respectively.\textsuperscript{227-229}

### VII. Miscellaneous non-steroidal inhibitors

Various non-steroidal inhibitors which could not be categoriesed under the above mentioned classes are discussed in this section as miscellaneous inhibitors.

Fan \textit{et al.} evaluated a series of 7-hydroxycoumarin derivatives as 5AR-1 inhibitors on prostate cancer cell line (LNCaP). The coumarin skeleton was considered as a mimic of transition state of the natural substrate T and as well as bioisostere of quinolin-2-one. Compound 1',1'-dimethylallyloxycoumarin (266) showed potent inhibitory activity (IC\textsubscript{50}=1.3 μM) for the 5AR-1. Compound 8-allyl-7-hydroxycoumarin (267) also exhibited potent inhibitory activity (IC\textsubscript{50}=0.99 μM) against 5AR-1. Introduction of a carbonyl
group at 7-position (268) resulted in only a slight increase in 5AR-1 inhibitory activity (IC$_{50}$=0.49 µM).\textsuperscript{230}

\begin{center}
\includegraphics[width=\textwidth]{images}
\end{center}

Chen \textit{et al.} screened several isoflavonoids as potential nonsteroidal 5ARIs. The compounds (269-272) were inhibitors of rat 5AR in the range of 27-49 µM.\textsuperscript{231}

\begin{center}
\includegraphics[width=\textwidth]{images}
\end{center}

Hosoda \textit{et al.} in 2007 designed and synthesized 5AR-1 inhibitors using 3, 3-diphenylpentane skeleton as a substitute for the usual steroid skeleton. Among the series 4-(3-(4-(N-Methylacetamido) phenyl) pentan-3-yl) phenyldibenzylcarbamate (273) was found to be a competitive 5AR-1 inhibitor with an IC$_{50}$ value of 0.84 µM.\textsuperscript{232}

\begin{center}
\includegraphics[width=\textwidth]{images}
\end{center}
1.1.3.3. Phytotherapy

Several studies have shown the use of phytotherapeutic agents for the treatment of BPH. These are mainly extracts of different plants and their parts. It still remains unresolved as to which components of the extracts are responsible for symptomatic relief of male LUTS. Phytosterols, β-sitosterol, fatty acids, lectins etc. have been considered as active components.\textsuperscript{233} \textit{In vitro} studies have shown that these plant extracts have anti-inflammatory, antiandrogenic, or oestrogenic effects; decrease sexual hormone binding globulin; inhibit aromatase, lipoxigenase, growth-factor stimulated proliferation of prostatic cells, α-adrenoceptors, 5ΑR, muscarinic cholinceptors; improve detrusor function; neutralise free radicals.\textsuperscript{234} However, most \textit{in vitro} effects have not been confirmed \textit{in vivo} and the precise mechanisms of action of plant extracts remain unclear.

These herbal formulations are prepared using roots, seeds, pollen, bark, or fruits of a single plant (monopreparations) or extracts of two or more plants to make a single preparation (combination preparations). The most widely plants used are as follows.

A. \textit{Cucurbita pepo} (pumpkin seeds)

Pumpkin seed (\textit{Cucurbita pepo} L.) oil has been used to treat urinary tract problems since the end of 19\textsuperscript{th} century. The seeds of the plant contains about 30\% to 51\% of the oil. Main components of the oil are unsaturated fatty acids such as linoleic acid (43-55\%) and oleic acid (27-38\%). Other chemical substances reported in the seeds of \textit{C. pepo} includes tocopherols and sterols in free and glucosidic forms.

It also contain minor amount of Δ\textsuperscript{5} and Δ\textsuperscript{1}-sterols. β-Sitosterol, which is the main component of \textit{C. pepo} oil, has also been shown to be a strong inhibitor of prostaglandin biosynthesis in prostatic tissue of patients with BPH and exert a marked anti-inflammatory effect.\textsuperscript{235}

B. \textit{Hypoxis rooperi} (South African star grass)

Reports have shown the use of South African star grass in the treatment of BPH and prostate adenoma. β-Sitosterol present in the extract exerts action on prostate adenoma and other component rooperol possess anti-inflammatory action probably due to its interference with prostaglandin metabolism.\textsuperscript{236} The use of this agent is limited because of its uncertainty about its long term effectiveness, safety and ability to prevent BPH complications.\textsuperscript{234}
C. *Pygeum africanum* (bark of the African plum tree)

Studies have shown the use of African plum tree (*Pygeum africanum*) bark extract in France and throughout Europe since 1969 to treat BPH. It has been considered as second most popular herbal preparation used worldwide for BPH. The extract contains phytosterols, pentacyclic triterpenes and ferulic acid esters. The phytosterols, especially β-sitosterol, have antiinflammatory properties which indirectly inhibits the prostaglandin synthesis which is responsible for the swelling of the prostate gland. The sterolic portion of *pygeum* is thought to help in preventing the accumulation of T in prostate.

