3. EXPERIMENTAL

3.1. Computer aided design of 5ARIs

**Hardware:** All molecular modeling studies were performed on Intel® Core™2 Quad CPU Q8400@2.66GHz platform running under Ubuntu 12.0.4.2 LTS operating system.

**Software:** The molecular modeling software package SYBYL 8.0 and X1.2 was used for CoMFA and CoMSIA studies. Schrodinger molecular modeling suite 2012 was used for docking and ADME prediction studies. Following modules were employed for this work: Ligprep, Macromodel, Glide, Prime, Qikprop.

3.1.1. 3D QSAR CoMFA and CoMSIA studies on 6-azasteroidal 5ARIs

**A. Data set**

All important prerequisite for choosing a QSAR dataset were taken into account. The biological data should have been determined in just one laboratory using the same method to avoid systematic error. A dataset of sixty one molecules were chosen on the basis of structural diversity and a span of atleast 2-3 orders of magnitude in their biological activity. The biological data was expressed as pIC$_{50}$ where, pIC$_{50}$ is the negative logarithm of molar concentration in nanomoles of the inhibitors producing 50% inhibition of 5AR-2 isozyme. It was divided into a training set of 51 molecules and a test set of 10 molecules to assess the predictivity of the models. The test set was judiciously chosen so that it covered almost entire range of biological activity and structural diversity. The general structure of the test and training set molecules along with the observed and predicted activity have been presented in Table 2-5.

**B. Compound generation and molecular alignment**

Compounds under investigation were built using the most active compound as template. The partial charges for all of the compounds were calculated using Gasteiger-Huckel method. The geometry of the molecules was optimised using Tripos force field with a distance dependent dielectric function and energy convergence criterion of 0.001 Kcal/mol Å with standard SYBYL settings and keeping maximum 1000 iterations.

In 3D QSAR studies, determination of biologically active conformation and molecular alignment of the compounds are the most important factors that affects quality of the model. The positioning of the molecular model within the fixed lattice is an important input variable in CoMFA, since the relative interaction energies depend strongly on the relative molecular positions.
Experimental

In the current study atoms and centroids based alignment was used to superimpose the molecules over the most active one. The SYBYL conventional fit atom alignment rule was applied. The atom based alignment module adjusts the geometry in such a way that its steric and electrostatic fields matched the template molecule. Compound A-2 from the series was selected as the template molecule, which was most potent inhibitor of the enzyme 5AR. The lowest energy conformer of the compound was obtained using Multisearch option in SYBYL. The atoms (Centroid C1, C2 and atom '*') used for alignment of the compounds under study were as shown in Figure 18A. Superimposition of all the molecules under study on the template has been shown in Figure 18B.

C. CoMFA

The steric (Lennard–Jones potential) and electrostatic fields (Coulombic potentials) were calculated at each lattice intersection for the aligned molecules kept in a 3D cubic lattice with a grid spacing of 2.0 Å in all the three coordinates. The van der Waals potentials and coulombic terms representing the steric and electrostatic fields, respectively, were calculated using standard Tripos force fields.

A sp³ carbon atom having a charge of +1 and a radius of 1.52 Å was used as a probe to calculate the steric and electrostatic fields. The steric and electrostatic fields were truncated at 0.3 kcal mol⁻¹. The other parameters were used as default because, the effect of altering the lattice spacing, column filtering and energy cut off values in the CoMFA process appears to be minimal and thus the use of the default settings for these parameters seems justified.

D. CoMSIA

CoMSIA fields (steric, electrostatic, hydrophobic, hydrogen bond donor and hydrogen bond acceptor) were calculated at each lattice intersection of the same lattice box used for CoMFA calculations. In the present study, standard settings of CoMSIA (probe with charge +1, radius 1 Å and hydrophobicity +1, attenuation factor of 0.3 and grid spacing 2 Å) were used to calculate the steric, electrostatic, hydrophobic, donor and acceptor fields. The equation used to calculate the similarity indices is as follows:

\[
A_F^q(j) = \sum_{l} w_{probe,k} w_{ik} e^{-ar_l^2}
\]
where A is the similarity index at grid point q, summed over all atom i of the molecule j under investigation, $w_{\text{probe,k}}$ is the probe atom with radius 1 Å, charge +1, hydrophobicity +1, hydrogen bond donating +1 and hydrogen bond accepting +1, $w_k$ is the actual value of physicochemical property k of atom I, $r_{iq}$ is the mutual distance between the probe atom at grid point q and atom i of the test molecule, and $\alpha$ is the attenuation factor.

E. Partial least square (PLS) analysis

PLS method\cite{315} was used to correlate 5AR inhibitory activity with the CoMFA and CoMSIA fields to derive 3D QSAR models. Internal cross validation analysis was performed internally using leave one out (LOO) method in which one compound is removed from the dataset and its activity is predicted using the model derived from the rest of the dataset.\cite{316} The cross validation $r^2$ that resulted in optimum numbers of components and lowest standard error of prediction were considered for further analysis. The external validation of various models was performed using a test set of 10 molecules. The analysis was carried out with the column filtering value of 2.0 kcal/mol to speed up the calculation and reduce the noise. Final analysis was performed to calculate non cross validated $r^2$ using the optimum number of components. The cross validation $r^2$, Fischer’s statistic (F-test), SEE, and predicted $r^2$ ($r^2_{pred}$) were calculated.

3.1.2. 3D QSAR CoMFA and CoMSIA studies on 4-azasteroidal 5ARIs

A. Dataset

A set of 52 substituted 4-azasteroidal inhibitors of the enzyme human 5AR were selected from literature\cite{389} on the basis of diversity in structural motif and variation in the biological activity. The biological activity was reported for human 5AR, which showed some assay to assay variability, so the inhibition is expressed as a ratio of the inhibitor IC$_{50}$ value to the IC$_{50}$ value of the compound B-44 from the series. Where IC$_{50}$ is the molar concentration in nanomoles of the inhibitors producing 50% inhibition of human 5AR enzyme. The activity was converted into negative logarithm. The dataset was divided into a training set of 42 molecules and a test set of 10 molecules which were used to assess the predictivity of the models. The test set was judiciously chosen so that it covered almost entire range of biological activity and structural diversity. The general structure of the test and training set molecules along with the obtained and predicted activity have been presented in Table 7-8.
Experimental

B. Compound generation and molecular alignment

Since the crystal structure of enzyme 5AR in complex with 4-azasteroid inhibitor is not reported, so the most active compound from the dataset was subjected to conformational search using OPLS_2005 forcefield using water as a solvent in MacroModel.\textsuperscript{309} The global minimum conformation obtained from the search was used as a template to build the other molecules. The partial charges for all of the compounds were calculated using Gasteiger-Huckel method. The geometry of the molecules was optimised using Tripos force field with a distance dependent dielectric function and energy convergence criterion of 0.001 Kcal/mol Å with standard SYBYL settings and keeping maximum 1000 iterations.

The optimized structures of the dataset molecules were aligned using maximum common substructure methodology implemented in SYBYL. Superimposition of all the molecules under study on the template has been shown in Figure 23(B). The test set molecules were also processed in a similar fashion as training set molecules.

C. CoMFA

CoMFA fields were calculated as discussed in section 3.1.1.

D. CoMSIA

CoMSIA fields were calculated as discussed in section 3.1.1.

E. Partial least square (PLS) analysis

The models were developed using PLS methodology to correlate 5AR inhibitory activity with the CoMFA and CoMSIA fields. Internal cross validation analysis was performed using LOO method. The models were also subjected to external cross validation using a test set of ten molecules. The cross validation $r^2$ that resulted in optimum numbers of components and lowest standard error of prediction were considered for further analysis.

The external validation of various models was performed using a test set of 10 molecules. The analysis was carried out with the column filtering value of 2.0 kcal/mol to speed up the calculation and reduce the noise. Final analysis was performed to calculate non cross validated $r^2$ using the optimum number of components. The cross validation $r^2$, Fischer’s statistic (F-test), SEE and predicted $r^2$ were calculated.
3.1.3. Docking studies

Docking studies were carried out using standard Glide\textsuperscript{311} molecular docking module. As the crystal structure of 5AR is not available, recently a docking study has been reported using 5β-reductase as a surrogate to the receptor on the basis that the substrate for both of the enzymes is same thus they have same or at least similar enzymatic functions when metabolizing steroid hormones.\textsuperscript{144} The protein structure PDB code: 3G1R\textsuperscript{317} was retrieved from Protein Data Bank (www.rcsb.com) and used for docking studies. The enzyme 5β-reductase is a dimer containing identical chains A and B. As the ligand was present in chain B in co-crystallized form, the monomer B was kept and treated using protein preparation wizard of the Schrödinger suite. Following hydrogen bonding assignment optimization, removal of water molecules and the protein ligand complex was energy minimised until an rmsd of 0.30Å. The docking studies were carried out in presence of cofactor NADPH to elucidate its potential role. The docking was performed using Glide extra precision mode (XP). The receptor grid was generated using the centroid of the co-crystallized ligand and a maximum size of 20Å. To mimic the flexibility of the protein structure, a scaling van der Walls radii for non-polar parts in the binding site and the ligand was carried out.

Further Prime MM-GBSA approach was used to predict the free energy of binding for the receptor-inhibitor complex\textsuperscript{318} using prime module of Schrodinger molecular modeling suite.\textsuperscript{312} The docked complexes were taken from pose viewer files of initial Prime XP docking. The MM-GBSA approach employs molecular mechanics, the generalized Born model and solvent accessibility method to elicit free energies from structural information circumventing the computational complexity of free energy simulations.\textsuperscript{319} The binding free energy of each ligand was calculated using following equation:

\[
\Delta G_{bin} = \Delta E_{mm} + \Delta G_{tol} + \Delta G_{SA}
\]

Where \(\Delta E_{mm}\) is the difference in the minimized energies between the receptor-ligand complex and the sum of energies of unliganded receptor and ligands. \(\Delta G_{tol}\) is the the difference in the GBSA solvation energy of the receptor ligand complex and the sum of energies of unliganded receptor and ligands. \(\Delta G_{SA}\) is the difference in surface area energies for the receptor ligand complex and the sum of energies of unliganded receptor and ligands.
3.2. Synthetic studies

All the chemicals and reagents were obtained from S.D. Fine Chem Ltd., E. Merck (India) Pvt. Ltd, Sigma Aldrich, Loba Chemie, Himedia, and CDH. Solvent used were of LR (Laboratory reagent) grade and freshly distilled and dried according to the standard procedure. Melting points were determined using Veego melting point apparatus and are uncorrected. Ultraviolet spectra were recorded on Perkin–Elmer λ15 UV/Vis. spectrophotometer. The infrared spectra were recorded on Perkin-Elmer RX-1 FTIR spectrophotometer using potassium bromide pellets. $^1$H and $^{13}$C nuclear magnetic spectroscopy was performed using a Bruker Avance-II 400 MHz instrument using deuterated chloroform or deuterated dimethyl sulphoxide as solvent and tetramethylsilane as internal reference.

Deuterated water was used for deuterium exchange reaction. Only principle peaks of interest are reported and expressed in δ ppm. Spin multiplicities are indicated with symbols: s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), m (multiplet), and bs (broad singlet). Mass analysis was carried out using Waters® Micromass® Q-Tof micro™ mass spectrometer at special analytical instruments facility (SAIF), Panjab University, Chandigarh. Precoated plates with Silica Gel G E. Merck 60 F$_{254}$ (0.25 mm) were used for thin layer chromatography (TLC). Chromatographic spots were developed by exposure to iodine vapors. Anhydrous sodium sulphate was used as drying agent.

