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Original article

Synthesis and biological evaluation of novel unsaturated carboxysteroids as human 5α-reductase inhibitors: A legitimate approach

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ABSTRACT

In the present study, novel steroidal 17a-substituted 3-cyano-17a-aza-D-homo-3,5-androstadien-17-ones (**12**–**19**) and 17a-substituted 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acids (**20**–**26**) were synthesized from dehydroepiandrosterone acetate (**6**) along with 17-oxo-19-nor-3,5-androstadien-3-oic acid (**30**) through a multistep synthesis. Compounds were evaluated for their *in vitro* 5α-reductase inhibitory activity by measuring the conversion of [^{3}H] androstenedione in human embryonic kidney (HEK) cells. *In vivo* 5α-reductase inhibitory activity was also determined using rat prostate weighing method. Compounds **21**–**23** and **25** showed potent inhibition of 5α-reductase II enzyme with IC_{50} values of 54.1 ± 9.5, 22.1 ± 2.4, 72.8 ± 2.3 and 26.5 ± 4.4 nM respectively as compared to Finasteride (30.3 nM) along with a significant ($p < 0.05$) reduction in rat prostate weight.

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1. Introduction

5α-Reductase inhibition has emerged as a key target for the management of benign prostatic hyperplasia (BPH) as abnormally high activity of the enzyme in human's results in excessive dihydrotestosterone levels in peripheral tissues leading to hyperplasia. BPH is a noncancerous growth of the prostate gland resulting due to the over-proliferation of the stromal and glandular elements of the prostate [1]. About 60% of men aged over 50 years have histological evidence of BPH and, after the age of 70, the proportion increases to 80% [2]. It is a chronic, progressive and highly prevalent disease which clinically manifests as lower urinary tract symptoms (LUTS) which includes frequency, hesitancy, urgency, nocturia, slow urinary stream and incomplete emptying thereby causing socio-economic burden to the patients [3,4].

Normal growth, development and maintenance of the prostate are dependent on the testicular androgens [5–7]. Within the prostate, testosterone (T) is enzymatically converted to an active metabolite, 5α-dihydrotestosterone (DHT), by steroid 5α-reductase enzyme (3-oxo-steroid-4-ene dehydrogenase {E.C. 1.3.99.5}) which is a system of two membrane bound nicotinamide dinucleotide phosphate (NADPH) dependent enzymes. Thus 5α-reductase dictates the cellular availability of DHT to prostatic epithelial cells and consequently modulates its growth. Once formed, DHT can bind reversibly to the androgen receptor to regulate prostatic cellular proliferation and survival. The presence of DHT and its binding to androgen receptors can directly up-regulate expression of prostate-specific differentiation markers such as prostate-specific antigen (PSA) synthesized by the human prostatic epithelial cells and locally active growth factors – the andromedins which stimulates the proliferation of so-called transit amplifying (TA) cells within the prostate [8,9]. This has led to the development of steroidal and non-steroidal 5α-reductase inhibitors as they inhibit the conversion of T (**1**) to DHT (**2**) as shown in Fig. 1 [10–12].

There are mainly two types of 5α-reductase enzymes: The type I enzyme which is present mainly in the hair follicles and peripheral skin and is not the major species expressed in the prostate while type II 5α-reductase is the major isozyme in genital tissues and a deletion in the gene leads to male pseudohermaphroditism [13,14].

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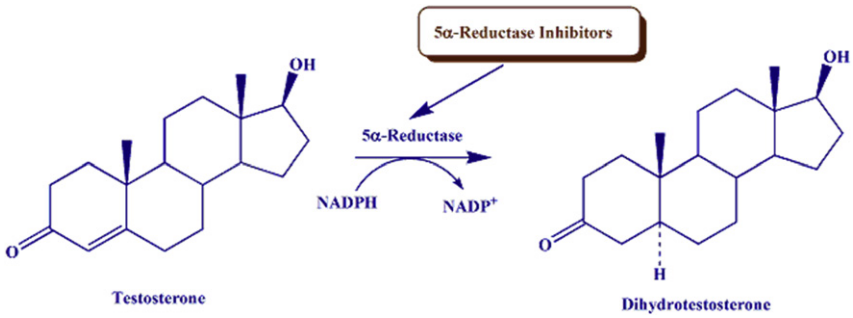


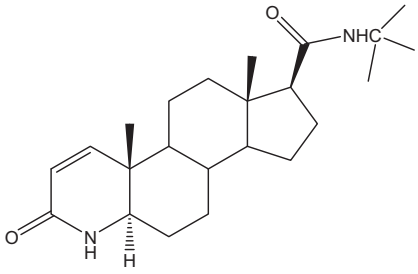
Fig. 1. Site of action of 5α-reductase inhibitors.

The type I isozymes is active at pH 6.0–8.5 while type II is active at pH 5.0–5.5. More recently with the development of genome-wide gene expression profile analyses a third type of 5α-reductase enzyme (type III) has been identified in hormone-refractory prostate cancer cells (HRPC) [15]. This enzyme also converts T (**1**) to DHT (**2**) in HRPC cells in a similar way to type I enzyme and was found to be active at pH 6.9 [16].

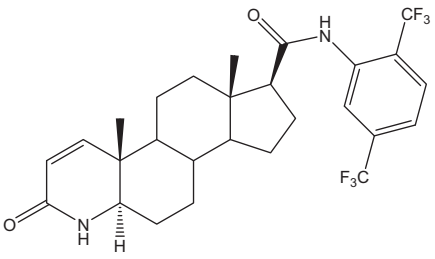
Finasteride (MK-906) was the first 5α-reductase inhibitor approved in U.S. for the treatment of BPH [17,18]. Finasteride (**3**) is a potent inhibitor of 5α-reductase type II with weak *in vitro* activity for 5α-reductase type I having IC_{50} value of 9.4 and 410 nM, respectively. At clinical dose, 5 mg/day, it caused 65–80% lowering of plasma DHT levels [19]. Dutasteride (GG745) (**4**) is another related drug approved by United States food and drug administration (U.S. FDA) in 2002 for the symptomatic treatment of BPH. Unlike Finasteride (**3**), Dutasteride (**4**) is a competitive inhibitor of both 5α-reductase type I and type II isozymes and it reduces DHT levels >90% following one year of oral administration [20]. It is a time dependent inhibitor as Finasteride (**3**) and forms a stable complex with a slow rate of dissociation constant and does not bind to the androgen receptor [21]. Epristeride (SK&F 105657) (**5**), a novel 5α-reductase inhibitor, is an interesting drug in the treatment of BPH. It belongs to class of carboxysteroids (Fig. 2). It is a potent inhibitor of 5α-reductase II while a weak inhibitor of 5α-reductase I. It has been shown to be an uncompetitive inhibitor against both T and NADPH. Because of presumably favourable electrostatic interaction between the carboxylate and the positively charged oxidized cofactor, the acrylate preferentially binds in a ternary complex with enzyme and $NADP^+$, which leads to the uncompetitive kinetic mechanism [22–24].

17a-aza-D-Homosteroids belongs to another category of compounds which have shown 5α-reductase inhibitory activity. The most interesting aspect concerning them is the possibility of "inverted action" or "back binding" as proposed by McDonald et al. Their proposition was based on the fact that the steroids have the potential to bind in two orientations at the active site of various metabolizing enzymes. Research on 5α-reductase inhibitors has shown that steroids without side chains can bind to enzymes with the A-ring of the substance simulating the D-ring of the substrate, while the D-ring emulates the A-ring. This could lead this compounds exhibiting same mechanism of action as 4-azasteroids [25].

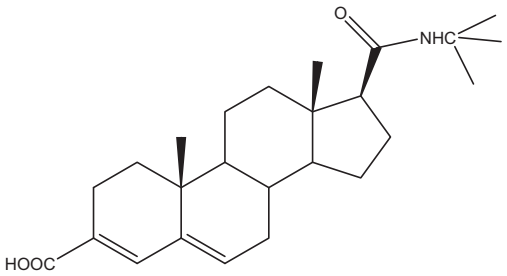
It is evident from the clinically used 5α-reductase inhibitors in the management of BPH that planarity is required in ring A of the steroidal nucleus so as to enter the active site of the enzyme. Planarity is provided by the presence of double bond between C-1 and C-2 in case of Finasteride (**3**) and Dutasteride (**4**), and conjugate double bonds at C-3 and C-5 in Epristeride (**5**). Therefore it was envisaged to synthesize Epristeride (**5**) related analogues having



Finasteride (3)



Dutasteride (4)



Epristeride (5)

Fig. 2. Structures of active molecules.

sp^2-hybridized 3,5-diene-3-oic acid moiety in androstane. In addition compounds would have 17-oxo-17a-aza-D-homo lactam in ring D instead of ring A as in Finasteride (**3**). Results of our previously published studies have indicated that the bulk around 17 position has to be increased and carboxylic group at position-3 as in Epristeride (**5**) has to be less bulky and unsubstituted one for better 5α-reductase inhibition [26]. This can be done by replacing a 5 membered D-ring with a 6 membered D-ring and substituting the lactam hydrogen with bulky groups.

Guarna et al. synthesized a novel class of compounds 19-nor-10-azasteroids which were found to be better inhibitors of the 5α-reductase enzyme [27,28]. Therefore, it was also envisaged to synthesize some molecules not having 19-methyl group and having rings A and B mimicking that of Epristeride (**5**).