D. *Secale cereale* (rye pollen)

Cemilton® is a commercial preparation which is made up of pollen extract from several plants including *Secale cereale*. It is manufactured by microbial digestion of the pollen followed by extraction with both water and organic solvents. Several *in vitro* studies have suggested that it also possesses anti-androgenic activity, 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 and thus relieves LUTS associated with BPH.

E. *Serenoa repens* (syn. *Sabal serrulata*; berries of the American dwarf palm, saw palmetto)

It is the most popular phytotherapeutic agent used in Europe and USA for the treatment of BPH. It is made up of extracts of the dried ripened fruits of *Serenoa repens*. The liposterolic extract obtained by hexane extraction of *Serenoa repens* is shown to possess antiandrogenic, antiestrogenic and anti-inflammatory activities. It also inhibits the 5AR-1 and 5AR-2 isozymes, prolactin and growth factor-induced cell proliferation. The ethanolic extract of saw palmetto inhibited 5AR-2 isozyme 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. It has been shown to be an effective dual inhibitor of 5AR isoenzyme in the prostate and induces its effects without interfering with the cellular capacity to secrete prostate-specific antigen (PSA).

F. *Urtica dioica* (roots of the stinging nettle)

*Urtica dioica* (stinging nettle) root extract have been shown to have limited clinical use in treatment of milder form of BPH. Various possible mechanisms of action
have been suggested including inhibition of prostatic growth factor interaction by blockage of conversion of T to DHT, inhibition of membrane sodium and potassium-adenosine triphosphatase in the prostate, resulting into suppression of prostate cell metabolism and growth.245, 246

However, despite several studies involving use of phytotherapeutic agents for the treatment of BPH, none of the formulation is approved by USFDA. This may be due to the unknown mechanism of action and safety and efficacy profile of these agents. These agents are generally used as an alternative therapy to the currently existing drugs with very less clinical evidences of their efficacy and safety.

1.1.3.4. Phosphodiesterase Inhibitors

A number of hypothesis have been proposed that include alteration of nitric oxide (NO)-cyclic guanosine monophosphate pathway, enhancement of RhoA-Rho-kinase contractile signalling, autonomic adrenergic hyperactivity, and pelvic atherosclerosis which may explain the action of PDE5 inhibitors on BPH-related LUTS.247

There are four PDE5 inhibitors, tadalafil (274), vardenafil, avanafil, and sildenafil clinically used in the treatment of erectile dysfunction.248 These PDE5 inhibitors mediates the smooth muscle relaxation in the lower urinary tract, thus making their potential use for management of BPH.249 Recently USFDA has approved daily tadalafil (5 mg) for the treatment of LUTS secondary to BPH with or without simultaneous erectile dysfunction and it is expected to be officially registered for the management of male LUTS in Europe.250

1.1.3.5. Leutenizing hormone releasing hormone (LHRH) antagonists

LHRH antagonists degarelix, tevarelix, cetrorelix (275) and ozarelix are mainly used in the treatment of prostate cancer. They competitively inhibit the gonadotropin releasing hormone receptor in the pituitary gland, thereby reducing the levels of LH
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which acts on the testes and, therefore, reducing the levels of T available for conversion to DHT.\textsuperscript{251} LHRH agonist/antagonists are used in the case where the patients are suffering from prostate cancer associated with significant LUTS.