3.2.1. SYNTHESIS OF 17α-SUBSTITUTED 3-CYANO-17α-AZA-D-HOMO-3,5-ANDROSTADIENES

1. 20-0X0-5,16-PREGNADIEN-3/7-YL ACETATE [16-DHYDROPREGNOLONE ACETATE (16-DPA), 502]

25(R)-5-Spirosten-3β-ol (501, 5 g, 12.5 mmol) and methylvamine hydrochloride (5 g) in pyridine (20 ml) were refluxed in acetic anhydride (20 ml) for 15 hrs. The reaction mixture was cooled and poured into ice-cold water (500 ml). The precipitated material was filtered, washed repeatedly with dilute hydrochloric acid, dried and dissolved in dichloromethane (50 ml) and glacial acetic acid (25 ml). The solution was cooled to -5 °C, stirred and to this was added dropwise cold solution of chromium trioxide [1.60 g of chromium trioxide in 20 ml of water and 15 ml of acetic acid (90% v/v)]. The reaction mixture was further stirred for 1 hr at -5 °C. Excess of chromium trioxide was destroyed with sodium metabisulphite (10%, 35 ml) at 0 °C. After separating the organic layer, the
aqueous layer was extracted with dichloromethane (3 × 50 ml). The combined organic layer was washed with water, sodium bicarbonate solution (5%) and water. The extract was dried, filtered and solvent removed under reduced pressure. The oily residue so obtained was refluxed with glacial acetic acid (50 ml) for 2 hr, cooled and poured into water (500 ml). It was extracted with DCM (3×50 ml), washed with water, aqueous sodium bicarbonate solution (5% w/v) and water. The extract was dried and the solvent was removed under vacuum to obtain a residue which was crystallized from methanol to yield 20-oxo-5,16-pregnadien-3β-yl acetate (502, 3.75 g, 87.21%), m. p. 176-177 °C (lit. 175-177 °C). Analysis:

Rf value: (CHCl₃: MeOH :: 9.0:1.0) 0.78
UV max (MeOH): 234.6 nm
IR (KBr): 2940, 1729, 1660, and 1243 cm⁻¹
¹H NMR (CDCl₃):
δ 0.92 (s, 3H, 18-CH₃), 1.07 (s, 3H, 19-CH₃), 2.04 (s, 3H, 21-CH₃), 2.26 (s, 3H, CH₂COO), 4.60 (m, 1H, 3α-H), 5.38 (bs, 1H, 6-vinylic), and 6.72 (bs, 1H, 16-vinylic) ppm.

II. 20-OXIMINO-5,16-PREGNADIEN-3β-YL ACETATE (503)

A solution of 20-oxo-5,16-pregnadien-3β-yl acetate (502, 5.02 g, 14.1 mmol) and hydroxylamine hydrochloride (2.36 g, 34 mmol) in pyridine (25 ml) was heated on water bath for 1 hr. The reaction mixture was poured into ice-cold water (500 ml) with stirring. The precipitated material was filtered, washed repeatedly to remove pyridine, dried and crystallized from acetone-hexane mixture to yield 20-oximino-5,16-pregnadien-3β-yl acetate (503, 4.60 g, 87.9%), m.p. 227-228 °C (lit. 228-230 °C). Analysis:

Rf value: (CHCl₃: MeOH :: 9.0:1.0) 0.76
IR (KBr): 3386, 2935, 1733, 1636, and 1244 cm⁻¹
¹H NMR (CDCl₃):
δ 0.95 (s, 3H, 18-CH₃), 1.06 (s, 3H, 19-CH₃), 2.01 (s, 3H, 21-CH₃), 2.04 (s, 3H,
Experimental

CH₃COO), 4.61 (m, 1H, 3α-H), 5.39 (bs, 1H, 6-vinylic), 6.07 (bs, 1H, 16-vinylic), and 8.1 (bs, 1H, 21-NO₂) ppm.

III. 17-OXO-5-ANDROSTEN-3β-YL ACETATE (504)

A cold solution of phosphorous oxychloride (4 ml) in dry pyridine (12 ml) was added dropwise to a stirred solution of 20-oximino-5,16-pregnandien-3β-y1 acetate (503, 0.9 g, 2.7 mmol) in pyridine (10 ml) below 0 °C. The reaction mixture was occasionally shaken further for 3 hrs at 0 °C and poured into mixture of crushed ice (50 g) and hydrochloric acid (30 ml). The resulting suspension was allowed to stand for 30 min. at room temperature and diluted with water, filtered, dried and crystallized from acetone-hexane to give 17-oxo-5-androsten-3β-y1 acetate (504, 0.65 g, 73.9%), m.p. 168-169 °C (lit. 169-170 °C).321

Analysis:
Rf value: (CHCl₃: MeOH :: 9.0: 1.0) 0.79
IR (KBr): 2949, 1738, and 1241 cm⁻¹
¹H NMR (CDCl₃):
δ 0.89 (s, 3H, 18-CH₃), 1.05 (s, 3H, 19-CH₃), 2.04 (s, 3H, CH₃COO), 4.60 (m, 1H, 3α-H), and 5.40 (bs, 1H, 6-vinylic) ppm.

IV. 17-OXIMINO-5-ANDROSTEN-3β-YL ACETATE (505)

17-Oxo-5-androsten-3β-y1 acetate (504, 4.95 g, 15 mmol) was dissolved in 95% ethanol (72 ml) and refluxed. To this was added aqueous solution of hydroxylamine hydrochloride (4.72 g, 68 mmol) and sodium acetate trihydrate (12.24 g, 90 mmol) in water (60 ml) and the reaction mixture was refluxed for 5 hrs. The solvent was partially removed by distillation and reaction mixture was poured into ice cold water (500 ml). The precipitated material was filtered, washed with water, dried and crystallized from methanol to yield 17-oximino-5-androsten-3β-y1 acetate (505, 5.07 g, 98.1%), m.p. 177-178 °C (lit. 178-180 °C).132

Analysis:
Rf value: (CHCl₃: MeOH :: 9.0: 1.0) 0.62
Experimental

IR (KBr): 3404, 2944, 1709, and 1268 cm⁻¹

¹H NMR (CDCl₃): δ 0.90 (s, 3H, 18-CH₃), 1.04 (s, 3H, 19-CH₃), 2.03 (s, 3H, CH₃COO), 4.59 (m, 1H, 3α-H), 5.39 (bs, 1H, 6-vinylic), and 8.50 (bs, 1H, 17-NOH) ppm.

V. 17-OXO-17a-AZA-D-HOMO-5-ANDROSTEN-3β-YL ACETATE (506)

A solution of thionyl chloride (2.5 ml) in dioxane (5 ml) was added to the stirring solution of 17-oximino-5-androsten-3β-yl acetate (505, 5.0 g, 14 mmol) in toluene (80 ml) and mixture cooled to 15 °C. The reaction mixture was kept at 20 °C for 20 minutes, cooled in ice bath and water (25 ml) was added. The solution was made alkaline with ammonia, extracted with chloroform (3 × 45 ml). Combined chloroform extract was washed with water (2 × 20 ml), dried, and solvent was removed under vacuum to obtain brownish residue which was crystallized from methanol to yield 17-Oxo-17a-aza-D-homo-5-androsten-3β-yl acetate (506, 3.25 g, 67%), m.p. 290-292 °C (lit. 289-292 °C).¹³²

Analysis:
Rf value: (CHCl₃: MeOH :: 9.0: 1.0) 0.70

IR (KBr): 3480, 2944, 1733, 1688, 1619, and 1247 cm⁻¹

¹H NMR (CDCl₃): δ 0.93 (s, 3H, 18-CH₃), 1.1 (s, 3H, 19-CH₃), 2.30 (s, 3H, CH₃COO), 4.6 (m, 1H, 3α-H), 5.40 (d, 1H, 6-vinylic), 6.8 (s, 1H, CONH, disappeared on deuterium exchange) ppm.

VI. 3β-HYDROXY-17a-AZA-D-HOMO-5-ANDROSTEN-17-ONE (507)

A solution of 17-oxo-17a-aza-D-homo-5-androsten-3β-yl acetate (506, 5 g, 14 mmol) in methanol containing potassium hydroxide (1 g, 18 mmol) was refluxed for 70 minutes. The resulting solution was concentrated, poured into ice-cold water (500 ml), and acidified with glacial acetic acid. The precipitated material was filtered, dried and crystallized from methanol to yield 3β-hydroxy-17a-aza-D-homo-5-androsten-17-one (507, 4.1 g, 90%), m.p. 270-272 °C (lit. 295-297 °C).¹³²

Analysis:
Rf value: (CHCl₃: MeOH :: 9.8: 0.2) 0.87
Experimental

IR (KBr): 3463, 2943, 2887, 1635, and 1253 cm⁻¹

¹H NMR (CDCl₃):
δ 1.07 (s, 3H, 18-CH₃), 1.30 (s, 3H, 19-CH₃), 3.25 (m, 1H, 3α-H), 3.60 (s, 1H, O-H disappeared on deuterium exchange), 5.38 (t, 1H, 6-vinylic), and 8.9 (s, 1H, NH, disappeared on deuterium exchange) ppm.

VII. 17a-AZA-D-HOMO-5-ANDROSTEN-3β-OL (508)

Sodium metal (40 g) was added slowly to a refluxing solution of 3β-Hydroxy-17a-aza-D-homo-5-androsten-17-one (507, 10 g, 32 mmol) in 1-pentanol (350 ml). The reaction mixture was thoroughly shaken vigorously to break sodium metal to small pieces to increase surface area. The reaction mixture was refluxed till the sodium metal has completely reacted. The hot solution was steam distilled, precipitates collected by filtration, washed with water, dried and crystallized from methanol to yield 17a-aza-D-homo-5-androsten-3β-ol (508, 8 g, 84%), m.p. 220-222 °C (lit. 231-233 °C).²

Analysis:
Rf value: (CHCl₃: MeOH :: 9.8: 0.2) 0.64
IR (KBr): 3481, 3234, 2934, 2876, and 1058 cm⁻¹
¹H NMR (CDCl₃):
δ 1.10 (s, 3H, 18-CH₃), 1.30 (s, 3H, 19-CH₃), 2.77 (t, 2H, 17-CH₂), 3.25 (m, 1H, 3α-H), 3.60 (s, 1H, O-H disappeared on deuterium exchange), 5.27 (t, 1H, 6-vinylic), and 6.30 (s, 1H, NH, disappeared on deuterium exchange) ppm.

VIII. 17a-AZA-D-HOMO-4-ANDROSTEN-3-ONE (509)

17a-Aza-D-homo-5-androsten-3β-ol (508, 8 g, 27.6 mmol) was dissolved in a mixture of toluene (300 ml), dry dioxane (150 ml) and cyclohexanone (50 ml). Traces of moisture were removed by azeotropic distillation of toluene (100 ml). The distillation was continued at a slow rate while adding dropwise a solution of aluminum isopropoxide (5 g) in dry toluene (50 ml). The mixture was refluxed for 6 hrs, and was allowed to stand at room temperature for overnight. The slurry was filtered, residue was washed with dry
Experimental

toluene, combined filtrate and washings steam distilled until the complete removal of organic solvents. The residual aqueous suspension was extracted with chloroform (3 × 50 ml), combined chloroform extract washed with water, dried, and the solvent removed to obtain a pale yellow solid which was crystallised from acetone to afford 17a-aza-D-homo-4-androsten-3-one (509, 6.2 g, 80%), m.p. 132-134 °C (lit. 136-138 °C).132

Analysis:

R$_1$ value: (CHCl$_3$: MeOH :: 9.8: 0.2)  0.76  
UV$_{\text{max}}$ (MeOH):  239 nm  
IR (KBr):  3541, 2926, 2848, 1669, and 1232 cm$^{-1}$  
$^1$H NMR (CDCl$_3$):  δ 1.02 (s, 3H, 18-C$_3$H$_3$), 1.32 (s, 3H, 19-C$_3$H$_3$), 5.60 (s, 1H, 4-vinylic), and 6.18 (s, 1H, NH, disappeared on deuterium exchange) ppm.

IX. 3-BROMO-17a-AZA-D-HOMO-3,5-ANDROSTADIENE (510, MK-201)

The compound 3-oxo-17a-aza-D-homo-4-androstene (509, 2 g, 7 mmol) was dissolved in glacial acetic acid (25 ml) in a 100 ml round bottom flask and to this, phosphorous tribromide (2 ml, 21 mmol) was added dropwise while stirring. The reaction mixture was protected from light and kept on stirring for 24 hrs. To this 50 ml of water was added, precipitates filtered, dried, and crude product crystallized from ethyl acetate to obtain 3-bromo-17a-aza-D-homo-3,5-androstadiene (510, 1.8 g, 74%), m.p. 260-261 °C.

Analysis:

UV$_{\text{max}}$ (MeOH):  234 nm  
R$_1$ value: (CHCl$_3$: MeOH :: 9.8: 0.2)  0.65  
IR (KBr):  3421, 2946, 1613, 1457, 1426, 1389, and 641 cm$^{-1}$  
$^1$H NMR (CDCl$_3$):  δ 0.92 (s, 3H, 18-C$_3$H$_3$), 1.20 (s, 3H, 19-C$_3$H$_3$), 2.84 (t, 2H, 17-C$_3$H$_2$), 5.37 (t, 1H, 6-vinylic), 6.28 (s, 1H, 4-vinylic), and 7.61 (s,
Experimental

1H, NH, disappeared on deuterium exchange) ppm

\[ ^{13} \text{C NMR (CDCl}_3\text{):} \delta 16.3 \text{ (C-18), 18.68 (C-19), 39.42 (C-17), 60.96 (C-13), 123.0 (C-6), 124.14 (C-3), 130.35 (C-4), and 142.48 (C-5) ppm} \]

Mass (ESI): m/z 351.08 [M+2]^+ and 350.06 [M+1]^+.