The present paper describes the synthesis of novel steroidal compounds which were evaluated for 5α-reductase inhibitory activity *in vitro* against 5α-reductase type I and II and *in vivo* by prostate weighing method.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of 17a-aza-D-homo-4-androstene-3,17-dione (*10*)

For the synthesis of proposed compounds, 17a-aza-D-homo-4-androstene-3,17-dione (**10**), was synthesized first as depicted in Fig. 3 using 17-oxo-5-androsten-3β-yl acetate (dehydroepiandrosterone acetate) (**6**) as the starting material by standard procedures. It was converted to its oxime (**7**) by refluxing in ethanol with hydroxylamine hydrochloride and sodium acetate trihydrate. 17-Oximino-5-androsten-3β-yl acetate (**7**) on Beckmann's rearrangement with thionyl chloride gave 17-oxo-17a-aza-D-homo-5-androsten-3β-yl acetate (**8**). The lactam (**8**) was hydrolyzed by refluxing with methanolic potassium hydroxide to obtain 3β-hydroxy-17a-aza-D-homo-5-androsten-17-one (**9**). Oppenaeur oxidation of compound **9** was carried out in a mixture of cyclohexanone and toluene to yield 17a-aza-D-homo-4-androstene-3,17-dione (**10**).

2.1.2. Synthesis of 17a-substituted 3-cyano-17a-aza-D-homo-3,5-androstadien-17-ones

17a-Aza-D-homo-4-androstene-3,17-dione (**10**) on treatment with phosphorus tribromide in glacial acetic acid gave 3-bromo-17a-aza-D-homo-3,5-androstadien-17-one (**11**) in 85.0% yield. The bromo compound (**11**) was refluxed with copper cyanide in dimethylformamide to yield 3-cyano-17a-aza-D-homo-3,5-androsta dien-17-one (**12**). The cyano compound (**12**) was treated with methyl iodide in the presence of sodium hydride in tetrahydrofuran to form 17a-methyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**13**) (Fig. 4a).

3-Cyano-17a-aza-D-homo-3,5-androstadien-17-one (**12**) was also treated with ethyl bromide, allyl bromide, benzyl chloride, acrylonitrile and acetyl chloride respectively to get 17a-ethyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**14**), 17a-allyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**15**), 17a-

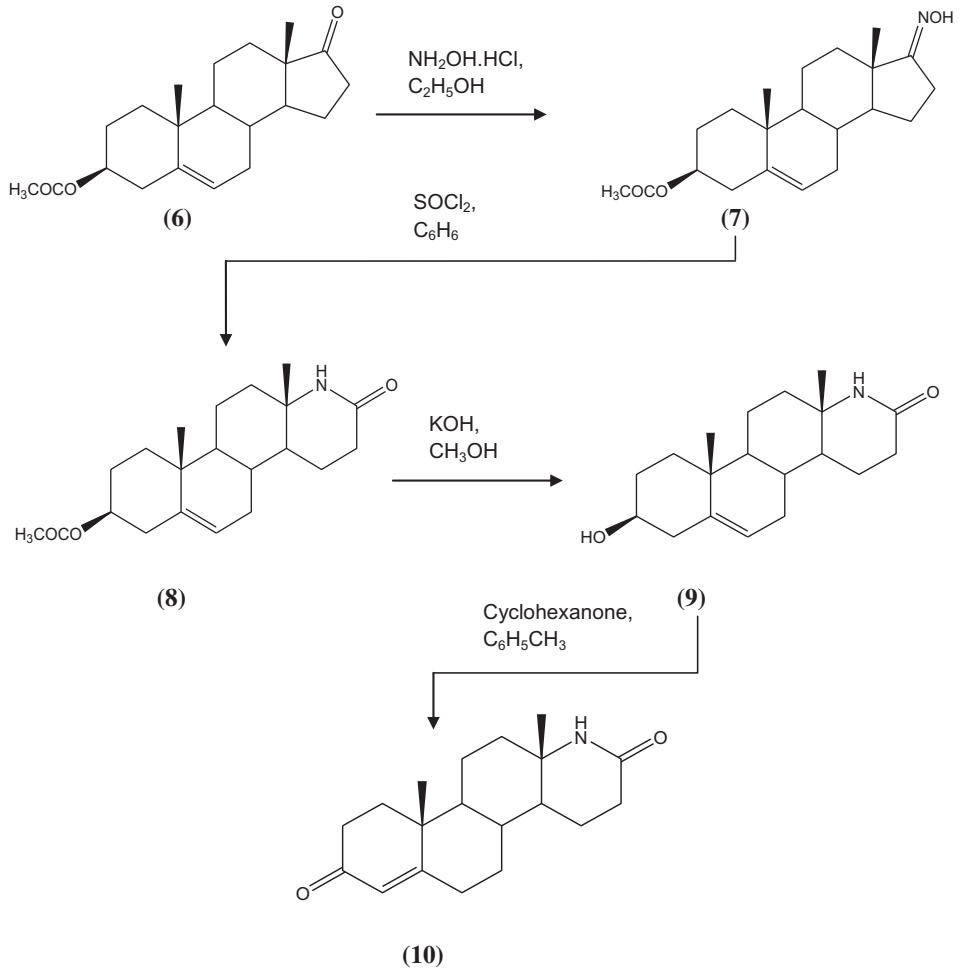


Fig. 3. Synthesis of 17a-aza-D-homo-4-androstene-3,17-dione (**10**).

benzyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**16**), 17a-cyanoethyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**17**) and 17a-acetyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**18**), 17a-benzoyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**19**) respectively (Fig. 4b).

2.1.3. Synthesis of 17a-substituted 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acids

3-Cyano-17a-aza-D-homo-3,5-androstadien-17-one (**12**) was hydrolyzed in the presence of ethanolic sodium hydroxide to obtain 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**20**). Compounds **14**–**18** were also hydrolyzed to obtain N-methyl (**21**), N-ethyl (**22**), N-allyl (**23**), N-benzyl (**24**) and N-17a-(2-propionoxy ethyl) (**25**) respectively. 17a-Acetyl-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**26**) was prepared by reacting 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**20**) directly with acetic anhydride in pyridine (Fig. 5) but the hydrolysis of 17a-benzoyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**19**) was not successful because of the N-benzoyl side chain breakdown in strong basic conditions.

*2.1.4. Synthesis of 17-oxo-19-nor-3,5-androstadien-3-oic acid (**30**)*

4-Androstene-19-nor-dione (**27**) was converted to 3-bromo-19-nor-3,5-androstadien-17-one (**28**) by treating with phosphorus tribromide in glacial acetic acid. The bromo compound was refluxed with copper cyanide in dimethylformamide to get 3-cyano-19-nor-3,5-androstadien-17-one (**29**). The cyano compound (**29**) was hydrolyzed in the presence of ethanolic sodium hydroxide to obtain 17-oxo-19-nor-3,5-androstadien-3-oic acid (**30**) (Fig. 6).

2.2. In vitro human 5α-reductase inhibitory activity against type I and type II 5α-reductase enzyme (HEK 293 cells method)

Compounds were evaluated *in vitro* for their inhibitory activity against human 5α-reductase enzyme I and II. For this purpose human embryonic kidney cell line (**HEK293**) which lacks endogenous 5α-reductase activity was transfected with the cDNAs encoding for both 5α-reductase type I and type II by inserting them into pRcCMV (Cytomegalovirus promoter of the eukaryotic expression vector). Single cell clones with substantially high enzymatic activity were selected and established as permanent cell

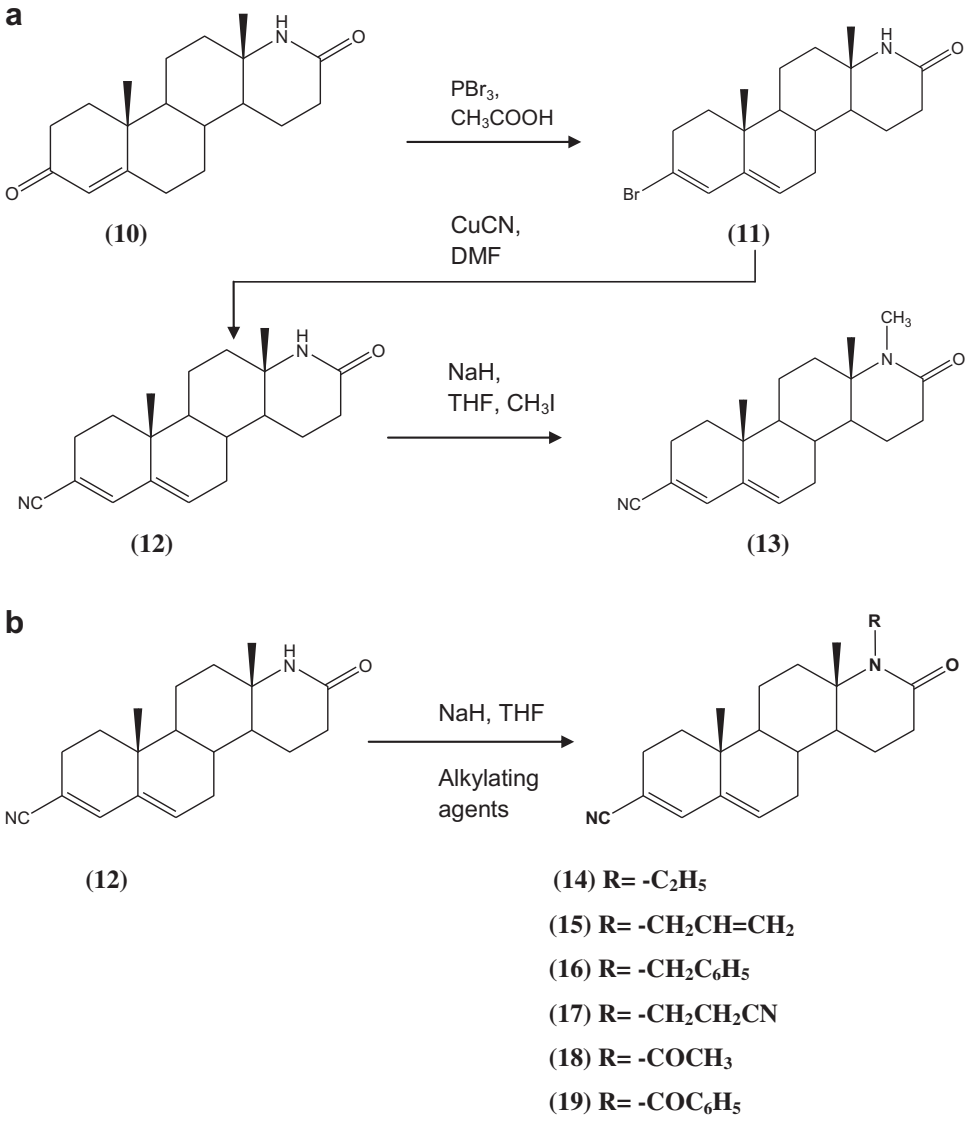


Fig. 4. (a and b) Synthesis of 17a-substituted 3-cyano-17a-aza-D-homo-3,5-androsta-dien-17-ones.