Either as a single in patients who cannot obtain radiation treatment or in patients who obtain medical castration treatment as part of neo adjuvant treatment in radical radiation treatment.\textsuperscript{252} Due to failure of a study, AEterma Zentaris terminated an agreement with Sanofi Aventis, USA for further development, commercialization and licensing of cetrorelix in the treatment of BPH.\textsuperscript{253} LHRH antagonists are promising agents in the management of both prostate cancer and BPH, however, further large Phase III, randomized, placebo and active control studies are needed to assess efficacy and tolerability.

\begin{center}
\includegraphics[width=0.7\textwidth]{cetrorelix.png}
\end{center}

\subsection{1.1.3.6. Muscarinic receptor antagonists}

Acetylcholine is the predominant neurotransmitter of the urinary bladder that stimulates the muscarinic receptors on the surface of detrusor smooth muscle cells. Out of five human muscarinic receptor subtypes (M1-M5), M2 and M3 are predominantly expressed in the detrusor and only M3 subtype is involved in bladder contractions in healthy humans.\textsuperscript{254} Inhibition of muscarinic receptors by antagonists decreases receptor stimulation and reduces the smooth muscle contractions of the bladder thus relieves LUTS. Various muscarinic receptor antagonists approved for the treatment of overactive bladder/storage symptoms in men and women are darifenacin hydrochloride (darifenacin), fesoterodine fumarate (fesoterodine), oxybutynin hydrochloride (oxybutynin), propiverine hydrochloride (propiverin), solifenacin succinate (solifenacin), tolterodine tartrate (tolterodine) and trospium chloride. However, this class of drug is only
recommended in the men with moderate to severe LUTS associated with predominant bladder storage symptoms.\textsuperscript{255}

1.1.4. Biological evaluation

Till date a number of \textit{in-vitro} and \textit{in-vivo} models are available for biological evaluation of 5AR inhibitory activity in both humans and animals. A brief summary of these methods has been described below.

1.1.4.1. \textit{In vitro} assay methods

\textbf{A. Inhibition of 5AR}

The enzyme 5AR can be obtained 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 5AR assay, reaction solutions are prepared in duplicate tubes containing 1 $\mu$M $[^{14}\text{C}]$ T. Further, the test compounds or standard drug as inhibitors are added in 5 $\mu$l 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 minutes and are stopped with addition of 2.0 ml of ethyl acetate containing T, 5α-dihydrotestosterone, and androstanedione (10 $\mu$g 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. Radioactivities of the products are combined for the calculation of the 5AR activity. IC$\textsubscript{50}$ values are calculated based on at least 5 dilutions of test preparations or standard.\textsuperscript{256}

\textbf{B. DU-145 assay method}

\textit{In vitro} inhibitory activity of the compounds can be evaluated using DU-145, PC-3 and SW-13 as a source of 5AR-1 and 5AR-2. It is carried out by incubating different concentrations of the compounds along with $[^1\text{H}]$ androstenedione.\textsuperscript{257, 258} 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.
C. *Penicillium crustosum* broth method

The *in vitro* biological activity of compounds can be determined by measuring the transformation of T to DHT produced by 5AR enzyme in *P. crustosum* broths. For evaluation of new compounds as 5ARIs, 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 or a combination of test steroids with T. It is determined by the reverse isotope dilution technique. This model determines the efficacy of the newly synthesized compounds as 5ARIs.259

D. HEK 293 cells method

In this method, human embryonic kidney cell line HEK293, which lacks endogenous 5AR activity was transfected with the cDNA for either of the isoforms i.e. 5AR-1 or 5AR-2. 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 used as control.

Using this method, dual and selective inhibitors of both of the isozymes could be identified. HEK293 cells (300,000/ well) transfected either with pRecCMV-I or with pRecCMV-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 $^3$H 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 under investigation.260,261
1.1.5. *In vivo* assay methods

**A. Chicken comb method**

This classical bioassay based on growth of the chicken comb has been used for evaluation of androgenic activity. Newly hatched chicks of either sex are used to study the growth of combs after systemic or local administration of novel compounds under investigations. 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 hrs after the last application, the animals are autopsied and 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 then established.\textsuperscript{262,263}

**B. Hamster flank organ test method**

In this method flank organs of the gonadectomized Syrian Golden male hamsters (150-200 g) are used for screening of new antiandrogenic agents. 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 T restored the original diameter of the spot. The flank organs can convert T to DHT in both intact and gonadectomized animal since the 5AR enzyme is present in this tissue. Varying doses of the test compound and the control i.e. T 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. The test compounds such as finasteride significantly reduces the diameter of the pigmented spot on the flank organs as compared to that of the T.