X. 3-CYANO-17a-AZA-D-HOMO-3,5-ANDROSTADIENE (511, MK-202)

A stirred mixture of 3-bromo-17a-aza-D-homo-3,5-androstadiene (510, 5 g, 14 mmol), cuprous cyanide (1.3 g, 15 mmol) and dimethylformamide (DMF, 25 ml) was heated to reflux for 3.5 hrs. The reaction was cooled, quenched into a solution of ammonia solution (25 ml) and water (50 ml) with stirring. The suspension was extracted with chloroform (2 x 50 ml), combined extract filtered through a pad of celite, washed with aqueous ammonium hydroxide followed by water, dried, concentrated under vacuum, and the residue crystallized from ethanol to give 3-cyano-17a-aza-D-homo-3,5-androstadiene (511, 1.25 g, 30%), m.p. 270-272 °C.

Analysis:

UV\(_{\text{max}}\) (MeOH): 239 nm

R\(_f\) value: (CHCl\(_3\): MeOH :: 9.8: 0.2) 0.69

IR (KBr): 3361, 2932, 2852, 2201, 1633, and 1599 cm\(^{-1}\)

\[ ^{1} \text{H NMR (CDCl}_3\text{):} \delta 0.93 \text{ (s, 3H, 18-CH}_3\text{), 1.15 (s, 3H, 19-CH}_3\text{), 2.84 (t, 2H, 17-CH}_2\text{), 5.70 (t, 1H, 6-vinylic), 6.40 (s, 1H, 4-vinylic), and 7.48 (s, 1H, NH, disappeared on deuterium exchange) ppm} \]

\[ ^{13} \text{C NMR (CDCl}_3\text{):} \delta 16.4 \text{ (C-18), 19.36 (C-19), 41.20 (C-17), 60.42 (C-13), 103.18 (C-3), 120.14 (C=N), 124.0 (C-6), 129.16 (C-4), and 141.83 (C-5) ppm} \]

XI. 17a-METHYL-3-CYANO-17a-AZA-D-HOMO-3,5-ANDROSTADIENE (512, MK-203)

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17a-aza-D-homo-3,5-androstadiene (511, 0.5 g, 1.7 mmol) in dried tetrahydrofuran (THF, 50 ml). After 30 minutes of stirring, methyl iodide (0.25 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 24 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 x 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17a-methyl-3-cyano-17a-aza-D-homo-3, 5-androstadiene (512, 0.4 g, 77%), m.p. 260-262 °C.

Analysis:

UV max (MeOH): 230 nm
Rf value: (CHCl₃: MeOH :: 9: 1) 0.67
IR (KBr): 3366, 2934, 2204, and 1449 cm⁻¹
¹H NMR (CDCl₃): \[ \delta 0.93 \text{ (s, 3H, 18-CH₃)}, 1.20 \text{ (s, 3H, 19-CH₃)}, 2.84 \text{ (t, 2H, 17-CH₂)}, 3.30 \text{ (s, 3H, N-CH₃)}, 5.78 \text{ (t, 1H, 6-vinyl)}, \text{ and 6.66 (s, 1H, 4-vinyl) ppm} \]
¹³C NMR (CDCl₃): \[ \delta 14.29 \text{ (C-18), 19.58 (C-19), 34.57 (N-CH₃), 49.42 (C-17), 60.96 (C-13), 107.24 (C-3), 120.0 (C=N), 123.0 (C-6), 131.35 (C-4), and 142.48 (C-5) ppm} \]

XII. 17a-ETHYL-3-CYANO-17a-AZA-D-HOMO-3,5-ANDROSTADIENE (513, MK-204)

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17a-aza-D-homo-3,5-androstadiene (511, 0.5 g, 1.7 mmol) in dried THF (50 ml). After 30 min of stirring, bromoethane (0.3 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 48 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 x 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17a-ethyl-3-cyano-17a-aza-D-homo-3, 5-androstadiene (513, 0.4 g, 77%), m.p. 260-262 °C.
Experimental

Sulphuric acid, poured into water, extracted with chloroform (3 × 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17α-ethyl-3-cyano-17α-aza-D-homo-3,5-androstadiene (513, 0.2 g, 36%), m.p. 274-275 °C.

Analysis:

UV<sub>max</sub> (MeOH): 233 nm

R<sub>f</sub> value: (CHCl<sub>3</sub>: MeOH :: 9: 1) 0.64

IR (KBr): 3364, 2920, 2204, 1638, and 1450 cm<sup>-1</sup>

<sup>1</sup>H NMR (CDCl<sub>3</sub>):

δ 0.90 (s, 3H, 18-CH<sub>3</sub>), 1.22 (s, 3H, 19-CH<sub>3</sub>), 1.03 (t, 3H, NCH<sub>2</sub>CH<sub>3</sub>), 2.64 (q, 2H, N-CH<sub>2</sub>CH<sub>3</sub>), 5.77 (t, 1H, 6-vinylic), and 6.65 (s, 1H, 4-vinylic) ppm

<sup>13</sup>C NMR (CDCl<sub>3</sub>):

δ 12.4 (N-CH<sub>2</sub>CH<sub>3</sub>), 16.58 (C-18), 21.0 (C-19), 41.05 (N-CH<sub>2</sub>CH<sub>3</sub>), 47.6 (C-17), 52.5 (C-13), 106.74 (C-3), 120.32 (C=N), 123.04 (C-6), 132.48 (C-4), and 139.54 (C-5) ppm

Mass (ESI): m/z 325.19 [M+1]<sup>+</sup>

XIII. 17α-ALLYL-3-CYANO-17α-AZA-D-HOMO-3,5-ANDROSTADIENE (514, MK-205)

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17α-aza-D-homo-3,5-androstadiene (511, 0.5 g, 1.7 mmol) in dried THF (65 ml). After 30 min of stirring, allyl bromide (0.38 ml, 4 mmol) was added and the reaction mixture was kept on stirring for 48 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 × 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from ethanol to yield 17α-allyl-3-cyano-17α-aza-D-homo-3,5-androstadiene (514, 0.22 g, 36%), m.p. 280-282 °C.

Analysis:

UV<sub>max</sub> (MeOH): 231 nm

R<sub>f</sub> value: (CHCl<sub>3</sub>: MeOH :: 9: 1) 0.72
Experimental

IR (KBr): 3312, 2923, 2205, 1690, 1604, and 1446 cm⁻¹

¹H NMR (CDCl₃):
δ 0.92 (s, 3H, 18-CH₃), 1.19 (s, 3H, 19-CH₃), 3.74 (dd, 1H, N-H/CH), 4.33 (dd, 1H, N-H/HC), 5.07 (dd, 1H, N-CH₂CH=CH₂), 5.10 (dd, 1H, N-CH₂CH=CH₂), 5.87 (m, 1H, N-CH₂=CHH), 5.78 (t, 1H, 6-vinylic), and 6.67 (s, 1H, 4-vinylic) ppm

¹³C NMR (CDCl₃):
δ 19.98 (C-18), 21.38 (C-19), 48.12 (C-17), 52.0 (N-CH₂CHCH₂), 55.0 (C-13), 115.32 (N-CH₂CHCH₂), 108.21 (C-3), 120.03 (C≡N), 126.0 (C-6), 128.53 (N-CH₂CHCH₂), 131.54 (C-4), and 141.29 (C-5) ppm

Mass (ESI): m/z 337.21 [M+1]+.

XIV. 17a-BENZYL-3-CYANO-17a-AZA-D-HOMO-3,5-ANDROSTADIENE (515, MK-206)

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17a-aza-D-homo-3,5-androstadiene (511, 0.5 g, 1.7 mmol) in dried THF (65 ml). After 30 min of stirring, benzyl chloride (0.5 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 24 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 x 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from ethanol to yield 17a-benzyl-3-cyano-17a-aza-D-homo-3,5-androstadiene (515, 0.22 g, 35%), m.p. 263-264 °C.

Analysis:

UV max (MeOH): 234 nm
R₇ value: (CHCl₃: MeOH :: 9: 1) 0.61
IR (KBr): 3312, 2923, 2205, 1690, 1604, and 1446 cm⁻¹

¹H NMR (CDCl₃):
δ 0.92 (s, 3H, 18-CH₃), 1.19 (s, 3H, 19-CH₃), 4.26 (m, 1H, PhC/H-N), 5.0 (m, 1H, PhCHHH-N), 5.78 (t, 1H, 6-vinylic), 6.67 (s,
Experimental

1H, 4-vinylic), and 7.22-6.94 (5H, CH-aromatic) ppm

\[^{13}\text{C}\] NMR (CDCl\textsubscript{3}): \(\delta 18.28\) (C-18), 18.79 (C-19), 56.14 (C-17), 59.7 (NCH\textsubscript{2}Ph), 60.54 (C-13), 120.0 (C=N), 123.0 (C-6), 124.8, 127.9, 128.7 (CH aromatic), 127.25 (C-3), 130.54 (C-4), and 139.29 (C-5) ppm

Mass (ESI): \(\text{m/z 373.17 [M+1]}^+\).

XV. 17a-BUTYL-3-CYANO-17a-AZA-D-HOMO-3,5-ANDROSTADIENE (516, MK-207)

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17a-aza-D-homo-3,5-androstadiene (511, 0.5 g, 1.7 mmol) in dried THF (65 ml). After 30 min of stirring, butyl bromide (0.4 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 48 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 \times 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from ethanol to yield 17a-butyl-3-cyano-17a-aza-D-homo-3,5-androstadiene (516, 0.3 g, 50%), m.p. 271-273 °C.

Analysis:

UV\textsubscript{max} (MeOH): 230 nm

R\textsubscript{f} value: (CHCl\textsubscript{3}: MeOH :: 9: 1) 0.63

IR (KBr): 3312, 2923, 2205, 1628, 1604, 1538, and 1249 cm\textsuperscript{-1}

\[^{1}\text{H}\] NMR (CDCl\textsubscript{3}): \(\delta 0.92\) (s, 3H, 18-CH\textsubscript{3}), 0.92 (t, 3H, N-CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 1.19 (s, 3H, 19-CH\textsubscript{3}), 3.74 (t, 2H, N-CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 5.96 (t, 1H, 6-vinylic), and 6.76 (s, 1H, 4-vinylic) ppm

\[^{13}\text{C}\] NMR (CDCl\textsubscript{3}): \(\delta 12.61\) (N-CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 17.37 (C-18), 19.08 (N-CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 26.59 (C-19),
Experimental

33.96 (N-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 48.16 (C-17), 55.19 (N-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 108.25 (C-3), 119.26 (C≡N), 124.21 (C-6), 130.29 (C-4), and 140.58 (C-5) ppm

Mass (ESI): m/z 353.18 [M+H]<sup>+</sup>.

**XVI. 17a-ISOBUTYL-3-CYANO-17a-AZA-D-HOMO-3,5-ANDROSTADIENE (517, MK-208)**

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17a-aza-D-homo-3,5-androstadiene (511, 0.5 g, 1.7 mmol) in dried THF (65 ml). After 30 min of stirring, isobutyl bromide (0.4 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 24 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 × 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17a-isobutyl-3-cyano-17a-aza-D-homo-3,5-androstadiene (517, 0.25 g, 42%), m.p. 216-218 °C.

**Analysis:**

UV<sub>max</sub> (MeOH): 235 nm

R<sub>f</sub> value: (CHCl<sub>3</sub>: MeOH :: 9: 1) 0.63

IR (KBr): 3323, 2963, 2207, 1618, 1578, and 1256 cm<sup>-1</sup>

<sup>1</sup>H NMR (CDCl<sub>3</sub>):

δ 0.89 (m, 6H, NCHHCH(CH<sub>3</sub>)<sub>2</sub>), 0.93 (s, 3H, 18-CH<sub>3</sub>), 1.21 (s, 3H, 19-CH<sub>3</sub>), 1.70 (m, 1H, NCHHCH(CH<sub>3</sub>)<sub>2</sub>), 2.27 (dd, 1H, NCH/CH(CH<sub>3</sub>)<sub>2</sub>), 2.56 (dd, 1H, NCH/CH(CH<sub>3</sub>)<sub>2</sub>), 5.78 (t, 1H, 6-vinylc), and 6.67 (s, 1H, 4-vinylc) ppm

<sup>13</sup>C NMR (CDCl<sub>3</sub>):

δ 18.28 (C-18), 20.6 [NCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>], 25.79 (C-19), 26.2 [NCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>], 47.14 (C-17), 58.0 [NCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>], 59.2 (C-13), 107.25 (C-3), 121.35 (C≡N), 127.0 (C-6), 131.63 (C-4), and 141.29 (C-5) ppm
3.2.2. SYNTHESIS OF 17α-SUBSTITUTED 17α-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACIDS

I. 17α-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (518, MK-209)

3-Cyano-17α-aza-D-homo-3,5-androstadiene (511, 0.25 g, 0.8 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 × 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered, dried and crystallized from methanol to get 17α-aza-D-homo-3,5-androstadien-3-oic acid (518, 0.12 g, 46%), m.p. 261-263 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9.5: 0.5) 0.82

IR (KBr): 3460, 3320, 2924, 2858, 1721, 1618, 1572, and 1260 cm⁻¹

¹H NMR (CDCl₃): δ 0.83 (s, 3H, 18-CH₃), 1.09 (s, 3H, 19-CH₃), 5.70 (t, 1H, 6-vinylic), 6.99 (bs, 1H, 4-vinylic), 7.34 (1H, NH, disappeared on deuterium exchange), and 8.21 (1H, COOH, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃): δ 14.1 (C-18), 18.54 (C-19), 43.8 (C-17), 117.94 (C-3), 123.62 (C-6), 132.85 (C-4), and 177.85 (COOH) ppm

Mass (ESI): m/z 316.21 [M+1]^⁺.
II. 17a-METHYL-17a-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (519, MK-210)

17a-Methyl-3-cyano-17a-aza-D-homo-3,5-androstadiene (512, 0.25 g, 0.8 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 × 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered, dried and crystallized from methanol to get 17a-methyl-17a-aza-D-homo-3,5-androstadien-3-oic acid (519, 0.14 g, 53%), m.p. 278-279 °C.