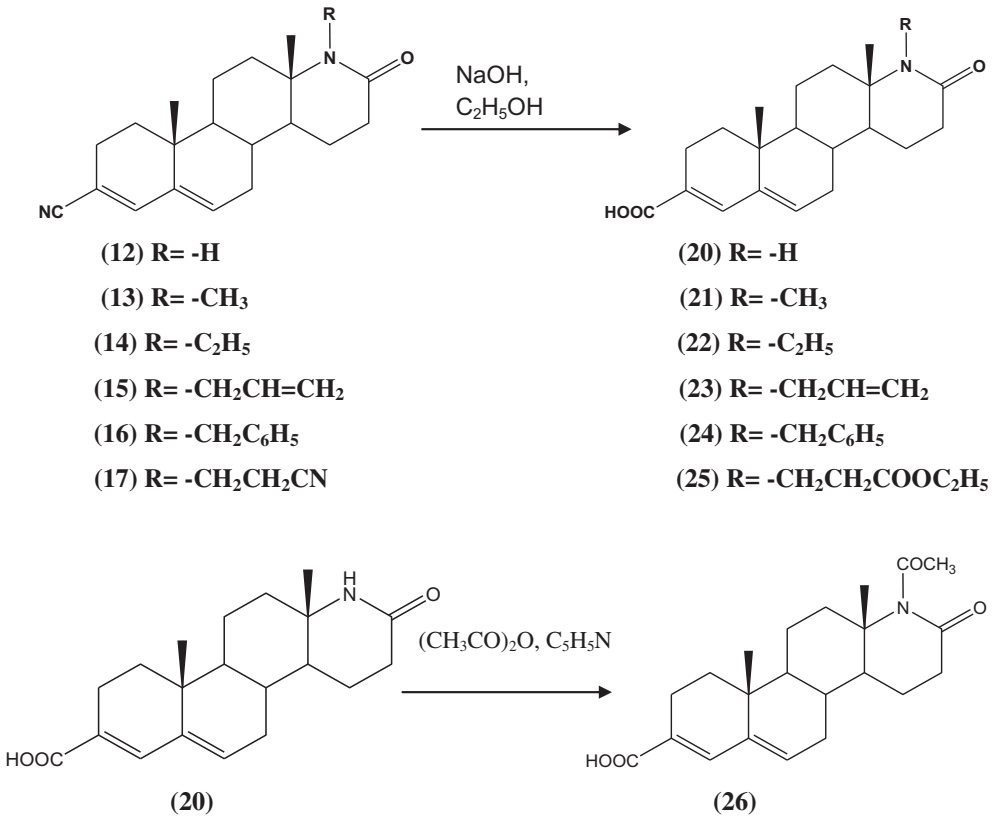


Fig. 5. Synthesis of 17a-substituted 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acids.

lines. The cell lines were used to test selected synthesized compounds as well as the clinically used steroidal inhibitor Finasteride (**3**) by measuring the conversion of [^{3}H] androstenedione [29,30]. Reaction products were quantified by a HPLC reversed phase technique. By the development of this assay method the conventional BPH microsomal assay and the DU-145 cell free assay were replaced. Compared to use of microsomal enzymes, whole cell systems are closer to the *in vivo* situation. The main disadvantages of the earlier methods were that in those methods the substrates used were different i.e. in BPH microsomal assay the substrate used was T, in DU-145 cell free assay it was androstenedione. In the present assay, androstenedione is used as substrate for both types of isozymes. Since source of enzyme is same, better comparable inhibition values for both isozymes can be obtained in the new

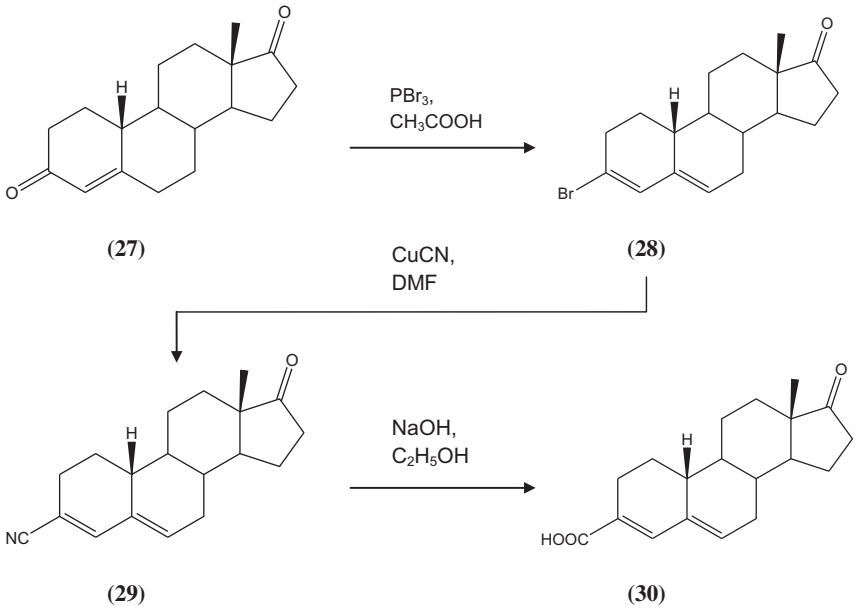


Fig. 6. Synthesis of 17-oxo-19-nor-3,5-androstadien-3-oic acid (**30**).

assay as processing method is similar. In case of earlier BPH microsomal assay homogenate was prepared from the prostate tissue of BPH patient. It was cumbersome to seek approval from patient every time and also individual fluctuations among the patients were there which in new HEK-II assay has been replaced. Therefore, this whole cell system gives better and faster results.

Another reason for the use of new assay was that in prostate gland both types of isozymes of 5α-reductase are present; therefore, homogenate prepared from prostatic tissue of BPH patient contained not only 5α-reductase II but also smaller quantity of 5α-reductase I due to which inhibition values of the selective inhibitors could be easily falsified. In HEK-II cells, it is only active 5α-reductase II which is in form of an enzyme preparation from these defined cells in high quantity. Similarly, in DU-145 cells apart from the 5α-reductase I there is also some amount of 5α-reductase II and 17β-hydroxysteroid dehydrogenase which can convert T and androstenedione. In addition, the 5α-reductase I activity is much smaller in DU145 cells than in HEK-I cells, so that for one HEK-I homogenate assay much less cells for the same enzyme yield would be required which in turn finally save time and costs. For example in order to obtain an androstenedione conversion about 25% cells must be incubated for 5 h in previous assay while in HEK-I cells time is 30 min. Hence new assay method provides better, faster and selective results.

Lysates are obtained after harvesting and resuspending 80% confluent cells in homogenate buffer (containing 300 mM 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 [29,30].

Finasteride (**3**), a clinically used drug, was used as the standard in the assay. It showed IC_{50} value of 453.0 nM for 5α-reductase I enzyme while for 5α-reductase II enzyme the value was 30.3 nM in the present assay. Epristeride (**5**) although not tested in assay has a literature IC_{50} value of 58–60 nM [31]. The results of 5α-reductase inhibitory activity of our synthesized compounds **11**–**19** at 10 μM are reported in Table 1. Out of the series 17a-methyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**13**) emerged as the most potent selective inhibitor of 5α-reductase type I enzyme. It possesses an additional 17a-methyl group as compared to the unsubstituted compound (**12**) which also possesses 3-cyano-3,5-diene and D-homo system. The observation is in concordance with the previously reported SAR studies [32,33] concluding that an N–CH_3 group is required to have higher potency against 5α-reductase I enzyme due to tighter binding at the active site but the compound showed less potency towards 5α-reductase II inhibition. Further increase in chain length from methyl to ethyl (**14**) resulted in reduction of 5α-reductase type I inhibition although there was an improvement in 5α-reductase II inhibition. Substitutions like allyl (**15**), benzyl (**16**), cyanoethyl (**17**), acetyl (**18**) and benzoyl (**19**) resulted in decrease of inhibition against 5α-reductase type I as well as 5α-reductase type II. In conclusion, substitution beyond methyl group leads to loss of activity against both types of 5α-reductase isozymes. In general 3-cyano derivatives were not active against the enzyme probably due to the lack of charge replacement of the enolate oxyanion at the active site as all other features such as sp^2-hybridized centre at C-3, C-4 and bulk in ring D were present in the molecules as those present in active molecule Epristeride (**5**).

Table 1
In vitro 5α-reductase inhibitory activities of bromo (**11**) and cyano compounds (**12**–**19**).