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 incorporation of
radioactive sodium acetate into lipids under culture condition can also be evaluated to establish the efficacy of the test compound relative to known compounds.\textsuperscript{170, 180, 264}

C. Scrotal incision method

In this method, male rats are castrated by scrotal incision under ether anesthesia. Varying doses of the test compounds in oil, finasteride (1mg/kg) and testosterone propionate (1mg/kg) are given to the animals by subcutaneous (s.c.) injections once daily for 4 days. Twenty four hrs after the last treatment, the animals are sacrificed by CO\textsubscript{2} inhalation, and the ventral prostates are removed. The mean percentage 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 methods are applied to determine statistical significant difference between the control and the test compounds.\textsuperscript{119, 199, 201}

D. Seminal vesicles test method

In this test, the effect of steroids on seminal vesicles obtained from castrated male hamsters is determined. The animals are administered with s.c. injections of the compounds under investigation 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. The results obtained are analyzed for significant difference between the known and test compounds using one-way analysis of variance.\textsuperscript{170, 174}

E. Effect on androgen level

In this method circulating levels of T and DHT hormone level or tissue concentration are measured after administering 5ARIs using radioimmunoassay or ELISA giving an indication of level of inhibition by the compound and can be compared with that of standard.\textsuperscript{256, 265}

F. Change in rat prostate weight method

In this method change in prostate weight of rat is monitored. Compounds under study are dissolved in 0.5% methylcellulose and administered orally to 9 weeks old
mature male rats for 14 days. On day 15, animals are sacrificed and body weight is recorded. After this, the adrenal glands, liver, ventral prostate, seminal vesicles and right testicle are removed, cleared of adherent tissue and weighed. The change in prostate weight after treatment with the compounds under investigation are then compared with control using ANOVA.229,266

1.1.5. Computer aided drug design

Drug design is basically design of small molecules that will activate/inhibit the functions of biomolecules which may result in desired therapeutic benefit. The ultimate understanding of drug action requires detailed mechanism of microscopic chemical processes at the level of actual motion and rearrangement of individual molecular fragments, contemporary theoretical approaches require identification of main active centers and the desired shape and electronic features governs activity, pharmacokinetic and pharmacodynamic profiles.267 CADD methods have their foundation in the correlation of computed and experimentally determined properties of molecules. The outcome of such studies helps in understanding the behavior of molecules prior to their synthesis thus saves lot of manpower, time and money.

Such studies comes under the preview of rational drug design. With tremendous upsurge in the computational power of machines, it is now possible to use them for easier task, like drawing a 2D structure to the herculean task, such as screening of a trillion of compounds against a suitable target. Molecular modeling has emerged as a valuable tool for rational design of inhibitors that specifically target the enzyme in question and, thereby, are more likely effective at low doses and devoid of negative side effects.267

These methods could be broadly classified into two types i.e. structure based drug design and ligand based drug design methods.

A. 3D QSAR: Comparative Molecular Field Analysis and Comparative Molecular Similarity Indices Analysis

Quantitative structure activity relationship (QSAR) is a relationship between the variables which describes the structural variation within the group of compounds under investigation and those which describes bioactivities. Traditionally 2D QSAR was used to correlate various structural parameters known as molecular descriptors derived from 2D structure of molecules under study with biological activity. Due to several disadvantages of 2D QSAR like non-consideration of configurational and conformational effects and
drug receptor interactions led to development of 3D QSAR. In 3D QSAR, 3D properties of a molecule are considered as a whole rather than individual substituents or moieties.

The basic philosophy of 3D QSAR is assumption that important features that contribute to the activity lies in its overall size, shape and electrostatic property. In 1988, Cramer et al. introduced the CoMFA method. The basic principle of CoMFA is that there is a relation between binding affinities of ligands and strength of non-covalent interaction fields surrounding the molecules. These fields were of steric and electrostatic nature first, but were later extended by hydrophobic fields and hydrogen bond accepting and hydrogen bond donating fields.

To calculate these fields value, the 3D structure of the molecule is locked into a suitably sized preconstructed 3D lattice or grid. The intersection of this lattice are called as grid points and these define 3D space around the molecule, and is denoted by a suitable number. After placing the molecule into the lattice, the steric and electrostatic fields around it can be measured by placing a probe atom such as proton or a sp^3 hybridised carbocation at each of the grid point in turn and using the software to calculate the steric and electrostatic interaction between the probe and the molecule. These values at each grid point are tabulated with certain cut off values. The field values are then used to generate the equations that will correlate fields with biological activity using partial least square method. The beauty of CoMFA lies in displaying results of these equations as visual graphic representation in 3D contour map which shows the individual contribution of fields towards binding affinity. The QSAR CoMFA equation are used for predicting property values of new compounds provided that there should be structural similarity between the compounds used for model development and new molecules.