Analysis:

Rf value: (CHC13: MeOH :: 9.5: 0.5) 0.73

IR (KBr): 3328, 2964, 2206, 1718, 1456, and 1260 cm⁻¹

¹H NMR (CDCl₃): δ 0.83 (s, 3H, 18-CH₃), 1.09 (s, 3H, 19-CH₃), 3.47 (s, 3H, N-CH₃), 5.7 (bs, 1H, 6-vinylic), 6.99 (bs, 1H, 4-vinylic), and 7.04 (1H, COOH, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃): δ 14.1 (C-18), 18.54 (C-19), 34.57 (N-CH₃), 43.8 (C-17), 61.38 (C-13), 107.94 (C-3), 123.62 (C-6), 141.23 (C-5), 132.85 (C-4), and 177.85 (COOH) ppm


III. 17a-ETHYL-17a-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (520, MK-211)

17a-Ethyl-3-cyano-17a-aza-D-homo-3,5-androstadiene (513, 0.25 g, 0.8 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was
Experimental

added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 × 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered, dried and crystallized from methanol to get 17α-ethyl-17α-aza-D-homo-3,5-androstadien-3-oic acid (520, 0.18 g, 66%), m.p. 281-283 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9.5: 0.5) 0.63

IR (KBr): 3321, 2924, 2858, 1715, 1456, and 1260 cm⁻¹

¹H NMR (CDCl₃):
δ 0.83 (s, 3H, 18-CH₃), 1.09 (s, 3H, 19-CH₃), 1.17 (q, 3H, NCH₂CH₃), 2.83 (q, 2H, N-CH₂CH₃), 5.92 (t, 1H, 6-vinylic), 6.73 (s, 1H, 4-vinylic), and 7.17 (1H, COOH, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃):
δ 13.24 (N-CH₂CH₃), 17.27 (C-18), 21.02 (C-19), 42.64 (N-CH₂CH₃), 48.62 (C-17), 51.53 (C-13), 107.74 (C-3), 121.30 (C-6), 133.24 (C-4), 141.39 (C-5), and 176.32 (COOH) ppm


IV. 17α-ALLYL-17α-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (521, MK-212)

17a-Allyl-3-cyano-17a-aza-D-homo-3,5-androstadiene (514, 0.25 g, 0.8 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 × 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was
filtered, dried and crystallized from methanol to get 17a-allyl-17a-aza-D-homo-3,5-androstadien-3-oic acid (521, 0.15 g, 57%), m.p. 269-270 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9.5: 0.5) 0.60
IR (KBr): 3326, 2924, 2858, 2206, 1718, 1693, 1456, and 1260 cm⁻¹

¹H NMR (CDCl₃): δ 0.93 (s, 3H, 18-CH₃), 1.21 (s, 3H, 19-CH₃), 3.81 (dd, 1H, N-H), 4.37 (dd, 1H, N-HHC), 5.21 (dd, 1H, N-CH₂CH=CHH), 5.17 (dd, 1H, N-CH₂CH=CHH), 5.61 (m, 1H, N-CH₂CH=CHH), 5.87 (t, 1H, 6-vinyllic), 6.72 (s, 1H, 4-vinyllic), and 7.02 (1H, COOH, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃): δ 20.17 (C-19), 22.72 (C-18), 48.21 (C-17), 53.60 (N-CH₂CHCH₂), 54.59 (C-13), 108.12 (C-3), 116.23 (N-CH₂CHCH₂), 127.02 (C-6), 129.27 (N-CH₂CHCH₂), 130.45 (C-4), 140.10 (C-5), and 178.75 (COOH) ppm


V. 17a-BENZYL-17a-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (522, MK-213)

17a-Benzyl-3-cyano-17a-aza-D-homo-3,5-androstadiene (515, 0.25 g, 0.7 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 × 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was
Experimental

filtered, dried and crystallized from methanol to get 17a-benzyl-17a-aza-D-homo-3,5-androstadien-3-oic acid (522, 0.15 g, 55%), m.p. 278-279 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9.5: 0.5) 0.81
IR (KBr): 3318, 2928, 2832, 2202, 1701, 1689, 1459, and 1261 cm⁻¹

¹H NMR (CDCl₃):
δ 0.91 (s, 3H, 18-CH₃), 1.21 (s, 3H, 19-CH₃), 4.62 (m, 1H, PhCH₂NH), 5.02 (m, 1H, PhCH₂NH), 5.83 (t, 1H, 6-vinylic), 6.72 (s, 1H, 4-vinylic), 7.23-6.83 (m, 5H, CH-aromatic), and 7.08 (1H, COO⁻H, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃):
δ 19.72 (C-18), 20.63 (C-19), 58.41 (C-17), 60.02 (NCH₂Ph), 61.72 (C-13), 126.71 (C-3), 125.1, 125.6, 128.2 (CH-aromatic), 129.63 (C-6), 140.94 (C-5), 131.96 (C-4), and 178.57 (COOH) ppm


VI. 17α-BUTYL-17α-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (523, MK-214)

17α-Butyl-3-Cyano-17α-aza-D-homo-3,5-androstadiene (516, 0.25 g, 0.7 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 × 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered, dried and crystallized from methanol to get 17α-buty1-17α-aza-D-homo-3,5-androstadien-3-oic acid (523, 0.12 g, 46%), m.p. 281-283 °C.
Analysis:

Rf value: (CHCl₃: MeOH :: 9.5: 0.5) 0.79

IR (KBr):
3320, 2926, 2292, 1708, 1631, 1539, and 1251 cm⁻¹

¹H NMR (CDCl₃):
δ 0.91 (s, 3H, 18-CH₃), 0.94 (t, 3H, N-CH₂CH₂CH₂CH₃), 1.09 (s, 3H, 19-CH₃), 3.62 (t, 2H, N-CH₂CH₂CH₂CH₃), 5.98 (t, 1H, 6-vinylic) and 6.67 (s, 1H, 4-vinylic), and 7.16 (1H, COOH, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃):
δ 12.61 (N-CH₂CH₂CH₂CH₃), 17.83 (C-18), 22.87 (N-CH₂CH₂CH₂CH₃), 26.63 (C-19), 34.01 (N-CH₂CH₂CH₂CH₃), 50.61 (C-17), 55.26 (N-CH₂CH₂CH₂CH₃), 109.62 (C-3), 125.12 (C-6), 131.92 (C-4), 141.85 (C-5), and 179.53 (COOH) ppm

Mass (ESI):
m/z 372.19 [M+1]^+. 

VII. 17a-ISOBUTYL-17a-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID 
(524, MK-215)

17a-Isobutyl-3-cyano-17a-aza-D-homo-3,5-androstadiene (517, 0.25 g, 0.7 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 x 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered, dried and crystallized from methanol to get 17a-isobutyl-17a-aza-D-homo-3,5-androstadien-3-oic acid (524, 0.12 g, 46%), m.p. 279-281 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9.5: 0.5) 0.81
IR (KBr): 3324, 2954, 2208, 1712, 1456, and 1261 cm⁻¹

¹H NMR (CDCl₃):
δ 0.90 [m, 6H, NCHHCH(CH₃)₂], 0.91 (s, 3H, 18-CH₃), 1.23 (s, 3H, 19-CH₃), 1.72 [m, 1H, NCHHCH(CH₃)₂], 2.25 [dd, 1H, NCH/CH(CH₃)₂], 2.62 [dd, 1H, NCH/CH(CH₃)₂], 5.87 (t, 1H, 6-vinylic), 6.36 (s, 1H, 4-vinylic), and 6.82 (1H, COOH, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃):
δ 19.82 (C-18), 20.41 [NCH₂CH(CH₃)₂], 26.93 (C-19), 27.32 [NCH₂CH(CH₃)₂], 48.41 (C-17), 57.42 (C-13), 59.02 [NCH₂CH(CH₃)₂], 109.21 (C-3), 127.03 (C-6), 132.17 (C-4), 140.31 (C-5), and 179.58 (COOH) ppm


3.2.3. SYNTHESIS OF 17-SUBSTITUTED 3-CYANO-17-AZA-D-HOMO-3,5-
ANDROSTADIEN-16,17α-DIONES

I. 17-OXO-5-ANDROSTEN-3-β-OL (525)

A solution of 17-oxo-5-androsten-3-β-yl acetate (504, 5 g, 15 mmol) in methanol (150 ml) containing potassium hydroxide (1 g, 18 mmol) was refluxed for 30 minutes. The resulting solution was concentrated and poured into ice-cold water (500 ml), acidified with glacial acetic acid, the precipitated material filtered, dried, and crystallized from methanol to yield 17-oxo-5-androsten-3-β-ol (525, 4.1 g, 95%), m.p. 138-140 °C (lit. 135-137 °C).³²²

Analysis:
Rₓ value: (CHCl₃; MeOH :: 9.8: 0.2) 0.87
IR (KBr): 3463, 2934, 1731, and 1635 cm⁻¹
¹H NMR (CDCl₃):
δ 0.9 (s, 3H, 18-CH₃), 1.16 (s, 3H, 19-CH₃), 3.43 (m, 1H, 3α-H), 3.60 (bs, 1H, OH,
disappeared on deuterium exchange), and 5.38 (t, 1H, 6-vinylic) ppm.

II. 16-OXIMINO-17-OXO-5-ANDROSTEN-3β-OL (526)

To a stirred solution of 17-oxo-5-androsten-3β-ol (525, 2 g, 7 mmol) in sodium tert-butoxide [prepared by dissolving sodium metal (1 g) in tert-butanol (17 ml)] was added isoamyl nitrite (1.8 ml, 14 mmol) at room temperature under nitrogen atmosphere. The reaction mixture, after being stirred overnight, was diluted with water (100 ml), acidified, the resulting yellow aqueous suspension extracted with chloroform (4 × 100 ml), combined chloroform extract washed with sodium bicarbonate solution (10% w/v), and extracted with sodium hydroxide solution (10% w/v, 4 × 100 ml). The combined alkaline extract was washed with chloroform (100 ml), acidified while cold, precipitated product filtered, washed, dried, and crystallized from methanol to afford 16-oximino-17-oxo-5-androsten-3β-ol (526, 1 g, 45%), m.p. 258-259 °C (lit 250-251 °C).323,324

Analysis:

\begin{align*}
R_f \text{ value: (CHCl}_3: \text{MeOH :: 9.8: 0.2)} & \quad 0.63 \\
\text{UV}_{\text{max}} \text{(MeOH):} & \quad 239 \text{ nm} \\
\text{IR (KBr):} & \quad 3378, 3198, 1730, 1631, 1455, 1300, 1143 \\
& \quad \text{and } 1045 \text{ cm}^{-1} \\
\text{^1H NMR (CDCl}_3): & \quad \delta 0.98 \text{ (s, 3H, 18-CH}_3), 1.13 \text{ (s, 3H, 19-CH}_3), 3.86 \text{ (bs, 1H, OH, disappeared on deuterium exchange), 5.40 (t, 1H, 6-vinylic),} \\
& \quad \text{and 11.53 (s, 1H, NOH, disappeared on deuterium exchange) ppm.}
\end{align*}

III. 16,17a-DIOXO-17-AZA-D-HOMO-5-ANDROSTEN-3β-YL ACETATE (527)

A mixture of 16-oximino-17-oxo-5-androsten-3β-ol (526, 1 g, 3.2 mmol), acetic anhydride (15 ml), and glacial acetic acid (10 ml) was refluxed for 18 hrs under anhydrous conditions. The solid residue, obtained after removing solvent under reduced pressure, was washed with petroleum ether (60-80 °C) and water. The material obtained was crystallized from ethanol to afford 16,17a-dioxo-17-aza-D-homo-5-androsten-3β-yl acetate (527, 0.45 g, 40%), m.p. 253-254 °C (lit 257-259 °C).324
Experimental

Analysis:
Rf value: (CHCl₃: MeOH :: 9.5: 0.5) 0.68
IR (KBr): 3384, 1725, 1713, 1693, 1442, 1370, 1260, and 1026 cm⁻¹
¹H NMR (CDCl₃): δ 1.01 (s, 3H, 18-CH₃), 1.19 (s, 3H, 19-CH₃), 2.03 (s, 3H, OOC-CH₃), 3.68 (m, 1H, 3α-H), 5.7 (t, 1H, 6-vinylic), and 8.47 (1H, NH, disappeared on deuterium exchange) ppm.