Compound code	Activity code	Type-I 5α-reductase percentage inhibition at 10 μM	Type-II 5α-Reductase percentage inhibition at 10 μM
11	SAR-16	34.5 ± 1.7	45.6 ± 5.2
12	SAR-1	27.6 ± 9.3	9.9 ± 5.9
13	SAR-2	**93.0 ± 3.1 (64.0 ± 10.1 at 2 μM)**	13.5 ± 7.1
14	SAR-5	56.1 ± 3.7	48.9 ± 2.7
15	SAR-7	20.8 ± 3.9	3.3 ± 5.7
16	SAR-9	0.0 ± 0.0	34.7 ± 8.6
17	SAR-10	0.0 ± 0.0	9.9 ± 10.8
18	SAR-12	2.2 ± 3.0	23.2 ± 9.8
19	SAR-13	0.0 ± 0.0	11.4 ± 3.9
3 (Finasteride)		**453.0 nM (IC_{50})**	**30.3 nM (IC_{50})**

Bold values represents to signify the important results.

With the aim of improving potency and providing charge replacement of the enolate oxyanion at the active site the above compounds were hydrolyzed and a carboxylic acid group was introduced as in Epristeride (**5**). The results of 5α-reductase inhibitory activity of compounds **20**–**26** at 10 and 2 μM against both type of isozymes along with IC_{50} against type II enzyme is reported in Table 2. Out of the series four compounds were found to be potent inhibitors of 5α-reductase showing inhibition around or better than the clinically used drug Finasteride (**3**). 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**20**) obtained from compound **12** showed better inhibition than the latter one towards 5α-reductase II enzyme. Increase in chain length to methyl (**21**) leads to increase in potency with strong inhibition of 5α-reductase II with an IC_{50} value of 54.1 nM which was near to that of the standard drug Finasteride (**3**) (30.3 nM).

Further increasing the chain length to ethyl (**22**) led to the increase in the activity as the compound **22** exhibited an IC_{50} value of 22.1 nM against 5α-reductase II which was better than the standard drug Finasteride (**3**) (IC_{50} = 30.3 nM). It emerged as the most potent compound of the series. Further substitution with an allyl group (**23**) led to the retention of 5α-reductase II activity (IC_{50} value = 72.8 nM). Finally, 17a-(2-propionoxy ethyl)-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**25**) derivative also showed potent inhibition against 5α-reductase II (IC_{50} value = 26.5 nM) while two synthesized derivatives benzyl (**24**) and acetyl (**26**) were not evaluated because of being unstable on storage. All the compounds showed very less inhibition against 5α-reductase I enzyme suggesting that the carboxylic group at position-3 provides the selective inhibition towards 5α-reductase II.

Among 19-nor compounds, the carboxy derivative (**30**) proved to be potent 5α-reductase II inhibitor having IC_{50} in nanomolar range (212.9 nM) showing that removal of 19-methyl group doesn't have a drastic effect on the 5α-reductase inhibitory activity although the cyano compound (**29**) was found to be inactive showing only 1% inhibition against type II enzyme. Results of 5α-reductase inhibitory activity of compounds **29**–**30** are reported in Table 3.

The synthesized compounds are expected to be uncompetitive inhibitors as Epristeride (**5**) due to presence of the acrylate in A and B ring which could lead to formation of ternary complex with the positively charged cofactor, enzyme and $NADP^+$ thereby possibly demonstrating uncompetitive mechanism.

Table 2
In vitro 5α-reductase inhibitory activities of carboxy compounds (**20**–**26**).

Compound code	Activity code	Type-I 5α-reductase percentage inhibition at 10 μM	Type-II 5α-reductase percentage inhibition at 10 μM	Type-II 5α-reductase percentage inhibition at 2 μM	IC_{50} of type-II enzyme (nM)
20	SAR-4	0.0 ± 0.0	**62.9 ± 2.2**	n.d.	n.d.
21	SAR-3	18.9 ± 1.1	**100.0 ± 0.0**	**99.0 ± 2.1**	**54.1±9.5**
22	SAR-6	32.3 ± 1.5	**100.0 ± 0.0**	**100.0 ± 0.0**	**22.1 ± 2.4**
23	SAR-8	0.4 ± 0.5	**100.0 ± 0.0**	**99.7 ± 0.5**	**72.8 ± 2.3**
24	–	n.d.	n.d.	n.d.	n.d.
25	SAR-11	8.5 ± 3.0	**100.0 ± 0.0**	**100.0 ± 0.0**	**26.5 ± 4.4**
26	–	n.d.	n.d.	n.d.	n.d.
3 (Finasteride)		**453.0 nM** (**IC_{50}**)	–	–	**30.3 nM**

n.d. = not determined.
Bold values represents to signify the important results.

2.3. *In vivo* 5α-reductase inhibitory activity (change in rat prostate weight in mature male rats)

5α-Reductase enzyme plays a crucial role in human prostate growth which is an age-related and androgen dependent process being mediated by DHT [34]. As there are many similarities between human and rat prostate growth, rat prostate is commonly used as the growth model although histological and biochemical differences still exist. 5α-Reductase enzyme has been isolated and shown to be expressed in the ventral prostate of the rats [35,36]. Although the rat enzyme has showed similarities to human enzymes in amino acid sequences (approximately 60% homology) and activities but in some cases different pharmacological responses were also shown. For each inhibitor to be evaluated, mature male wistar rats were domesticated and were given once daily dose of 1 mg/kg of compounds under test for 14 days and the inhibitory effects on the growth of prostate and other androgen-target organs was examined on 15th day after 24 h of last dose when they were sacrificed and weighed. The following organs were removed, free of adherent tissue and weighed: ventral prostate, dorsal prostate, testis (both), vas deferens, epididymis, liver, adrenal and seminal vesicles. Values are reported as mean ± S.E.M. for actual weights and percentage of vehicle control and were obtained by using the analysis of variance feature [37,38].

Among the synthesized compounds, active compounds based on their *in vitro* 5α-reductase inhibitory activity along with standard drug Finasteride (**3**) were selected for activity. One group received only vehicle (0.50% CMC) and served as control. The results of the activity are given in Table 4. The percentage decrease in the organ weights (ventral prostate, dorsal prostate, vas deferens and epididymis) of importance is reported in Table 5. In other organs the decrease was not significant. Treatment with any compound did not induce any mortality.

Animals treated (2 weeks) with Finasteride (**3**) or a 5α-reductase inhibitor were expected to have prostate weights significantly smaller than those of control animals. Consistent with this expectation, Finasteride (**3**) produced a statistically significant decrease in ventral and dorsal prostate where it reduces the weight to around 44% as compared to control.

Finasteride (**3**) also reduced the weights of seminal vesicle, vas deferens and epididymis on expected lines as these organs are also partially dependent on DHT for their homeostasis but the reduction was less significant. There were no significant differences between treatment groups in adrenal weight, body weight gain, liver weight, or testicular weights of rats at the conclusion of these studies. The suppression of prostate and other organs growth in the animals treated with Finasteride (**3**) can be explained as a consequence of an inhibition of DHT formation in these organs.

17a-Methyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**13**) was found to be 5α-reductase I active *in vitro* and hence is likely to reduce weights of organs where there is presence of 5α-reductase I and have lower effect on prostate where predominantly 5α-reductase II enzyme is present. The results of the study are in conformation with the same as the compound has miniscule effect on the reduction of prostate weight. Although it did reduce the weight of vas deferens and epididymis but the reduction was not significant.

In case of 17a-substituted-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acids which were found to be potent inhibitors of 5α-reductase II enzyme *in vitro*; there was a statistically significant reduction in the weight of ventral prostate and the magnitude of the reduction of these organ weights were almost equal with those of the standard drugs.

Although there was reduction in the weight of ventral prostate as in the case of 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**20**) but it was smaller as compared to the N-substituted compounds and was not statistically significant. Hence substitution at 17a-position leads to better inhibitory compounds with significant inhibition of enzyme in the ventral prostate. In case of 17a-methyl-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**21**) presence of N-methyl group increased the reduction (39.50%) over unsubstituted one (27.33%). N-ethyl (**22**), N-allyl (**23**), and N-(2-propionoxy ethyl) (**25**) derivatives lead to a statistically significant reduction of 39.35%, 35.34%, and 33.86%, respectively in the weights of ventral prostate. In all the compounds there was a reduction in the weights of dorsal prostate, vas deferens, epididymis and seminal vesicles on the expected lines. N-methyl (**21**) derivative showed statistically significant reduction (31.75%) in weight of epididymis suggesting greater reduction of epididymal 5α-reductase enzyme while N-(2-propionoxy ethyl) (**25**) and 19-nor carboxy compound (**30**) showed significant reduction in the weight of vas deferens (37.55% and 34.80% respectively).

Table 3
In vitro 5α-reductase inhibitory activities of 19-nor compounds (**29**–**30**).

Compound code	Activity code	Type-I 5α-reductase percentage inhibition at 10 μM	Type-II 5α-reductase percentage inhibition at 10 μM	Type-II 5α-reductase percentage inhibition at 2 μM	IC_{50} of type-II enzyme (nM)
29	SAR-17	0.0 ± 0.0	1.2 ± 2.1	n.d.	n.d.
30	SAR-19	82.1 ± 10.0	100.0 ± 0.0	89.5 ± 7.6	**212.9 ± 28.9**

Bold values represents to signify the important results.

Table 4
Effect of once daily 14-day oral treatment with compounds on body weight and organ weights in adult male rats.