One of the most important prerequisite for this type of analysis is alignment of the set of molecules. Various types of alignment methods are used such as maximum common substructure based, atom and centroid based which uses a template molecule for alignment. The template molecule could be among (a) the most active molecule, (b) lead molecule, and (c) molecule containing the largest number of functional groups. The other alignment methods are pharmacophore based and docking based.

For calculating the steric contributions, the Lennard-Jones potential is used, and for calculating the electrostatic contributions, the Coulomb potential is used. CoMSIA is an extension of the CoMFA methodology developed by Klebe et al.
Similarity is compared in terms of similarity indices. Its advantages over the standard CoMFA technique are reported to be a greater robustness regarding both region shifts and small shifts within the alignment, and more intuitively interpretable contour maps. This is a result of the application of similarity indices calculated by using a Gaussian-type distance dependence instead of the Lennard-Jones and the Coulomb potential which makes the more or less arbitrary application of cutoff values unnecessary.

Lack of knowledge about crystal structure of the 5AR-1 and 5AR-2 is the major hurdle in the discovery of novel 5ARIs. Not much studies have been reported involving use of CADD methods for design of 5ARIs. However the various studies are summarized below.

Ahmed et al. reported a modeling study using both steroidal and non-steroidal 5ARIs to elucidate the essential structural requirements for design of non-steroidal inhibitors. The results of the study revealed that there is a requirement for groups to mimic the steroid substrate (T) A-ring, in particular the C-3 carbonyl group; the area around C-3, C-4, C-5 and C-6 of T appears to be sterically hindered, presumably due to the binding of the NADPH moiety. It was also suggested that two isozymes possibly vary in the positioning of the reducing NADPH moiety. Studies has also shown that the area of the enzyme active site around the C-17 OH of the substrate does not appear to possess hydrogen bonding groups, and the volume of space available to groups about the steroid C-17 position is not restricted, suggesting that the exit/entry into the active site may exist about this area. Several non-steroidal inhibitors were designed on the basis of this study and further testing led to promising results.

Chen et al. developed a pharmacophore model for further investigation of active sites of 5AR. Sixteen structurally different non steroidal inhibitors were used as training set for the generation of pharmacophore model. The highest scoring hypothesis consisted of two hydrogen bond acceptors (HBA1 and HBA2) and three hydrophobic groups (HP1-3). The 3D pharmacophore model with distance constraints are shown in Figure 14. Further screening of an in house database consisting of 151 isoflavone compounds in the National Cancer Institute (NCI) database using the developed pharmacophore hypothesis identified 37 hits. In vitro testing of these hits identified 7 compounds with IC50 in pM range.
Faragalla *et al.* reported a comparison of pharmacophores for non-steroidal inhibitors of human 5AR-1, human 5AR-2 and rat 5AR-2 enzymes. The best pharmacophore for human 5AR-1 (hi) was consisting of essentially three features and one pseudo-feature: a HBA, a hydrophobic, a hydrophobic-aromatic feature and a HBA pseudo-feature. Figure 15 (a) depicted the mapping of compound 276 on the hypothesis hl. Two different hypotheses were produced for human 5AR-2 (hIIA and HIIb) (Figure 15 (b)) (The wire cage spheres represent the pharmacophore features. For hydrogen bond acceptor (HBA) and ring aromatic features the smaller sphere represents the area where the feature of the molecule lies during the mapping and the larger sphere represents the interaction point of the enzyme). Hypothesis IIA consisted of a hydrogen bond acceptor, negative ionisable group and two ring aromatic features, whereas hypothesis IIB consisted of a hydrogen bond acceptor, hydrophobic, and two ring aromatic features. It was hypothesised on the basis of validation studies that hypothesis IIB seems to be more appropriate for this enzyme.\(^{275}\)

The pharmacophores identified for rat 5AR-2 was categorized into two groups, one contained one HBA or negative ionizable feature while the other contained two HBA or negative ionizable features. The top scoring hypothesis has been shown in Figure 15 (c).
Figure 15. Hypotheses for inhibition of the rat and human 5AR enzymes [(a) human type 1, (b) human type 2 and (c) rat type 2] with the most active compounds 274 (hI), 275 (hII B), 276 (hII A), 277 (rII A), and 278 (rII B) from the training sets mapped onto them. The coloured circles on the two dimensional chemical structures show where a feature is mapped onto a compound. Colour coding: dark blue, negative ionisable; blue, hydrophobic aromatic; light blue, hydrophobic; orange, ring aromatic; green, HBA.