IV. 16,17a-DIOXO-17-AZA-D-HOMO-5-ANDROSTEN-3β-OL (528)
A mixture of 16,17a-dioxo-17-aza-D-homo-5-androsten-3β-yl acetate (527, 2 g, 5.6 mmol), potassium hydroxide (0.4 g) and methanol (100 ml) was refluxed for 30 min. After acidification with dilute hydrochloric acid, the reaction mixture was further refluxed for 15 min. Solvent removed, residue washed with water, dried, and crystallized from methanol to give 16,17a-dioxo-17-aza-D-homo-5-androsten-3β-ol (528, 1.1 g, 60%), m.p. 263-264 °C (lit. 258-260 °C).³²⁵

Analysis:
Rf value: (CHCl₃: MeOH :: 9.8: 0.2) 0.60
IR (KBr): 3433, 3327, 3158, 1714, 1687, 1375, 1291, and 1062 cm⁻¹
¹H NMR (CDCl₃/DMSO-d₆): δ 1.07 (s, 3H, 18-CH₃), 1.21 (s, 3H, 19-CH₃), 3.25 (m, 1H, 3α-H), 4.96 (br, 1H, OH disappeared on deuterium exchange), 5.38 (t, 1H, 6-vinylic), and 8.12 (1H, NH disappeared on deuterium exchange) ppm.

V. 17-AZA-D-HOMO-4-ANDROSTEN-3,16,17a-TRIONE (529)
16,17a-Dioxo-17-aza-D-homo-5-androsten-3β-ol (528, 2 g, 6.3 mmol) was dissolved in a mixture of cyclohexane (20 ml), toluene (100 ml) and dry dioxane (3 ml) and the solution was subjected to azeotropic distillation to remove traces of moisture. The distillation was continued at a slow rate, till the dropwise addition of a solution of
Experimental aluminum isopropoxide (2 g) in dry toluene (20 ml) was complete. The reaction mixture was refluxed for 3 hrs and then allowed to stand at room temperature for 12 hrs. The slurry was filtered and washed with toluene. The combined washings and filtrate were steam distilled until the complete removal of solvents was affected. The residual aqueous suspension was allowed to stand at room temperature to give a solid material which was recrystallized from aqueous ethanol to give 17-aza-D-homo-4-androsten-3,16,17a-trione (529, 1.2 g, 60%), m.p. 165-167 °C (lit 168-170 °C).325

Analysis:

Rf value: (CHCl3:MeOH::9.8:0.2) 0.60
IR (KBr): 3426, 3215, 2998, 1719, 1699, 1670, 1380, 1282, and 1112 cm⁻¹
¹H NMR (CDCl₃): δ 1.0 (s, 3H, 18-C/H₃), 1.23 (s, 3H, 19-C/H₃), 5.68 (s, 1H, 4-vinylic), and 8.54 (bs, 1H, OH disappeared on deuterium exchange) ppm.

VI. 3-BROMO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-16,17a-DIONE (530, MK-216)

The compound 17-aza-D-homo-4-androstene-3,16,17a-trione (529, 2 g, 6.3 mmol) was dissolved in glacial acetic acid (25 ml) in a 100 ml round bottom flask and to this, phosphorous tribromide (2 ml, 21 mmol) was added dropwise while stirring. The reaction mixture was protected from light and kept on stirring for 24 hrs. To this 50 ml of water was added, precipitates were filtered, dried, and crude product crystallized from ethyl acetate to obtain 3-bromo-17-aza-D-homo-3,5-androstadien-16,17-dione (530, 1.8 g, 78%), m.p. 281-283 °C.

Analysis:

UV max (MeOH): 240 nm
Rf value: (CHCl₃:MeOH :: 9.8:0.2) 0.63
IR (KBr): 3227, 2998, 1720, 1671, 1381, 1282, and 1110, and 653 cm⁻¹
¹H NMR (CDCl₃): δ 0.90 (s, 3H, 18-CH₃), 1.3 (s, 3H, 19-CH₃), 5.62 (t, 1H, 6-vinylic), 6.35 (s, 1H, 4-
Experimental

\[ \delta \ 19.3 \ (C-18), \ 21.83 \ (C-19), \ 27.62 \ (C-4), \ 124.04 \ (C-3), \ 124.12 \ (C-6), \ 140.84 \ (C-5), \ 160.21 \ (C-17a), \ and \ 173.26 \ (C-16) \ ppm \]


VII. 3-CYANO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-16,17a-DIONE (531, MK-217)

A stirred mixture of 3-bromo-17-aza-D-homo-3,5-androstadiene-16,17a-dione (531, 5 g, 13.3 mmol), cuprous cyanide (1.3 g, 15 mmol) and DMF (25 ml) was heated to reflux for 3.5 hrs. The reaction was cooled, quenched into a solution of ammonia solution (25 ml) and water (50 ml) with stirring. The suspension was extracted with chloroform (2 x 50 ml), combined extract filtered through a pad of celite, washed with aqueous ammonium hydroxide followed by water, dried, concentrated under vacuum, and the residue crystallized from ethanol to yield 3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (531, 1.6 g, 37%), m.p. 261-262 °C.

Analysis:

UV:\( \text{max} \): 232 nm

Rf value: (CHCl₃: MeOH :: 9.8: 0.2) 0.77

IR (KBr): 3425, 3184, 2983, 2257, 1721, 1674, 1357, 1282, and 1061 cm⁻¹

\(^1H\) NMR (CDCl₃):

\[ \delta \ 0.90 \ (s, \ 3H, \ 18-CH₃), \ 1.17 \ (s, \ 3H, \ 19-CH₃), \ 5.72 \ (t, \ 1H, \ 6-vinylic), \ 6.48 \ (s, \ 1H, \ 4-vinylic), \ 8.23 \ (bs, \ 1H, \ NH, \ disappeared \ on \ deuterium \ exchange) \ ppm \]

\(^{13}C\) NMR (CDCl₃):

\[ \delta \ 19.86 \ (C-18), \ 20.38 \ (C-19), \ 56.74 \ (C-13), \ 108.21 \ (C-3), \ 120.25 \ (C=N), \ 124.31 \ (C-6), \ 131.57 \ (C-4), \ 141.72 \ (C-5), \ 161.37 \ (C-17a), \ and \ 171.06 \ (C-16) \ ppm \]

Mass (ESI): m/z 325.08 [M+1]⁺.
VIII. 17-METHYL-3-CYANO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-16,17a-DIONE (532, MK-218)

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (531, 0.5 g, 1.5 mmol) in dry THF (50 ml). After 30 minutes of stirring, methyl iodide (0.25 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 24 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 × 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17-methyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (532, 0.3 g, 58%), m.p. 258-260 °C.

Analysis:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf value: (CHCl₃: MeOH :: 9: 1)</td>
<td>0.64</td>
</tr>
<tr>
<td>IR (KBr):</td>
<td>3425, 2934, 2206, 1718, 1662, 1637, and 1449 cm⁻¹</td>
</tr>
<tr>
<td>¹H NMR (CDCl₃):</td>
<td>δ 0.90 (s, 3H, 18-CH₃), 1.3 (s, 3H, 19-CH₃), 3.32 (s, 3H, N-CH₃), 5.78 (t, 1H, 6-vinyl), and 6.54 (s, 1H, 4-vinyl) ppm</td>
</tr>
<tr>
<td>¹³C NMR (CDCl₃):</td>
<td>δ 15.0 (C-18), 18.68 (C-19), 31.72 (N-CH₃), 61.36 (C-13), 108.17 (C-3), 119.28 (C≡N), 124.21 (C-6), 129.72 (C-4), and 141.92 (C-5) ppm</td>
</tr>
<tr>
<td>Mass (ESI):</td>
<td>m/z 339.07 [M+1]⁺</td>
</tr>
</tbody>
</table>

IX. 17-ETHYL-3-CYANO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-16,17a-DIONE (533, MK-219)

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (531, 0.5 g, 1.5 mmol) in dry THF (50 ml). After 30 minutes of stirring, bromoethane (0.3 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 24 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 × 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17-ethyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (533, 0.3 g, 58%), m.p. 258-260 °C.
Experimental

ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17-ethyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (533, 0.18 g, 33%), m.p. 273-275 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9: 1) 0.40

IR (KBr): 3264, 2920, 2206, 1723, 1690, and 1450 cm⁻¹

¹H NMR (CDCl₃):

δ 0.91 (s, 3H, 18-CH₃), 1.14 (t, 3H, NCH₂CH₃), 1.21 (s, 3H, 19-CH₃), 3.47 (q, 2H, N-CH₂CH₃), 5.64 (t, 1H, 6-vinyl), and 6.67 (d, 1H, 4-vinyl) ppm

¹³C NMR (CDCl₃):

δ 12.3 (N-CH₂CH₃), 17.83 (C-18), 21.1 (C-19), 36.15 (N-CH₂CH₃), 51.25 (C-13), 107.64 (C-3), 119.23 (CN), 124.30 (C-6), 131.24 (C-4), 140.63 (C-5), and 171.76 (C-16) ppm


X. 17-ALLYL-3-CYANO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-16,17a-DIONE (534, MK-220)

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (531, 0.5 g, 1.5 mmol) in dry THF (50 ml). After 30 minutes of stirring, allyl bromide (0.38 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 24 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 × 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17-allyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (534, 0.22 g, 41%), m.p. 279-280 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9: 1) 0.67
IR (KBr): 3432, 2913, 2205, 1714, 1691, 1614, and 1546 cm\(^{-1}\)

\(^1\)H NMR (CDCl\(_3\)): \(\delta\) 0.91 (s, 3H, 18-CH\(_3\)), 1.25 (s, 3H, 19-CH\(_3\)), 3.62 (dd, 1H, N-H/HC), 4.37 (dd, 1H, N-H/HC), 5.02 (dd, 1H, N-CH\(_2\)CH=CH/HC), 5.22 (dd, 1H, N-CH\(_2\)CH=CH/HC), 5.75 (t, 1H, 6-vinylic), 5.81 (m, 1H, N-CH\(_2\)CH=CH/HC), and 6.72 (s, 1H, 4-vinylic) ppm

\(^13\)C NMR (CDCl\(_3\)): \(\delta\) 16.83 (C-18), 19.73 (C-19), 40.37 (N-CH\(_2\)CHCH\(_2\)), 57.32 (C-13), 109.42 (C-3), 116.53 (N-CH\(_2\)CHCH\(_2\)), 121.03 (C=N), 124.60 (C-6), 128.82 (N-CH\(_2\)CHCH\(_2\)), 129.31 (C-4), 140.42 (C-5), 167.92 (C-17a), and 171.21 (C-16) ppm

Mass (ESI): m/z 365.10 [M+H]\(^+\).

XI. 17-BENZYL-3-CYANO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-16,17-DIONE (535, MK-221)

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (531, 0.5 g, 1.5 mmol) in dry THF (65 ml). After 30 minutes of stirring, benzyl chloride (0.5 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 24 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 x 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17-benzyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (535, 0.26 g, 48%), m.p. 271-272 °C.

Analysis:

R\(_f\) value: (CHCl\(_3\): MeOH :: 9: 1) 0.61

IR (KBr): 3416, 2936, 2208, 1710, 1667, 1605, and 1463 cm\(^{-1}\)
**Experimental**

$^1$H NMR (CDCl$_3$):  
δ 0.87 (s, 3H, 18-CH$_3$), 1.18 (s, 3H, 19-CH$_3$), 4.38 (dd, 1H, PhCH=H-N), 5.12 (dd, 1H, PhCH=H-N), 5.82 (t, 1H, 6-vinylic), 6.71 (s, 1H, 4-vinylic), and 7.42-6.98 (m, 5H, CH-aromatic) ppm

$^{13}$C NMR (CDCl$_3$):  
δ 18.21 (C-18), 21.09 (C-19), 47.34 (NCH$_2$Ph), 61.45 (C-13), 119.20 (C≡N), 121.21 (C-16), 123.71, 126.63, 129.82 (CH-aromatic), 124.21 (C-6), 128.91 (C-3), 129.73 (C-4), 141.85 (C-5), and 167.32 (C-17a) ppm

Mass (ESI):  
m/z 415.21 [M+1]$^+$.  