Compound tested	Body weight gain (g) % ± S.E	Ventral prostate mg/100 g ± S.E	Dorsal prostate mg/100 g ± S.E	Seminal vesicles mg/100 g ± S.E	Testis mg/100 g ± S.E	Adrenal mg/100 g ± S.E	Liver g/100 g ± S.E	Vas deferens mg/100 g ± S.E	Epididymis mg/100 g ± S.E
Control	9.412 ± 1.632	59.494 ± 4.733	50.325 ± 7.519	175.439 ± 12.833	1054.099 ± 55.648	14.762 ± 0.600	2.994 ± 0.254	67.613 ± 3.248	187.356 ± 5.961
Finasteride (**3**)	8.032 ± 2.748	32.866 ± 4.371*	27.519 ± 6.206*	105.510 ± 19.006	1058.022 ± 37.041	16.575 ± 1.704	3.5097 ± 0.12	57.843 ± 5.253	160.371 ± 17.802
13; SAR-2	6.767 ± 3.551	55.495 ± 5.497	54.386 ± 2.825	223.050 ± 38.305	932.988 ± 97.586	21.311 ± 1.948	4.861 ± 0.268	57.756 ± 10.594	168.407 ± 25.922
20; SAR-4	8.323 ± 1.416	43.232 ± 2.452	26.924 ± 3.837*	186.102 ± 5.643	993.729 ± 81.587	13.973 ± 1.289	2.713 ± 0.177	49.808 ± 4.697	161.159 ± 19.856
21; SAR-5	6.517 ± 2.389	35.994 ± 3.887*	30.217 ± 4.339	126.262 ± 19.216	956.392 ± 31.494	12.464 ± 1.974	3.224 ± 0.143	47.763 ± 4.259	127.864 ± 19.272*
22; SAR-6	5.409 ± 0.798	36.086 ± 2.465*	36.048 ± 2.095	151.015 ± 19.989	1128.214 ± 94.123	15.574 ± 2.661	2.91 ± 0.245	54.547 ± 10.144	155.039 ± 10.918
23; SAR-8	7.518 ± 4.042	38.471 ± 2.871*	34.599 ± 5.819	149.399 ± 23.904	1094.935 ± 99.486	17.967 ± 5.422	3.168 ± 0.336	48.757 ± 5.955	140.991 ± 17.825
25; SAR-11	15.618 ± 3.184	39.347 ± 1.618*	35.857 ± 6.134	136.605 ± 34.854	1075.498 ± 44.159	17.522 ± 2.253	3.542 ± 0.438	42.223 ± 2.733*	139.747 ± 9.688
30; SAR-19	22.992 ± 1.323*	46.074 ± 2.744	42.464 ± 1.268	167.370 ± 15.751	926.533 ± 33.952	15.019 ± 1.451	3.078 ± 0.057	44.085 ± 0.921*	131.382 ± 6.244

*$P < 0.05$: Dunnett's *t*-test (control used as reference group); $n = 5$ rats for each treatment group.

Table 5
Percentage change in organ weights after treatment with compounds.

Compound code	Ventral prostate	Dorsal prostate	Vas deferens	Epididymis
Finasteride (**3**)	44.76	45.32	14.45	14.40
13; SAR-2	6.72	−8.07	14.58	10.11
20; SAR-4	27.33	46.50	26.33	13.98
21; SAR-3	39.50	39.96	29.36	31.75
22; SAR-6	39.35	28.37	19.32	17.25
23; SAR-8	35.34	31.25	27.89	24.75
25; SAR-11	33.86	28.75	37.55	25.41
30; SAR-19	22.56	15.62	34.80	29.88

Positive values indicate percentage decrease; negative values indicate percentage increase.

3. Conclusion

In the present study a series of eight novel 17a-substituted 3-cyano-17a-aza-D-homo-3,5-androstadien-17-ones (**12**–**19**), seven 17a-substituted 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acids (**20**–**26**) and 17-oxo-19-nor-3,5-androstadien-3-oic acid (**30**) have been synthesized as novel human 5α-reductase inhibitors through multistep synthesis. *In vitro* biological evaluation against HEK cells revealed that most of the synthesized compounds showed potent inhibition when compared to standard drug Finasteride (**3**). The most potent compounds **21**–**23**, and **25** showed 5α-reductase II inhibition with IC_{50} being **54.1**, **22.1**, **72.8**, and **26.5** nM respectively as compared to Finasteride (**30.3** nM) with significant ($p < 0.05$) reduction in rat prostate weights. Also compound **13** was found to be potent inhibitor of 5α-reductase I. The study concludes that D-homo ring is well tolerated at the enzyme site and substitution of 17a-aza position with alkyl groups enhances the activity. The results may prove as potential lead for the development of compounds to be used in the treatment of BPH.

4. Experimental

4.1. Chemistry

For the synthesis of 17-*oximino*-5-*androsten*-3*β*-*yl acetate* (**7**), 17-*oxo*-17*a*-*aza*-*D*-*homo*-5-*androsten*-3β-*yl acetate* (**8**), 3*β*-*hydroxy*-17*a*-*aza*-*D*-*homo*-5-*androsten*-17-*one* (**9**) *and* 17*a*-*aza*-*D*-*homo*-4-*androstene*-3,17-*dione* (**10**) refer to supplementary material.

4.1.1. 3-Bromo-17a-aza-D-homo-3,5-androstadien-17-one (*11*)

17a-Aza-D-homo-4-androstene-3,17-dione (**10**) (3.33 g, 9.16 mmol) was dissolved in glacial acetic acid (25.0 ml) and 1.61 ml of phosphorus tribromide (4.60 g, 17.0 mmol) was added drop wise to the mixture at room temperature. The dark solution was kept at 8–10 °C for 24 h during which the product got precipitated from the solution. The solid product was filtered and washed with cold glacial acetic acid, followed by cold water (3 × 50.0 ml). The product was dried under vacuum to afford 3-bromo-17a-aza-D-homo-3,5-androstadien-17-one (**11**) (3.42 g, 85.0%): mp 239–241 °C; UV_{max} (MeOH): 241.4 nm (log & 4.28); IR (KBr, cm^{-1}): 3184, 2946 and 1645; ^1H NMR (400 MHz, DMSO-d_6): δ 0.95 (s, 3H, 19-C*H*$_3$), 1.24 (s, 3H, 18-C*H*$_3$), 5.41 (br s, 1H, 6-vinylic), 6.28 (br s, 1H, 4-vinylic) and 7.61 ppm (1H, N*H*); ^{13}C NMR (400 MHz, DMSO-d_6): δ 167.52 (C-17), 116.18 (C-3), 135.86 (C-4), 125.71 (C-5), 118.60 (C-6), 29.57 (C-1), 25.19 (C-2), 26.03 (C-7), 27.94 (C-8), 42.83 (C-9), 30.14 (C-10), 14.57 (C-11), 33.97 (C-12), 49.68 (C-13), 42.26 (C-14), 15.95 (C-15), 27.04 (C-16), 16.88 (C-18) and 13.79 (C-19) ppm; Mass (APCI): 364.47 $[M]^+$, 366.20 $[M+2]^+$.

4.1.2. 3-Cyano-17a-aza-D-homo-3,5-androstadien-17-one (*12*)

3-Bromo-17a-aza-D-homo-3,5-androstadien-17-one (**11**) (1.08 g, 3.0 mmol) was dissolved in dimethylformamide (25.0 ml) and to this

solution was added cuprous cyanide (0.27 g, 3.0 mmol). The reaction mixture was refluxed for 5 h and allowed to cool to around 100 °C and quenched with stirring into a solution of 20.0 ml of concentrated aqueous ammonia and 40.0 ml of water. The resulting suspension was extracted twice with 100.0 ml of dichloromethane and organic phase was washed with three 50.0 ml portions of 50/50 v/v concentrated aqueous ammonia/water followed by water. The organic phase was concentrated under vacuum and crystallised from absolute ethanol. The solid product was filtered and dried under vacuum to afford 3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**12**) (0.52 g, 56.5%): mp 288–290 °C; UV_{max} (MeOH): 262.2 nm (log *&* 4.42); IR (KBr, cm^{-1}): 3189, 2945, 2203 and 1662; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (s, 3H, 19-C*H*$_3$), 1.22 (s, 3H, 18-C*H*$_3$), 5.79 (br s, 1H, 6-vinylic), 6.67 (br s, 1H, 4-vinylic) and 7.07 ppm (1H, N*H*, disappeared on D_2O exchange); ^{13}C NMR (400 MHz, $CDCl_3$): δ 171.94 (C-17), 120.14 (CN), 107.01 (C-3), 142.79 (C-4), 139.45 (C-5), 131.64 (C-6), 32.32 (C-1), 24.21 (C-2), 30.64 (C-7), 31.92 (C-8), 47.88 (C-9), 34.57 (C-10), 19.88 (C-11), 39.16 (C-12), 54.03 (C-13), 47.12 (C-14), 20.71 (C-15), 31.43 (C-16), 22.02 (C-18) and 18.79 (C-19)ppm; Mass (APCI): 311.40 $[M+1]^+$.

4.1.3. 17a-Methyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (*13*)

3-Cyano-17a-aza-D-homo-3,5-androstadien-17-one (**12**) (0.31 g, 1.0 mmol) was dissolved in anhydrous tetrahydrofuran (65.0 ml) and stirred with sodium hydride (60% dispersion in mineral oil; 0.64 g, 1.74 mmol) which was previously washed three times under nitrogen atmosphere with hexane to remove paraffin oil. The reaction mixture was allowed to stir for 10 min, 1.2 ml of methyl iodide was added drop wise and the mixture was kept on stirring at room temperature for 24 h. The progress of reaction was monitored with the help of TLC. After the completion of the reaction excess of hydride was neutralized with methanol. The resulting solution was concentrated, acidified with dilute hydrochloric acid, poured into water and extracted with chloroform (3 × 50.0 ml). The combined organic layer was washed with water, dried and solvent removed under reduced pressure to get a residue which was crystallised from methanol to give 17a-methyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**13**) (0.29 g, 90.6%): mp 233–235 °C; UV_{max} (MeOH): 262.0 nm (log *&* 4.41); IR (KBr, cm^{-1}): 2949, 2916, 2205 and 1631; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (s, 3H, 19-C*H*$_3$), 1.23 (s, 3H, 18-C*H*$_3$), 2.91 (s, 3H, N-C*H*$_3$), 5.79 (br s, 1H, 6-vinylic) and 6.67 (br s, 1H, 4-vinylic) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 170.13 (C-17), 120.14 (*C*N), 107.12 (C-3), 142.74 (C-4), 139.40 (C-5), 131.71 (C-6), 32.42 (C-1), 24.25 (C-2), 31.59 (C-7), 32.37 (C-8), 48.94 (C-9), 34.50 (C-10), 19.49 (C-11), 37.08 (C-12), 58.66 (C-13), 46.44 (C-14), 21.19 (C-15), 31.67 (C-16), 18.73 (C-18), 17.95 (C-19) and 26.81 (N–*C*H$_3$) ppm; Mass (APCI): 325.33 $[M+1]^+$.