Pharmacophore rII A contains one HBA group, a ring aromatic feature and two hydrophobic features of unequal weighing. The pharmacophoric model rII B contained a
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HBA feature, a negative ionizable feature, a hydrophobic feature and a hydrophobic-aromatic feature also of unequal weighing.

In order to identify common pharmacophore for inhibition of 5AR by 3-O-acylated (-)-epigallocatechins, Lin et al. subjected them to pharmacophore model generation based on common chemical features using Catalyst/HipHop approach. The most reasonable pharmacophore model contained ten common features as shown in Figure 16A that included four hydrogen bond donors, one hydrophobic group, and five excluded volumes. The most potent compound mapped onto model is shown in Figure 16B.

![Figure 16](image)

**Figure 16.** (A) Pharmacophore model for inhibition of 5AR by 3-O-acylated (-)-epigallocatechins. Color coding: hydrogen-bond donor, light red; hydrophobic, light blue; excluded volume, black. (B) Most active compound mapped onto the pharmacophore.

Wikel et al. reported a QSAR study of benzoquinolones as inhibitors of human type 1 5AR. The various models were developed using the regression analysis indicated two features: substituent lipophilic property that describes a localized feature of the structure around the aromatic ring substituent. A second feature, the energy of HOMO, was determined from a visualization technique and was considered as a global descriptive molecular feature that differentiated the two structural subtypes in the study. The importance of HOMO is warranted in this series since this molecular orbital would be
involved in a normal reduction reaction as performed by the enzyme in these kind of substrates.\textsuperscript{189}

An extensive work has been carried by Kurup \textit{et al.} to provide a rational basis for design of potent inhibitors of 5AR and 3-BHSD (3-$\beta$ hydroxysteroid dehydrogenase). Apart from 5AR, the other enzyme 3-BHSD is a potent enzyme involved in biosynthesis of steroidal hormones and inhibitors of this might be an attractive target for reduction of substrate concentration i.e. T. The similarity in transition state by reaction catalyzed by 5AR and 3-BHSD makes this as important selectivity criteria for 5AR inhibition. QSAR studies has been performed on the assumption that parameters used for model development can also account for variation in the steric, hydrophobic, and electronic properties of the major differences in the members of a set of congeners. Based on these QSAR studies the following generalizations have been reported. (1) four or six azasteroids are suitable molecules to inhibit all three types of enzymes. (2) substituents at C-17 interact with the hydrophobic region while it is mostly steric interaction at other positions. Clog\textit{P} appeared to be the most important for the activity thus confirms the usefulness of lipophilic group at C-17. (3) A conjugated 3-ene-4-one as well as the second conjugation at C-5 imparted both 5AR-1 and 5AR-2 inhibitory activity. A double bond at C-1 in addition to C-4 seems to be highly detrimental to the activity. (4) Substituents at C-4 hindered binding to the receptor sterically. (5) Substituents at C-6 and C-7 causes steric hindrance and are detrimental to the activity as far as 5AR-1 is concerned whereas substituent at C-6 is not good for 5AR-2 inhibitory activity. (6) The presence of –CON group at the C-17 is useful for the activity.\textsuperscript{277}

To quantitatively rationalize the SAR of a series of benzo[c]quinolizin-3-one derivatives, Occhiatio \textit{et al.} reported a 3D QSAR study employing CoMFA methodology. The best CoMFA model was obtained by taking into consideration both the DFT-calculated dipole moment and log \textit{P} value along with steric and electrostatic fields using partial least square (PLS) methodology. The best QSAR model has an optimal number of components equal to 3 and descriptive and predictive abilities evaluated by robust statistical parameters ($r^2 = 0.841$, $s = 0.262$, $q^2 = 0.682$, $s_{cross} = 0.041$ and $r^2_{pred} = 0.676$).\textsuperscript{278} The steric and electrostatic contour plots are presented in \textbf{Figure 17}. 