**XII. 17-BUTYL-3-CYANO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-16,17a-DIONE (536, MK-222)**

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (531, 0.5 g, 1.5 mmol) in dried THF (65 ml). After 30 minutes of stirring, butyl bromide (0.4 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 24 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 × 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17-butyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (536, 0.2 g, 37%), m.p. 269-270 °C.

**Analysis:**

R$_t$ value: (CHCl$_3$: MeOH :: 9: 1) 0.70

IR (KBr):  
3416, 2928, 2209, 1713, 1691, 1607, and 1253 cm$^{-1}$

$^1$H NMR (CDCl$_3$):  
δ 0.91 (s, 3H, 18-CH$_3$), 0.94 (t, 2H, N-CH$_2$CH$_2$CH$_2$CH$_3$), 1.20 (s, 3H, 19-CH$_3$), 3.91 (t, 2H, N-CH$_2$CH$_2$CH$_2$CH$_3$), 5.61 (t,
1H, 6-vinylic), and 6.72 (s, 1H, 4-vinylic) ppm

$^{13}$C NMR (CDCl$_3$):

δ 11.26 (N-CH$_2$CH$_2$CH$_2$CH$_3$), 18.46 (N-CH$_2$CH$_2$CH$_2$CH$_3$), 19.29 (C-18), 21.25 (C-19), 32.19 (N-CH$_2$CH$_2$CH$_2$CH$_3$), 54.71 (N-CH$_2$CH$_2$CH$_2$CH$_3$), 109.82 (C-3), 119.62 (C=N), 124.12 (C-6), 131.09 (C-4), 141.54 (C-5), 168.34 (C-17a), and 170.21 (C-16) ppm

Mass (ESI): m/z 381.09 [M+1]$^+$. 

XIII. 17-ISOBUTYL-3-CYANO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-16,17a-DIONE (537, MK-223)

Hexane washed sodium hydride (0.12 g, 5.1 mmol) was added to a stirred solution of 3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (531, 0.5 g, 1.5 mmol) in dry THF (50 ml). After 30 minutes of stirring, isobutyl bromide (0.4 ml, 4 mmol) was added dropwise and the reaction mixture was kept on stirring for 24 hrs. After completion of reaction excess of hydride was neutralized with methanol. The resulting solution was acidified with dilute sulphuric acid, poured into water, extracted with chloroform (3 × 50 ml), combined extract was washed, dried, solvent removed, and crude product crystallized from methanol to yield 17-isobutyl-3-cyano-17-aza-D-homo-3,5-androstadiene-16,17a-dione (537, 0.25 g, 46%), m.p. 275-276 °C.

Analysis:

R$_f$ value: (CHCl$_3$: MeOH :: 9: 1) 0.80

IR (KBr):

3391, 2962, 2202, 1716, 1695, 1603, and 1473 cm$^{-1}$

$^1$H NMR (CDCl$_3$):

δ 0.91 [m, 6H, NCH$_2$CH(CH$_3$)$_2$], 1.02 (s, 3H, 18-CH$_3$), 1.32 (s, 3H, 19-CH$_3$), 2.73 (dd, 1H, NCH$\equiv$CH(CH$_3$)$_2$), 2.31 (dd, 1H, NCH$\equiv$CH(CH$_3$)$_2$), 5.68 (t, 1H, 6-vinylic), and 6.86 (s, 1H, 4-vinylic) ppm
**3.2.4. SYNTHESIS OF 17-SUBSTITUTED 16,17a-DIOXO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACIDS**

**I. 17-AZA-16,17a-DIOXO-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (538, MK-224)**

3-Cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (531, 0.25 g, 0.8 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 × 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered, dried and crystallized from methanol to get 17-aza-16,17a-dioxo-D-homo-3,5-androstadien-3-oic acid (538, 0.19 g, 73%), m.p. 281-282 °C.

**Analysis:**

R_f value: (CHCl₃: MeOH :: 9.5: 0.5) 0.63

IR (KBr): 3427, 3349, 2943, 2858, 1721, 1690, 1456, 1364, and 1260 cm⁻¹

^1^H NMR (CDCl₃):

\[ \delta 0.91 \text{ (s, } 3\text{H, } 18-\text{CH}_3\text{)}, 1.30 \text{ (s, } 3\text{H, } 19-\text{CH}_3\text{)}, 5.8 \text{ (t, } 1\text{H, } 6\text{-vinyleic)}, 7.19 \text{ (s, } 1\text{H, } 4\text{-vinyleic)}, 7.38 \text{ (1H, } NH\text{, disappeared on deuterium exchange)}, \text{ and } 8.13 \text{ (1H, } COOH\text{, disappeared on deuterium exchange) ppm} \]
**Experimental**

$^{13}$C NMR (CDCl$_3$):

$\delta$ 18.21 (C-18), 19.48 (C-19), 52.67 (C-13), 107.82 (C-3), 126.43 (C-6), 130.62 (C-4), 140.71 (C-5), 160.96 (C-17a), 171.24 (C-16), and 176.98 (COOH) ppm


**II. 17-METHYL-16,17a-DIOXO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (539, MK-225)**

17-Methyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (532, 0.25 g, 0.7 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 × 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered, dried, and crystallized from methanol to get 17-methyl-16,17-dioxo-17-aza-D-homo-3,5-androstadien-3-oic acid (539, 0.18 g, 69%), m.p. 274-275 °C.

**Analysis:**

$R_f$ value: (CHCl$_3$; MeOH :: 9.5: 0.5) 0.70

IR (KBr): 3322, 2928, 2861, 1721, 1658, 1460, and 1262 cm$^{-1}$

$^1$H NMR (CDCl$_3$):

$\delta$ 0.89 (s, 3H, 18-CH$_3$), 1.10 (s, 3H, 19-CH$_3$), 3.67 (s, 3H, N-CH$_3$), 5.69 (t, 1H, 6-vinyl), 7.19 (s, 1H, 4-vinyl), and 8.17 (1H, COOH, disappeared on deuterium exchange) ppm

$^{13}$C NMR (CDCl$_3$):

$\delta$ 18.41 (C-18), 19.85 (C-19), 33.45 (N-CH$_3$), 107.94 (C-3), 128.36 (C-6), 131.28
Experimental

(C-4), 169.21 (C-17a), 170.82 (C-16), and 172.78 (COOH) ppm

Mass (ESI): m/z 358.07 [M+H]+.

III. 17-ETHYL-16,17a-DIOXO-17-aza-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (540, MK-226)

17-Ethyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (533, 0.25 g, 0.7 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 x 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered, dried and crystallized from methanol to get 17-ethyl-17-aza-16,17a-dioxo-D-homo-3,5-androstadien-3-oic acid (540, 0.14 g, 54%), m.p. 259-261 °C.

Analysis:

R_f value: (CHC13: MeOH :: 9.5: 0.5) 0.64

IR (KBr):

3318, 2919, 2854, 1715, 1690, 1452, and 1261 cm⁻¹

^1H NMR (CDCl₃):

δ 0.91 (s, 3H, 18-CH₃), 1.18 (t, 3H, NCH₂CH₃), 1.25 (s, 3H, 19-CH₃), 3.64 (q, 2H, N-CH₂CH₃), 5.67 (t, 1H, 6-vinylic), 6.98 (s, 1H, 4-vinylic), and 8.03 (s, 1H, COOH, disappeared on deuterium exchange) ppm

^13C NMR (CDCl₃):

δ 12.13 (N-CH₂CH₃), 18.13 (C-18), 20.15 (C-19), 36.71 (N-CH₂CH₃), 113.07 (C-3), 122.09 (C-6), 138.32 (C-4), 167.23 (C-17a), 169.01 (C-16), and 171.98 (COOH) ppm

IV. 17-ALLYL-16,17a-DIOXO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (541, MK-227)

17-Allyl-3-cyano-17-aza-D-homo-3,5-androstadiene-16,17a-dione (534, 0.25 g, 0.7 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 × 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered, dried and crystallized from methanol to get 17-allyl-16,17a-dioxo-17-aza-D-homo-3,5-androstadien-3-oic acid (541, 0.13 g, 50%), m.p. 254-257 °C.

Analysis:

Rf value: (CHCl3: MeOH :: 9.5: 0.5) 0.82

IR (KBr): 3321, 2928, 2849, 1719, 1691, 1458, and 1261 cm⁻¹

¹H NMR (CDCl₃):
δ 0.83 (s, 3H, 18-CH₃), 1.09 (s, 3H, 19-CH₃), 3.52 (dd, 1H, N-H/C), 4.29 (dd, 1H, N-CH₂C=CHH₂), 5.12 (dd, 1H, N-CH₂CH=CHH₂), 5.31 (dd, 1H, N-CH₂CH=CHH₂), 5.90 (m, 1H, N-CH₂CH=CHH₂), 5.69 (t, 1H, 6-vinylic), 7.01 (s, 1H, 4-vinylic), and 8.13 (s, 1H, COO⁻H, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃):
δ 16.17 (C-18), 19.85 (C-19), 51.19 (N-CH₂CHCH₂), 109.74 (C-3), 116.38 (C-16), 117.65 (N-CH₂CHCH₂), 128.71 (C-6), 128.82 (N-CH₂CHCH₂), 131.28 (C-4), 168.69 (C-17a), and 173.78 (COOH) ppm.

V. 17-BENZYL-16,17a-DIOXO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (542, MK-228)

17-Benzyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (535, 0.25 g, 0.6 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 x 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5°C. The precipitated compound was filtered, dried and crystallized from methanol to get 17-benzyl-16,17a-dioxo-17-aza-D-homo-3,5-androstadien-3-oic acid (542, 0.1 g, 38%), m.p. 262-263 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9.5: 0.5) 0.78
IR (KBr): 3325, 2942, 2859, 1721, 1691, 1450, and 1256 cm⁻¹

¹H NMR (CDCl₃): δ 0.83 (s, 3H, 18-CH₃), 1.09 (s, 3H, 19-CH₃), 4.48 (dd, 1H, PhCH=H-N), 5.13 (dd, 1H, PhCH=H-N), 5.86 (t, 1H, 6-vinylic), 6.92 (s, 1H, 4-vinylic) and 7.53-6.92 ppm (m, 5H, aromatic protons), and 8.29 (1H, COOH, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃): δ 17.31 (C-18), 19.85 (C-19), 46.63 (NCH₂Ph), 121.36 (C-6), 121.95 (C-3), 131.28 (C-4), 169.61 (C-17a), 172.22 (C-16), and 178.78 (COOH) ppm

Mass (ESI): m/z 434.27 [M+1]⁺.
VI. 17-BUTYL-16,17a-DIOXO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-3-OIC ACID (543, MK-229)

17-Butyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (536, 0.25 g, 0.7 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 x 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered and dried under vacuum to get 17-butyl-16,17a-dioxo-17-aza-D-homo-3,5-androstadien-3-oic acid (543, 0.15 g, 58%), m.p. 249-251 °C.