4.1.4. 17a-Ethyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (*14*)

Following similar procedure as mentioned above using 3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**12**) (0.31 g, 1.0 mmol) and bromoethane (1.5 ml) with crystallization from methanol yielded 17a-ethyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**14**) (0.19 g, 55.9%): mp 208–210 °C; UV_{max} (MeOH): 261.6 nm (log *&* 3.87); IR (KBr, cm^{-1}): 2943, 2891, 2203 and 1634; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (s, 3H, 19-C*H*$_3$), 1.15 (s, 3H, 18-C*H*$_3$), 1.18 (t, 3H, *CH*$_3$CH$_2$N–), 3.08 (dq, 1H, CH$_3$CH*H*–N), 3.72 (dq, 1H, CH$_3$CH*H*–N), 5.80 (br s, 1H, 6-vinylic) and 6.67 (br s, 1H, 4-vinylic) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 169.78 (C-17), 120.14 (*C*N), 107.05 (C-3), 142.76 (C-4), 139.37 (C-5), 131.78 (C-6), 32.47 (C-1), 24.25 (C-2), 31.63 (C-7), 32.36 (C-8), 49.17 (C-9), 34.50 (C-10), 19.54 (C-11), 37.29 (C-12), 59.39 (C-13), 46.47 (C-14), 21.14 (C-15), 31.85 (C-16), 18.71 (C-18), 15.54 (C-19), 36.34 (N–*C*H$_2$CH$_3$) and 19.22 (N–CH$_2$*C*H$_3$) ppm; Mass (APCI): 339.53 $[M+1]^+$.

4.1.5. 17a-Allyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (*15*)

3-Cyano-17a-aza-D-homo-3,5-androstadien-17-one (**12**) (0.34 g, 1.0 mmol) and allyl bromide (1.5 ml) were reacted using procedure as mentioned above and crystallization from ethanol yielded 17a-allyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**15**) (0.23 g, 60.5%) : mp 179–180 °C; UV_{max} (MeOH): 258.4 nm (log *&* 4.17); IR (KBr, cm^{-1}): 2945, 2877, 2209, 1630 and 1613; ^1H NMR (400 MHz, $CDCl_3$): 0.90 (s, 3H, 19-C*H*$_3$), 1.21 (s, 3H, 18-C*H*$_3$), 3.74 (dd, 1H, N–H*H*C), 4.33 (dd, 1H, N–*H*HC), 5.07 (dd, 1H, N–CH$_2$CH=C*H*H), 5.10 (dd, 1H, N–CH$_2$CH=CH*H*), 5.87 (ddq, 1H, N–CH$_2$*C*H=CHH), 5.79 (br s, 1H, 6-vinylic) and 6.67 (br s, 1H, 4-vinylic) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 170.06 (C-17), 120.14 (*C*N), 107.07 (C-3), 142.75 (C-4), 139.36 (C-5), 131.71 (C-6), 32.34 (C-1), 24.23 (C-2), 31.51 (C-7), 32.56 (C-8), 49.11 (C-9), 34.46 (C-10), 19.65 (C-11), 37.30 (C-12), 59.63 (C-13), 46.37 (C-14), 21.11 (C-15), 31.59 (C-16), 19.42 (C-18) 18.70 (C-19), 135.90 (N–CH$_2$*C*H=CH$_2$), 115.51 (N–CH$_2$CH=*C*H$_2$) and 43.74 (N–*C*H$_2$CH=CH$_2$) ppm; Mass (APCI): 351.33 $[M+1]^+$.

Similarly, *17a-benzyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one* (**16**) *and 17a-cyanoethyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one* (**17**) were synthesized. The spectral data is reported in the supplementary material.

4.1.6. 17a-Acetyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (*18*)

3-Cyano-17a-aza-D-homo-3,5-androstadien-17-one (**12**) (1.02 g, 3.0 mmol) was dissolved in pyridine (10.0 ml) and acetic anhydride (5.0 ml) was added to the mixture. The solution was refluxed for 5 h at about 120 °C. The excess of acetic anhydride was destroyed with methanol after cooling to room temperature and poured into crushed ice. The resulting precipitate was filtered, washed thoroughly with water and dried to yield 17a-acetyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**18**) (0.95 g, 93.1%): mp 191–193 °C; UV_{max} (MeOH): 261.8 nm (log *&* 4.26); IR (KBr, cm^{-1}): 2942, 2855, 2203, 1727 and 1659; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (s, 3H, 19-C*H*$_3$), 1.45 (s, 3H, 18-C*H*$_3$), 2.34 (s, 3H, N-COC*H*$_3$), 5.78 (br s, 1H, 6-vinylic) and 6.67 (br s, 1H, 4-vinylic) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 173.43 (C-17), 120.12 (*C*N), 107.26 (C-3), 142.66 (C-4), 139.47 (C-5), 131.36 (C-6), 32.84 (C-1), 24.22 (C-2), 31.63 (C-7), 32.41 (C-8), 49.00 (C-9), 34.45 (C-10), 19.83 (C-11), 35.46 (C-12), 62.10 (C-13), 46.51 (C-14), 21.09 (C-15), 32.33 (C-16), 20.04 (C-18), 18.68 (C-19), 29.46 (N–CO–*C*H$_3$) and 179.04 (N–CO–CH$_3$); Mass (APCI): 353.20 $[M+1]^+$, 311.33 $[M-42]^+$.

4.1.7. 17a-Benzoyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (*19*)

3-Cyano-17a-aza-D-homo-3,5-androstadien-17-one (**12**) (0.34 g, 1.0 mmol) was dissolved in 10.0 ml of dry benzene and benzoyl chloride (0.35 ml; 3.0 mmol) was added to it. The reaction mixture was refluxed under anhydrous conditions for 18 h. Then, the solvent was removed under vacuum, the residue was diluted with diethyl ether and filtered to give 17a-benzoyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**19**) (0.32 g, 71.1%): mp 240–241 °C; UV_{max} (MeOH): 259.2 nm (log *&* 4.39); IR (KBr, cm^{-1}): 2954, 2198, 1710 and 1644; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (s, 3H, 19-C*H*$_3$), 1.59 (s, 3H, 18-C*H*$_3$), 7.8 (d, 2H), 7.52 (t, 1H), 7.41 (t, 2H) (aromatic hydrogens), 5.81 (br s, 1H, 6-vinylic) and 6.68 (br s, 1H, 4-vinylic) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ173.08 (C-17), 120.12 (*C*N), 107.28 (C-3), 142.69 (C-4), 139.50 (C-5), 131.37 (C-6), 32.49 (C-1), 24.24 (C-2), 31.61 (C-7), 32.35 (C-8), 48.54 (C-9), 34.50 (C-10), 20.26 (C-11), 35.65 (C-12), 61.44 (C-13), 46.57 (C-14), 20.86 (C-15), 31.92 (C-16), 21.13 (C-18), 18.75 (C-19) 128.68, 128.98, 133.02, 135.86 (aromatic carbons) and 176.28 (N–*C*O*C*$_6$H$_5$); Mass (APCI): 415.20 $[M+1]^+$, 311.47 $[M-COC_6H_5]^+$.

*4.1.8. 17-Oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**20**)*

3-Cyano-17a-aza-D-homo-3,5-androstadien-17-one (**12**) (0.31 g, 1.0 mmol) was dissolved in 20.0 ml of absolute alcohol, to the solution 50% aqueous sodium hydroxide (2.0 ml) was added and allowed to reflux for 24 h. The reaction was monitored with the help of TLC, after completion of reaction it was cooled to 50 °C and to this was added with stirring mixture of 50% hydrochloric acid (5.0 ml) and dichloromethane (50.0 ml). The aqueous phase pH was kept between pH 1.5–2.0. The aqueous layer was extracted with dichloromethane (3 × 50.0 ml), combined organic layers were washed with water and dried. The dichloromethane was removed under vacuum and ethyl acetate (30.0 ml) was added. This was refluxed for 2 h, and then kept at 0–5 °C. The precipitated compound was filtered and dried under vacuum to afford 17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**20**) (0.20 g, 60.6%): mp 264–265 °C; UV_{max} (MeOH): 268.0 nm (log & 4.48); IR (KBr, cm^{-1}): 3464, 3216, 2939, 1723, 1687, 1635, 1608 and 1184; ^1H NMR (400 MHz, DMSO-d_6): δ 0.89 (s, 3H, 19-C*H*$_3$), 1.19 (s, 3H, 18-C*H*$_3$), 5.79 (br s, 1H, 6-vinylic), 6.99 (br s, 1H, 4-vinylic) and 7.04 (1H, N*H*, disappeared on D_2O exchange) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 171.23 (*C*OOH), 169.43 (C-17), 126.46 (C-3), 137.65 (C-4), 140.86 (C-5), 130.39 (C-6), 33.09 (C-1), 21.69 (C-2), 30.85 (C-7), 32.05 (C-8), 47.98 (C-9), 34.83 (C-10), 19.96 (C-11), 39.21 (C-12), 53.95 (C-13), 47.37 (C-14), 20.92 (C-15), 31.56 (C-16), 22.05 (C-18), 18.81 (C-19); Mass (APCI Mode): 330.40 $[M+1]^+$, 344.33 $[M+15].^+$