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Figure 17. (a) Steric CoMFA STDEV*COEFF contour maps. The regions where increasing the molecular volume increases 5AR-1 inhibitory activity are green, and the regions where increasing the volume decreases the activity are yellow. (b) Electrostatic CoMFA STDEV*COEFF contour maps. The regions where increasing the positive charges increases 5AR-1 inhibitory activity are red, and the regions where increasing the negative charges increases the activity are blue.

In order to optimize the structural features required for potent inhibition of 5AR, several 3D-QSAR studies have been reported from our laboratory involving use of Self Organizing Molecular Filed Analysis (SOMFA) as summarized below. Similar to both CoMFA and molecular similarity studies Robinson et al. reported a novel 3D QSAR technique called SOMFA.\textsuperscript{279} It is also a grid-based approach however, no probe interaction energies are required to be calculated. It generates intrinsic molecular properties, such as the molecular shape and electrostatic potential, which are used to develop QSAR models.\textsuperscript{280}

Series of compounds belonging to unsaturated 3-carboxysteroid derivatives\textsuperscript{281} unsaturated 4-azasteroids\textsuperscript{282} and pregnane derivatives\textsuperscript{283} were subjected to SoMFA analysis and the contribution of shape and electrostatic potential were evaluated. The statistical results of all the studies demonstrated robust predictive ability. The overall results of these studies indicated that bulkiness at C-17 is favorable for activity whereas at C-3 steric bulk is detrimental for activity. Presence of electronegative groups at C-3 and C-4 were favorable for activity while electropositive groups are nonfavourable for activity at these positions.
2. RESUMÉ AND DISCUSSION

5ARIs have been intensively investigated for their potential use in the management of BPH. It reduces the free circulating level of more active androgen DHT by inhibiting the conversion of T into DHT. DHT has been proposed as a causative factor in BPH. There has been intense interest in developing inhibitors of steroidal 5AR to lower the levels of DHT synthesized in the body. During last three decades, several steroidal and non-steroidal molecules have been evaluated as potential 5ARIs, however only finasteride and dutasteride are the drugs used clinically. Epristeride had entered clinical trials, but could not come to clinics probably due to availability of other more effective drugs. Therefore, it was considered of interest to exploit steroidal nucleus for possible modifications with respect to the treatment of BPH.

Epristeride is a potent inhibitor of 5AR-2 while a weak inhibitor of 5AR-1. Activity was found to be enhanced in analogues possessing an additional unsaturation at C-5 along with Δ3-unsaturation.150-152

The main focus of the current study is to synthesize novel steroidal 5ARIs. In absence of the crystal structure of enzyme 5AR, ligand based 3D QSAR studies involving CoMFA and CoMSIA were employed to design these steroidal molecules. Docking studies were also performed using crystal structure of 5β-reductase which was considered as a surrogate to the 5AR enzyme.

The considerable role of sp² hybridized 3,5-diene-3-oic acid in inhibiting the enzyme 5AR led us to design several carboxylic acid derivatives with additional nitrogen in ring D. Certain oxime derivatives were also prepared on the basis of recent literature reports161,162 that these contain potential pharmacophoric features necessary for inhibition of 5AR.
The work carried out is discussed under the following heads:

2.1. Computer aided design of 5ARIs
   2.1.1. 3D QSAR CoMFA and CoMSIA studies on 6-azasteroidal 5ARIs
   2.1.2. 3D QSAR CoMFA and CoMSIA studies on 4-azasteroidal 5ARIs
   2.1.3. Docking studies

2.2. Synthetic studies
   2.2.1. Synthesis of 17a-substituted 3-cyano-17a-aza-D-homo-3,5-androstadienes
   2.2.2. Synthesis of 17a-substituted 17a-aza-D-homo-3,5-androsten-3-0ic acids
   2.2.3. Synthesis of 17-substituted 3-cyano-17-aza-D-homo-3,5-androsten-16,17a-diones
   2.2.4. Synthesis of 17-substituted 16,17a-oxo-17-aza-D-homo-3,5-androsten-3-0ic acids
   2.2.5. Synthesis of 5a-oxo-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-0ic acid derivatives
   2.2.6. Synthesis of 25(\(\overline{R}\))-5a-oxo-5-aza-B-homo-3,5-seco-4-nor-spirostan-3-0ic acid derivatives
   2.2.7. Synthesis of miscellaneous androstene derivatives

2.3. ADME predictive studies

2.4. Biological Evaluation
   2.4.1. In vitro 5AR inhibitory activity
   2.4.2. In vivo 5AR inhibitory activity