Analysis:

Rf value: (CHC13: MeOH :: 9.5: 0.5) 0.80
IR (KBr): 3324, 2938, 2861, 1718, 1688, 1462, and 1262 cm

$^1$H NMR (CDCl3): δ 0.92 (s, 3H, 18-CH3), 0.98 (t, 3H, N-CH2CH2CH2CH3), 1.3 (s, 3H, 19-CH3), 4.13 (t, 2H, N-CH2CH2CH2CH3), 5.62 (t, 1H, 6-vinyllic), 7.14 (s, 1H, 4-vinyllic), and 8.03 (1H, COOH, disappeared on deuterium exchange) ppm

$^{13}$C NMR (CDCl3): δ 12.30 (N-CH2CH2CH2CH3), 17.21 (C-18), 19.19 (N-CH2CH2CH2CH3), 20.15 (C-19), 33.60 (N-CH2CH2CH2CH3), 45.92 (N-CH2CH2CH2CH3), 106.97 (C-3), 121.63 (C-6), 136.32 (C-4), 168.83 (C-17a), 171.02 (C-16), and 174.78 (COOH) ppm

VII. 17-ISOBUTYL-16,17a-DIOXO-17-AZA-D-HOMO-3,5-ANDROSTADIEN-3-
OIC ACID (544, MK-230)

17-Isobutyl-3-cyano-17-aza-D-homo-3,5-androstadien-16,17a-dione (537, 0.25 g, 0.7 mmol) was dissolved in absolute ethanol (20 ml). To the solution, aqueous sodium hydroxide (2 ml, 50% w/v) was added and refluxed for 24 hrs. After completion of reaction it was cooled and to this a mixture of hydrochloric acid (5.0 ml, 50% v/v) and DCM (50 ml) was added with stirring. The aqueous phase pH was kept between 1.5-2.0. The aqueous layer was extracted with DCM (3 × 50 ml), combined organic layers washed with water, dried, solvent removed under vacuum to get a solid residue. Ethyl acetate (30 ml) was added to the residue, refluxed for 2 hrs, cooled and kept at 0-5 °C. The precipitated compound was filtered, dried and crystallized from methanol to get 17-isobutyl-16,17-dioxo-17-aza-D-homo-3,5-androstadien-3-oic acid (544, 0.10 g, 38%), m.p. 272-273 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9.5: 0.5) 0.60

IR (KBr): 3327, 2921, 2843, 1723, 1686, 1455, and 1261 cm⁻¹

¹H NMR (CDCl₃): δ 0.89 [m, 6H, NCH₂CH(CH₃)₂], 0.9 (s, 3H, 18-C₃H₃), 1.21 (s, 3H, 19-C₃H₃), 1.81 [m, 1H, NCH₂CH(CH₃)₂], 2.36 [dd, 1H, NCH(CH₃)CH₂], 2.83 [dd, 1H, NCH₂CH(CH₃)₂], 6.14 (t, 1H, 6-vinylc), 7.08 (s, 1H, 4-vinylc), and 8.07 (1H, COOH, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃): δ 18.76 (C-18), 21.28 (C-19), 21.31 [NCH₂CH(CH₃)₂], 25.82 [NCH₂CH(CH₃)₂], 50.27 [NCH₂CH(CH₃)₂], 118.45 (C-3), 128.36 (C-6), 131.28 (C-4), 160.85 (C-17a), 171.01 (C-16), and 178.75 (COOH) ppm

Mass (ESI): m/z 400.22 [M+1]⁺.
3.2.5. SYNTHESIS OF 5α-OXO-5-AZA-B-HOMO-3,5 SECO-4-NOR-CHOLESTAN-3-OIC ACID DERIVATIVES

I. CHOLEST-4-EN-3-ONE (546)

Cholesterol (545, 5 g, 13 mmol) and cyclohexanone (25 ml) were added to dry toluene (100 ml) from which 10 ml has been distilled off to remove traces of water as an azeotrope. As the distillation continued, a solution of aluminium isopropoxide (1.4 g) in dry toluene (20 ml) was added dropwise during 30 minutes. After distilling about 30 ml, the reaction mixture was refluxed for further 4 hrs. It was kept overnight and then filtered. The residue was washed with dry toluene (4 × 10 ml), combined filtrate and washings steam distilled, extracted with chloroform. The chloroform extract was dried, solvent removed, the residue crystallized from methanol to get cholest-4-en-3-one (546, 3.5 g, 70%), mp 76-78 °C (lit. 79-80 °C).326

Analysis:

UV max (MeOH): 239 nm
Rf value: (CHCl3: MeOH :: 9: 1) 0.71
IR (KBr): 2943, 1674, and 1614 cm⁻¹
¹H NMR (CDCl3): δ 0.83 (s, 3H, 18-CH₃), 0.96 (s, 3H, 19-CH₃), and 5.65 (t, 1H, 6-vinylic) ppm
¹³C NMR (CDCl3): δ 15.9 (C-18), 20.6 (C-19), 23.6 [(CH₃)₂], 123.6 (C-4), 171.82 (C-5), and 199.74 (C-3) ppm.

II. 5-OXO-3,5-SECO-4-NOR-CHOLESTAN-3-OIC ACID (547)

A solution of potassium carbonate (3.3 g) in water (65 ml) was added to a vigorously stirred solution of cholest-4-en-3-one (546, 4 g, 10 mmol) in t-butanol-water (9:1) azeotrope (250 ml). A solution of sodium metaperiodate (40 ml, 16 g in 200 ml water) and solution of potassium permanganate (0.8% w/v, 4 ml) were then added. The sodium metaperiodate solution was further added at a rate of 9 ml/min for the first 10 minutes and then 2-3 ml/min for the next 30 minutes. The permanganate solution was added whenever necessary to maintain the permanganate colour. The reaction mixture was stirred vigorously for 3 hrs. The excess of potassium permanganate was destroyed with sodium metabisulphite. The resulting iodine coloured solution was concentrated
under reduced pressure to remove t-butanol, cooled to room temperature, acidified with ice-cold sulphuric acid (50% v/v), and extracted with solvent ether (4 x 10 ml). The ether extract was washed with sodium metabisulphite solution (5% w/v, 2 x 20 ml) and then with water (2 x 10 ml), ether layer was extracted with sodium hydroxide (5% w/v), extract was acidified with dilute hydrochloric acid, and precipitates obtained were filtered, dried and recrystallized from acetone to obtain 5-oxo-3,5-seco-4-nor-cholestan-3-oic acid (547, 2.8 g, 70%), m.p. 150-152 °C (lit. 153-154 °C).327

Analysis:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Rf value (CHCl₃: MeOH :: 9: 1)</td>
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</tr>
<tr>
<td>IR (KBr)</td>
<td>3362, 2940, 1706, 1380, and 1298 cm⁻¹</td>
</tr>
<tr>
<td>¹H NMR (CDCl₃)</td>
<td>δ 0.64 (s, 3H, 18-CH₃), 0.79 [d, 6H, (CH₃)₂], 0.84 (d, 3H, 21-CH₃), 1.04 (s, 3H, 19-CH₃), and 8.17 (s, 1H, COOH, disappeared in deuterium exchange) ppm</td>
</tr>
<tr>
<td>¹³C NMR (CDCl₃)</td>
<td>δ 15.6 (C-18), 20.2 (C-19), 21.2 [(CH₃)₂], 178.8 (COOH), and 210.3 (C-5) ppm</td>
</tr>
</tbody>
</table>

III. METHYL 5-OXO-3,5-SECO-4-NOR-CHOLESTAN-3-OATE (548)

Sulphuric acid (0.5 ml) was added to a solution of 5-oxo-3,5-seco-4-nor-cholestan-3-oic acid (547, 1 g, 2.4 mmol) in methanol (5 ml). The reaction mixture was subjected to reflux for 3 hrs. The reaction mixture was then poured into ice-cold water (20 ml), extracted with chloroform, dried, and solvent removed to get the residue. Efforts were made to crystallize the material, but the compound 5-oxo-3,5-seco-4-nor-cholestan-3-oate (548) was obtained in viscous form (0.8 g, 80%).

Analysis:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf value (CHCl₃: MeOH :: 9: 1)</td>
<td>0.70</td>
</tr>
<tr>
<td>IR (KBr)</td>
<td>2943, 1737, 1706, 1257, and 1169 cm⁻¹</td>
</tr>
<tr>
<td>¹H NMR (CDCl₃)</td>
<td>δ 0.82 (s, 3H, 18-CH₃), 0.87 [d, 6H, (CH₃)₂], 0.91 (s, 3H, 21-CH₃), 1.11 (s, 3H, 19-CH₃), and 3.45 (s, 3H, OCOCH₃) ppm</td>
</tr>
</tbody>
</table>
IV. METHYL 5-OXIMINO-3,5-SECO-4-NOR-CHOLESTAN-3-OATE (549)

A solution of sodium acetate trihydrate (1.25 g, 15 mmol) and hydroxylamine hydrochloride (0.5 g, 7.2 mmol) in water (7 ml) was added to a refluxing solution of methyl 5-oxo-3,5-seco-4-nor-cholestan-3-oate (548, 0.5 g, 1.2 mmol) in ethanol (15 ml). After 4 hrs the refluxing solution was cooled and poured into ice-cold water, extracted with chloroform, dried, and solvent removed to obtain the residue. The oxime (549) obtained was in viscous form (0.4 g, 80%).

Analysis:

Rf value: (CHCl3: MeOH :: 9: 1) 0.80

IR (KBr): 3445, 2946, 1737, and 1172 cm⁻¹

1H NMR (CDCl3):
δ 0.89 (s, 3H, 18-CH₃), 0.90 [d, 6H, (CH₃)₂], 0.96 (s, 3H, 21-CH₃), 1.08 (s, 3H, 19-CH₃), 3.65 (s, 3H, COOCH₃), and 6.33 (bs, 1H, NOH, disappeared on deuterium exchange) ppm

13C NMR (CDCl₃):
δ 16.2 (C-18), 19.1 (C-19), 20.2 (C-21), 21.3 [(CH₃)₂], 51.55 (OCOCH₃), 174.49 (OCOCH₃), and 214.99 (C-5) ppm.

V. METHYL 5a-OXO-5-AZA-B-HOMO-3,5-SECO-4-NOR-CHOLESTAN-3-OATE (550)

A solution of thionyl chloride (0.25 ml) in dioxane (1.25 ml) was added dropwise to a stirred solution of methyl 5-oximino-3,5-seco-4-nor-cholestan-3-oate (549, 0.5 g, 1.1 mmol) in benzene (25 ml) at 10 °C. The temperature of the reaction mixture was maintained at 8-10 °C during the addition. The reaction mixture was allowed to stand with stirring at 20 °C for 45 minutes. Water (25 ml) was added followed by dilute ammonia solution (25 ml) till alkaline, benzene layer was separated and the aqueous layer extracted with chloroform (5 × 20 ml), combined organic layer washed with water, dried and solvent removed to get a residue which was crystallized from methanol to afford
Experimental

methyl 5a-oxo-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-oate (550, 0.37 g, 75%), m.p. 139-140 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9: 1) 0.62

IR (KBr): 3462, 2950, 1744, 1658, and 1176 cm⁻¹

¹H NMR (CDCl₃): δ 0.70 (s, 3H, 18-CH₃), 0.91 (s, 3H, 19-CH₃), 1.34 (s, 3H, C-21), 2.44 (m, 2H, 6-CH₂), 3.69 (s, 3H, OCOCH₃), and 5.81 (s, 1H, NH, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃): δ 14.5 (C-18), 19.1 (C-21), 21.4 (C-19), 22.9 [(CH₃)₂], 51.99 (OCOCH₃), 173.62 (OCOCH₃), and 178.27 (C-5a) ppm.

VI. 5a-OXO-5-AZA-B-HOMO-3,5-SECO-4-NOR-CHOLESTAN-3-OIC ACID (551)

Methyl 5a-oxo-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-oate (550, 1 g, 2.3 mmol) was dissolved in methanol (100 ml) and potassium hydroxide (0.4 g) was added and refluxed for 2-3 hrs. Reaction mixture was acidified with hydrochloric acid (10% w/v), poured into ice-cold water (200 ml), precipitates were filtered at pump, dried, and crystallized from methanol to obtain 5a-oxo-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-oic acid (551, 0.91 g, 88%), m.p. 163-164 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9: 1) 0.68

IR (KBr): 3476, 3329, 2944, 1715, 1656, and 1265 cm⁻¹

¹H NMR (CDCl₃): δ 0.82 (s, 3H, 18-CH₃), 1.16 (s, 3H, 19-CH₃), 1.38 (s, 3H, C-21), 2.31 (m, 2H, methylenic protons), 5.62 (s, 1H, NH, disappeared on deuterium exchange), and 7.97 (s, COOH, disappeared on deuterium exchange) ppm
VII. ETHYL 5α-OXO-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-oate (552, MK-231)

Sulphuric acid (0.5 ml) was added to a solution of 5α-oxo-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-oic acid (551, 0.1 g, 0.23 mmol) in methanol (25 ml). The reaction mixture was subjected to reflux for 3 hrs. The reaction mixture was then poured into ice-cold water (20 ml), extracted with chloroform, chloroform layer was washed with sodium carbonate (5% w/v), dried, and solvent removed to get the residue which on crystallization from methanol gave ethyl 5α-oxo-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-oate (552, 0.06 g, 56%), m.p. 189-191 °C.

Analysis:

1H NMR (CDCl3):
δ 0.80 (s, 3H, 18-CH3), 0.81 [d, 6H, (CH3)2], 1.07 (s, 3H, 19-CH3), 1.28 (3H, t, OCH2CH3), 1.46 (s, 3H, 21-CH3), 4.13 (m, 2H, CH2) 4.25 (2H, q, OCH2CH3), and 5.82 (s, 1H, NH, disappeared on deuterium exchange) ppm

13C NMR (CDCl3):
δ 14.9 (C-18), 15.6 (COOCH2CH3), 20.8 (C-21), 22.6 [(CH3)2], 60.8 (COOCH2CH3), 174.43 (COOCH2CH3), and 177.95 (C-5a) ppm

Mass (ESI): m/z 448.39 [M+1].