*4.1.9. 17a-Methyl-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**21**)*

17a-Methyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**13**) (0.32 g, 1.0 mmol) was dissolved in 20.0 ml of absolute alcohol, to the solution 50% aqueous sodium hydroxide (2.0 ml) was added and allowed to reflux for 24 h. Following similar procedure as mentioned above and crystallization from methanol yielded 17a-methyl-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**21**) (0.19 g, 55.9%): mp 238–240 °C; UV_{max} (MeOH): 267.0 nm (log & 4.27); IR (KBr, cm^{-1}): 3501, 2955, 1690, 1594 and 1235; ^1H NMR (400 MHz, $CDCl_3$): δ 0.89 (s, 3H, 19-C*H*$_3$), 1.20 (s, 3H, 18-C*H*$_3$), 2.92 (s, 3H, N-C*H*$_3$), 5.84 (br s, 1H, 6-vinylic), 7.14 (br s, 1H, 4-vinylic) and 7.8 (br s, 1H, COOH) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.04 (*C*OOH), 170.71 (C-17), 125.70 (C-3), 139.38 (C-4), 140.82 (C-5), 131.51 (C-6), 33.01 (C-1), 21.42 (C-2), 31.56 (C-7), 32.52 (C-8), 48.92 (C-9), 34.76 (C-10), 19.37 (C-11), 37.10 (C-12), 58.90 (C-13), 46.63 (C-14), 21.33 (C-15), 31.78 (C-16), 18.76 (C-18), 17.98 (C-19) and 26.96 (N–*C*H$_3$); Mass (APCI Mode): 344.40 $[M+1]^+$.

*4.1.10. 17a-Ethyl-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**22**)*

17a-Ethyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**14**) (0.34 g, 1.0 mmol) was hydrolyzed using similar procedure and after crystallization from methanol 17a-ethyl-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**22**) (0.20 g, 55.5%) was obtained: mp 249–250 °C; UV_{max} (MeOH): 267.2 nm (log & 4.34); IR (KBr, cm^{-1}): 3430, 2941, 1688, 1638, 1584 and 1241; ^1H NMR (400 MHz, $CDCl_3$): δ 0.82 (s, 3H, 19-C*H*$_3$), 1.13 (s, 3H, 18-C*H*$_3$), 1.18 (t, 3H, *C*H$_3$CH$_2$N-), 3.03 (dq, 1H, CH$_3$C*H*H–N), 3.67 (dq, 1H, CH$_3$CH*H*–N), 5.78 (br s, 1H, 6-vinylic) and 7.08 (br s, 1H, 4-vinylic) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.08 (COOH), 170.73 (C-17), 125.66 (C-3), 139.43 (C-4), 140.81 (C-5), 131.53 (C-6), 33.04 (C-1), 21.42 (C-2), 31.52 (C-7), 32.62 (C-8), 49.12 (C-9), 34.79 (C-10), 19.30 (C-11), 37.25 (C-12), 59.92 (C-13), 46.67 (C-14), 21.31 (C-15), 31.82 (C-16), 18.75 (C-18), 15.40 (C-19), 36.74 (N–*C*H$_2$CH$_3$) and 19.26 (N–CH$_2$*C*H$_3$) ppm; Mass (APCI Mode): 358.53 $[M+1]^+$.

*4.1.11. 17a-Allyl-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**23**)*

17a-Allyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**15**) (0.35 g, 1.0 mmol) was hydrolyzed to obtain 17a-allyl-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**23**) (0.25 g, 67.6%) via similar procedure: mp 194–196 °C; UV_{max} (MeOH): 266.2 nm (log & 4.11); IR (KBr, cm^{-1}): 3397, 2948, 1702, 1611 and 1259; ^1H NMR (400 MHz, $CDCl_3$): δ 0.81 (s, 3H, 19-C*H*$_3$), 1.15 (s, 3H, 18-C*H*$_3$), 3.69 (dd, 1H, N–H*H*C), 4.24 (dd, 1H, N–*H*HC), 5.03 (dd, 1H, N–CH$_2$CH=C*H*H), 5.07 (dd, 1H, N–CH$_2$CH=CH*H*), 5.82 (ddq, 1H, N–CH$_2$C*H*=CHH), 5.79 (br s, 1H, 6-vinylic) and 7.07 (br s, 1H, 4-vinylic) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.00 (COOH), 170.85 (C-17), 125.52 (C-3), 139.57 (C-4), 140.79 (C-5), 131.63 (C-6), 33.00 (C-1), 21.41 (C-2), 31.27 (C-7), 32.71 (C-8), 49.09 (C-9), 34.75 (C-10), 21.29 (C-11), 37.31 (C-12), 60.12 (C-13), 46.58 (C-14), 21.09 (C-15), 31.80 (C-16), 19.46 (C-18), 18.76 (C-19), 135.48 (N–CH$_2$CH=CH$_2$), 115.92 (N–CH$_2$CH=*C*H$_2$) and 44.02 (N–*C*H$_2$CH=CH$_2$) ppm; Mass (APCI): 370.60 $[M+1]^+$.

For synthesis and spectral data of 17*a-benzyl*-17*-oxo*-17*a-aza-D-homo*-3,5*-androstadien*-3*-oic acid* (**24**) refer to supplementary material.

*4.1.12. 17a-(2-Propionoxyethyl)-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**25**)*

17a-Cyanoethyl-3-cyano-17a-aza-D-homo-3,5-androstadien-17-one (**17**) (0.36 g, 1.0 mmol) was hydrolyzed by ethanolic sodium hydroxide using similar procedure to obtain 17a-(2-propionoxy ethyl)-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**25**) (0.21 g, 50.0%): mp 200–205 °C; UV_{max} (MeOH): 267.2 nm (log & 4.44); IR (KBr, cm^{-1}): 3424, 2951, 1730, 1610 and 1260; ^1H NMR (400 MHz, $CDCl_3$): δ 0.89 (s, 3H, 19-C*H*$_3$), 1.22 (s, 3H, 18-C*H*$_3$), 3.36 (m, 1H, CH$_3$CH$_2$COOCH$_2$C*H*H–N), 3.90 (m, 1H, CH$_3$CH$_2$COOCH$_2$ CH*H*–N), 2.84 (m, 1H, CH$_3$CH$_2$COOCH*H*CHH–N), 2.56 (m, 1H, CH$_3$CH$_2$COOC*H*HCHH–N), 5.86 (br s, 1H, 6-vinylic), 7.14 (br s, 1H, 4-vinylic), 4.14 (q, 2H, CH$_3$C*H*$_2$COOCH$_2$CHH–N), 1.24 (t, 3H, C*H*$_3$CH$_2$COOCH$_2$ CH$_2$–N) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.04 (COOH), 171.93 (C-17), 172.46 (N–CH$_2$CH$_2$*C*OOC$_2$H$_5$), 125.43 (C-3), 139.66 (C-4), 140.83 (C-5), 131.67 (C-6), 34.30 (C-1), 21.39 (C-2), 31.86 (C-7), 32.56 (C-8), 49.27 (C-9), 34.80 (C-10), 19.41 (C-11), 37.28 (C-12), 59.92 (C-13), 46.72 (C-14), 21.29 (C-15), 32.56 (C-16), 19.04 (C-18), 18.75 (C-19), 60.49 (N–*C*H$_2$CH$_2$ COO*C*H$_2$CH$_3$), 37.55 (N–*C*H$_2$CH$_2$COOCH$_2$CH$_3$), 14.22 (N–*C*H$_2$ CH$_2$COOCH$_2$*C*H$_3$); Mass (APCI): 430.27 $[M+1]^+$.

*4.1.13. 17a-Acetyl-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**26**)*

17-Oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**20**) (0.32 g, 1.0 mmol) was dissolved in pyridine (10.0 ml) and acetic anhydride (5.0 ml) was added to the mixture. The solution was refluxed for 5 h at about 120 °C. The excess of acetic anhydride was destroyed with methanol after cooling to room temperature and poured into crushed ice. The resulting precipitate was filtered, washed thoroughly with water and dried to yield 17a-acetyl-17-oxo-17a-aza-D-homo-3,5-androstadien-3-oic acid (**26**) (0.26 g, 81.2%): mp 198–200 °C; UV_{max} (MeOH): 265.2 nm (log & 4.32); IR (KBr, cm^{-1}): 3422, 2937, 1701, 1697, 1635 and 1200; ^1H NMR (400 MHz, DMSO-d_6): δ 0.85 (s, 3H, 19-C*H*$_3$), 1.40 (s, 3H, 18-C*H*$_3$), 2.34 (s, 3H, N-CO*C*H$_3$), 5.80 (br s, 1H, 6-vinylic) and 6.95 (br s, 1H, 4-vinylic) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 172.74 (COOH), 168.51 (C-17), 126.18 (C-3), 136.85 (C-4), 140.18 (C-5), 129.93 (C-6), 32.57 (C-1), 21.26 (C-2), 31.13 (C-7), 31.93 (C-8), 48.10 (C-9), 34.15 (C-10), 20.73 (C-11), 34.97 (C-12), 61.47 (C-13), 46.12 (C-14), 19.31 (C-15), 32.25 (C-16), 19.76 (C-18), 18.26 (C-19), 28.98 (N–CO–*C*H$_3$) and 178.75 (N–*C*O–CH$_3$); Mass (APCI): 372.05 $[M+1]^+$, 330.26 $[M-42]^+$.

*4.1.14. 3-Bromo-19-nor-3,5-androstadien-17-one (**28**)*

Following a similar procedure as for the synthesis of compound **11** and using 19-nor-4-androstene-3,17-dione (**27**) (2.49 g,

9.16 mmol) and 1.61 ml of phosphorus tribromide (4.60 g, 17.0 mmol) afforded 3-bromo-19-nor-3,5-androstadien-17-one (**28**) (2.70 g, 88.23%): mp 174–176 °C; UV_{max} (MeOH): 234.4 nm (log & 4.14); IR (KBr, cm^{-1}): 2927, 1732, 1608 and 1435; ^1H NMR (400 MHz, $CDCl_3$): δ 0.90 (s, 3H, 18-CH_3), 5.51 (br s, 1H, 6-vinylic) and 6.36 ppm (br s, 1*H*, 4-vinylic) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 221.06 (C-17), 36.02 (C-1), 28.89 (C-2), 122.92 (C-3), 136.50 (C-4), 131.61 (C-5), 123.80 (C-6), 30.13 (C-7), 36.21 (C-8), 50.97 (C-9), 40.00 (C-10), 21.70 (C-11), 31.32 (C-12), 47.81 (C-13), 43.72 (C-14), 25.89 (C-15), 35.82 (C-16) and 13.67 (C-18); Mass (APCI): 335.27 $[M]^+$ and 337.20 $[M+2]^+$.

*4.1.15. 3-Cyano-19-nor-3,5-androstadien-17-one (**29**)*

Following similar procedure as for compound **12** but using 3-bromo-19-nor-3,5-androstadien-17-one (**28**) (1.04 g, 3.0 mmol) and cuprous cyanide (0.27 g, 3.0 mmol) afforded 3-cyano-19-nor-3,5-androstadien-17-one (**29**) (0.54 g, 64.3%): mp 230–232 °C; UV_{max} (MeOH): 262.2 nm (log & 3.87); IR (KBr, cm^{-1}): 2925, 2202, 1730 and 1630 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.91 (s, 3H, 18-CH_3), 5.89 (br s, 1*H*, 6-vinylic) and 6.72 ppm (br s, 1*H*, 4-vinylic) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ 120.25 (CN), 220.67 (C-17), 35.74 (C-1), 26.23 (C-2), 108.46 (C-3), 143.53 (C-4), 135.22 (C-5), 132.20 (C-6), 27.38 (C-7), 35.99 (C-8), 50.85 (C-9), 40.06 (C-10), 21.63 (C-11), 30.61 (C-12), 47.74 (C-13), 43.48 (C-14), 25.65 (C-15), 31.20 (C-16) and 13.63 (C-18); Mass (APCI): 300.33 $[M + 18]^+$.

*4.1.16. 17-Oxo-19-nor-3,5-androstadien-3-oic acid (**30**)*

Following similar procedure as for compound **20** and hydrolyzing 3-cyano-19-nor-3,5-androstadien-17-one (**29**) (0.84 g, 3.0 mmol) by 50% aqueous sodium hydroxide solution (2.0 ml) afforded 17-oxo-19-nor-3,5-androstadien-3-oic acid (**30**) (0.60 g, 71.4%): mp 250–252 °C; UV_{max} (MeOH): 269.6 nm (log & 4.45); IR (KBr, cm^{-1}): 3450, 2931, 1732, 1675, 1627, 1279 and 1081 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.92 (s, 3H, 18-CH_3), 5.98 (br s, 1*H*, 6-vinylic), 7.20 ppm (br s, 1*H*, 4-vinylic); ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.61 (COOH), 221.03 (C-17), 126.88 (C-3), 140.73 (C-4), 136.59 (C-5), 132.49 (C-6), 35.79 (C-1), 25.80 (C-2), 26.81 (C-7), 36.14 (C-8), 50.95 (C-9), 40.64 (C-10), 21.66 (C-11), 30.86 (C-12), 47.81 (C-13), 43.70 (C-14), 24.56 (C-15), 31.27 (C-16) and 13.64 (C-18); Mass (APCI): 300.53 $[M^+]$.

4.2. Biological activity

4.2.1. In vitro human 5α-reductase inhibitory activity against type I and type II 5α-reductase enzyme

HEK-I and HEK-II served cell lines were used as a source of 5α-reductase enzyme. HEK 293 cells are human embryonic kidney cells which were made cultivatable for 5α-reductase I and II when cDNAs encoding 5α-reductase type I and type II were inserted into pRcCMV vector and expressed in them.

4.2.1.1. Preparation of solutions used in inhibitory assay. Inhibitor preparation: A stock solution of 10 mM in DMSO was prepared. Before the test it was diluted to a concentration which is 50 times more concentrated than the concentration in the test. In the next step one volume of this solution was diluted with 2.5 volumes of tris buffer (so that it's now 20 times more concentrated than that would be used in the test). This results in a test concentration of 2% DMSO. As controls 2% DMSO (without inhibitor) was used. From this 20 times compound solution, 25 μl is used in a total test volume of 500 μl.

NADPH regenerating system: NADP was dissolved in tris buffer to a concentration of 22 mM while in the test the concentration used is 0.55 mM. Also glucose-6-phosphate was dissolved in tris buffer to a concentration of 100 mM while in the test the end concentration used is 5 mM. In case of glucose-6-phosphate dehydrogenase 5 μl of the sample obtained from Sigma (G8404) was directly used and it was diluted in 1 ml tris buffer. A 1:2:1 mixture of this regenerating system (NADP: glucose: glucose-6-P-dehydrogenase) was stored in the fridge and 50 μl of it was used in the end assay.

Androstenedione: A concentrated stock solution of 1 mM in methanol was used for the assay. For the test from the stock solution a 5 μM solution is prepared by first diluting it 1:1 in methanol and then 1 ml of this solution is diluted to 100 ml in tris buffer. 50 μl of it is used in the inhibitory assay i.e. an end concentration of 500 nM.

Master mix: A master mix of 124.7 μl tris buffer, 50 μl androstenedione solution and 50 μl regenerating system was made and mixed with 0.3 μl [^3H] androstenedione. 25 μl of the compound solution to be tested was added to the solution prepared so as to get a final test volume of 250 μl.

Standard: Finasteride, a clinically used drug, was used as standard.

Reversed phase HPLC: HPLC analyses were performed by the use of a high pressure solvent delivery pump (Waters M6000A, Milford, USA), a radioactivity detector (LB506C, Berthold, Wildbad, Germany) and an autosampler system (851-AS, Jasco, Tokyo, Japan). Nucleosil 120-3-C_8 was applied as stationary phase using prepacked columns (125 × 4 mm; Macherey–Nagel, Duren, Germany). Following parameters were used for analysis: Flow rate: 0.325 ml/min; Run time: 11 min; Additive flow for Scintillator: 1.2 ml from 3.5 to 10 min, Solvent System: 28% MeOH: 72% water.

Inhibition assay: Lysates were obtained after harvesting and resuspending 80% confluent cells in homogenate buffer (containing 300 mM 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 assay to 250 μl of master mix prepared above 250 μl of the cell suspension was added so as to make a total volume of 500 μl. After an incubation of 30 min at 37 °C, the reaction was stopped by the addition of 750 μl of ether. The steroids were extracted by shaking the reaction mixture for 10 min, then centrifuging it for further 10 min. and finally freezing the aqueous phase (in an ethanol-dry ice bath) to collect the ether phase containing the steroids. The latter phase is dried in a speed vacuum, resuspended again in 35 μl of methanol and then transferred to HPLC vials for 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.

4.2.2. In vivo 5α-reductase inhibitory activity

Male wistar rats were used in the study in accordance with a protocol (6598/PS dated 21.08.2010) approved by the Institutional Animal Ethical Committee (IAEC) at the central animal house facilities of Panjab University, Chandigarh, India. The experiments were conducted as per CPCSEA (Committee for Prevention, Control and Supervision of Experimental Animals) guidelines. All rats were housed under standard conditions with free access to food and water. The control diet for the rats contained 50.0% nitrogen free extract, 20% protein, 8% fat, 4% cellulose, 1–1.5% of each mineral and vitamin mix. The control diet was obtained from Ashirwad Industries (Chandigarh, India). Finasteride was procured from Dr Reddy's Laboratories, Hyderabad as gift sample. Mature male wistar rats were domesticated for a month and oral administration was started after 9 weeks. Following this varying amounts of drug suspension at a dose of 5 ml/kg was administered orally once daily for 14 consecutive days. All the test and reference compounds were suspended in 0.50% carboxymethylcellulose sodium salt (Himedia) solution. Rats were weighed and sacrificed by ether anaesthesia on the 15th day after 24 h of last dosing. The following organs were

identified, removed and after removal of adhering fat and connective tissue weighed: ventral prostate, dorsal prostate, seminal vesicles, testes, epididymis, vas deferens, liver and adrenal glands. Organ weights were recalculated (mg/100 g body weight) i.e. dividing the weight of the tissue by the body weight so as to remove variation due to the body weights among the groups. All weighing of the organs were made on Shimazdu AW 220 balance. Animals were divided into groups of 5 animals each. The following groups were taken in the study: Control (animals receiving only vehicle i.e. 0.50% CMC), others were being Finasteride (animals receiving 5 ml/kg Finasteride) and synthesized compounds (animals receiving 5 ml/kg compound). Results are expressed as mean ± standard error of mean (SEM) of 5 animals per group. Statistical significance of differences between groups was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test. For the statistical determination, statistical computerized software SIGMASTAT 3.5 was used. A probability (P) value of less than 0.05 indicates a statistically significant difference between the treatment groups.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.06.026.

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