VIII. ISOPROPYL 5a-OXO-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-oate (553, MK-232)

Sulphuric acid (0.5 ml) was added to a solution of 5a-oxo-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-oic acid (551, 0.1 g, 0.23 mmol) in methanol (25 ml). The reaction mixture was subjected to reflux for 3 hrs. The reaction mixture was then poured...
into ice-cold water (20 ml), extracted with chloroform, chloroform layer was washed with sodium carbonate (5% w/v), dried, solvent removed to get the residue which on crystallization gave isopropyl 5α-oxo-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-oate (553, 0.05 g, 51%), m.p. 216-219 °C.

Analysis:

<table>
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<th>Rf value: (CHCl₃: MeOH :: 9: 1)</th>
<th>0.80</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR (KBr):</td>
<td>3459, 2947, 1716, 1680, and 1204 cm⁻¹</td>
</tr>
<tr>
<td>¹H NMR (CDCl₃):</td>
<td>δ 0.71 (s, 3H, 18-CH₃), 0.83 [d, 6H, (CH₃)₂], 1.01 (s, 3H, 19-CH₃), 1.23 [6H, d, OCH(CH₃)₂], 1.42 (s, 3H, 21-CH₃) 5.01 [1H, m, OCH(CH₃)₂], and 5.96 (s, 1H, NH, disappeared on deuterium exchange) ppm</td>
</tr>
<tr>
<td>¹³C NMR (CDCl₃):</td>
<td>δ 14.8 (C-18), 19.8 (C-21), 20.6 (C-19), 20.4 [COCH(CH₃)₂], 22.2 [(CH₃)₂], 69.40 [COCH(CH₃)₂], 177.84 (C-5a), and 173.99 [C-3] ppm</td>
</tr>
<tr>
<td>Mass (ESI):</td>
<td>m/z 462.41 [M+1]⁺</td>
</tr>
</tbody>
</table>

IX. 5-aza-B-homo-3,5-seco-4-nor-cholestan-3-ol (554, MK-233)

A suspension of methyl 5α-oxo-5-aza-B-homo-3,5-seco-4-nor-cholestan-3-oate (550, 0.5 g, 2.3 mmol) and lithium aluminium hydride (1 g) in dry diethyl ether (50 ml) was stirred under nitrogen for 72 hrs. The reaction flask was cooled with an ice-water bath and water (10 ml) was added carefully. The resulting mixture was refluxed for 20 min. and the warm suspension was filtered through a pad of celite. The celite was washed with hot chloroform, the filtrates were combined, solvents removed to give a residue which on crystallization from methanol gave 5-aza-B-homo-3,5-seco-4-nor-cholestan-3-ol (554, 0.16 g, 38%), m.p. 188-190 °C.

Analysis:

<table>
<thead>
<tr>
<th>Rf value: (CHCl₃: MeOH :: 9: 1)</th>
<th>0.80</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR (KBr):</td>
<td>3406, 3328, 2943, 1564, and 1224 cm⁻¹</td>
</tr>
</tbody>
</table>
Experimental

$^1$H NMR (CDCl$_3$):

$\delta$ 0.76 (s, 3H, 18-CH$_3$), 0.81 [d, 6H, CH$_3$)$_2$]
0.96 (s, 3H, 19-CH$_3$), 1.46 (s, 3H, 21-CH$_3$),
2.44-2.57 (m, 2H, 5a-CH$_2$), 3.61 (s, 1H, OH, disappeared on deuterium exchange), and
6.03 (s, 1H, NH, disappeared on deuterium exchange) ppm

$^{13}$C NMR (CDCl$_3$):

$\delta$ 14.6 (C-18), 19.3 (C-21), 20.8 (C-19), 22.5
[CH$_3$)$_2$], 41.43 (C-5a), and 65.89 (CH$_2$OH)
ppm

Mass (ESI):

m/z 392.58 [M+1]$^+$. 

3.2.6. SYNTHESIS OF 25(R)-5a-OXO-5-AZA-B-HOMO-3,5-SECO-4-NOR-
SPIROSTAN-3-OIC ACID DERIVATIVES

I. 25(R)-4-SPYROSTEN-3-ONE (555)

Diosgenin (551, 5 g, 12.5 mmol) and cyclohexanone (50 ml) were added to dry
toluene (80 ml). Traces of moisture were removed by azeotropic distillation of toluene
(20 ml). The distillation was continued at a slow rate while adding a solution of
aluminium isopropoxide (4 g) in dry toluene (40 ml) dropwise. The reaction mixture was
refluxed continuously for 4 hrs, cooled, filtered at the pump and the residue was washed
with dry toluene (40 ml). The combined filtrate and the washings were steam distilled
until the complete removal of organic solvents was affected. The reaction mixture was
then allowed to cool, extracted with chloroform, dried, solvent removed to get the
residue, which on crystallization from petroleum ether (40-60 °C) gave 25(R)-4-spirosten-
3-one (114, 3.5 g, 70%), m.p. 180-182 °C (lit. 186-188 °C).328

Analysis:

UV$_{\text{max}}$ (MeOH):

241 nm

R$_f$ value: (CHCl$_3$: MeOH :: 9: 1) 0.60

IR (KBr):

2940, 1670, 1450 1220, and 1060 cm$^{-1}$

$^1$H NMR (CDCl$_3$):

$\delta$ 0.71 (d, 3H, 27-CH$_3$), 0.81 (s, 3H, 18-
CH$_3$), 1.14 (s, 3H, 19-CH$_3$), 3.27-3.42 (m,
II. 25(R)-5-OXO-3,5-SECO-4-NOR-SPIROSTAN-3-OIC ACID (556)

A solution of potassium carbonate (2.8 g) in water (80 ml) was added to a vigorously stirred solution of 25(R)-4-spirosten-3-one (555, 5 g, 12 mmol) in t-butanol-water (9:1) azeotrope (300 ml). A solution of sodium metaperiodate (50 ml, 4% w/v) and potassium permanganate (5 ml, 0.8% w/v) were then added. The sodium metaperiodate solution was further added at a rate of 11 ml/min for first 10 minutes and then 2-3 ml/min for the next 30 minutes. The permanganate solution was added whenever necessary to maintain the permanganate colour. After 5 hrs the excess permanganate was destroyed with sodium metabisulphite and the resulting iodine coloured solution was concentrated under reduced pressure to 420 ml, cooled to 4 °C, acidified with ice-cold sulphuric acid (50%) and extracted with ether (3 × 20 ml). The ether extract was washed free of iodine with aqueous sodium metabisulphite (5% w/v) followed by water, ether layer was extracted with sodium hydroxide (5% w/v), extract was acidified with dilute hydrochloric acid, and precipitates were filtered, dried, and recrystallized from acetone to obtain 25(R)-5-oxo-3,5-seco-4-nor-spirostan-3-oic acid (556, 3.75 g, 75%), m.p. 208-210 °C (lit.³²⁹ 210-213 °C).

Analysis:

Rf value: (CHCl₃: MeOH :: 9: 1) 0.65
IR (KBr): 3361, 2942, 1715, 1379, and 1248 cm⁻¹

¹H NMR (CDCl₃):
δ 0.73 (d, 3H, 27-CH₃), 0.87 (s, 3H, 18-CH₃), 0.91 (d, 3H, 21-CH₃), 1.0 (s, 3H, 19-CH₃), 3.40 (m, 2H, 26-CH₂), 4.35 (m, 1H, 16-CH), and 8.36 (s, 1H, COOH, disappeared on deuterium exchange) ppm

³¹C NMR (CDCl₃):
δ 8.6 (C-16), 15.3 (C-21), 15.8 (C-18), 19.2 (C-19), 109.26 (C-22), 171.19 (C-5), and 199.54 (C-3) ppm.
Experimental

$^{13}$C NMR (CDCl$_3$):

δ 12.9 (C-16), 15.6 (C-18), 15.9 (C-21), 19.7 (C-19), 109.36 (C-22), 179.31 (C-3), and 209.6 (C-5) ppm.

III. METHYL (25R)-5-OXO-3,5-SECO-4-NOR-SPIROSTAN-3-OATE (557)

Sulphuric acid (0.5 ml) was added to a solution of 25(R)-5-oxo-3,5-seco-4-nor-spirostan-3-oic acid (556, 1 g, 2.3 mmol) in methanol (5 ml). The reaction mixture was subjected to reflux for 3 hrs. The reaction mixture was then poured into ice-cold water (20 ml), extracted with chloroform, dried, and solvent removed to get an off-white residue. The residue was recrystallized from methanol to obtain methyl 25(R)-5-oxo-3,5-seco-4-nor-spirosten-3-oate (557, 0.8 g, 77%), m.p. 88-90 °C.

Analysis:

R$_f$ value: (CHC$_3$: MeOH :: 9: 1) 0.72

IR (KBr): 2951, 1740, 1711, 1255, and 1176 cm$^{-1}$

$^1$H NMR (CDCl$_3$):

δ 0.73 (d, 3H, 27-CH$_3$), 0.77 (s, 3H, 18-CH$_3$), 0.91 (d, 3H, 21-CH$_3$), 1.07 (s, 3H, 19-CH$_3$), 3.39 (s, 3H, OCOCH$_3$), 3.41 (m, 2H, 26-CH$_2$), and 4.36 (m, 1H, 16-CH) ppm

$^{13}$C NMR (CDCl$_3$):

δ 17.1 (C-18), 19.6 (C-19), 20.4 (C-21), 51.59 (OCOCH$_3$), 174.41 (C-3), and 214.49 (C-5) ppm.

IV. METHYL 25(R)-5-OXIMINO-3,5-SECO-4-NOR-SPIROSTAN-3-OATE (558)

A solution of sodium acetate trihydrate (1.25 g, 15 mmol) and hydroxylamine hydrochloride (0.5 g, 7.2 mmol) in water (7 ml) was added to a refluxing solution of methyl 25(R)-5-oxo-3,5-seco-4-nor-spirostan-3-oate (557, 0.5 g, 1.1 mmol) in ethanol (15 ml). After 4 hrs the refluxing solution was cooled, separated crystals were filtered, washed with aqueous ethanol (30% v/v), dried, and recrystallized from methanol to give methyl 25(R)-5-oximino-3,5-seco-4-nor-spirostan-3-oate (558, 0.4 g, 76%), m.p. 184-185 °C.

Analysis:

R$_f$ value: (CHCl$_3$: MeOH :: 9: 1) 0.80
Experimental

IR (KBr): 3432, 2951, 1742, 1261, and 1171 cm$^{-1}$

$^1$H NMR (CDCl$_3$):  
\[ \delta 0.73 \text{ (d, 3H, 27-CH$_3$)}, 0.83 \text{ (s, 3H, 18-CH$_3$)}, 0.90 \text{ (d, 3H, 21-CH$_3$)}, 1.02 \text{ (s, 3H, 19-CH$_3$)}, 3.29 \text{ (m, 2H, 26-CH$_2$)}, 4.34 \text{ (m, 1H, 16-CH)}, 4.59 \text{ (s, 3H, OCOCH$_3$)}, \text{ and 7.4 (s, 1H, NOH, disappeared on deuterium exchange) ppm} \]

$^{13}$C NMR (CDCl$_3$):  
\[ \delta 2.9 \text{ (C-19), 15.8 (C-18), 21.2 (C-21), 51.55 (OCOCH$_3$), 163.7 (C-5a), and 174.8 (C-3) ppm} \]

V. METHYL 25($R$)-5a-OXO-5-AZA-B-HOMO-3,5-SECO-4-NOR-SPIROSTAN-3-OATE (559)

A solution of thionyl chloride (0.25 ml) in dioxane (1.25 ml) was added dropwise to a stirred solution of methyl 25($R$)-5-oximino-3,5-seco-4-nor-spirostan-3-oate (558, 0.5 g, 1.1 mmol) in benzene (25 ml) at 10 °C. The temperature of the reaction mixture was maintained at 8-10 °C during the addition. The reaction mixture was allowed to stand with stirring at 20 °C for 45 min. Water (25 ml) was added followed by dilute ammonia solution (25 ml) till alkaline. The benzene layer was separated and the aqueous layer extracted with chloroform (5 x 20 ml). The combined organic layer washed with water, dried, and solvent removed to get a residue which was crystallized from methanol to afford methyl 25($R$)-5a-oxo-5-aza-B-homo-3,5-seco-4-nor-spirostan-3-oate (559, 0.37 g, 73%), m.p. 152-154 °C.

Analysis:

\[ \text{Rf value: (CHCl$_3$: MeOH :: 9: 1) 0.68} \]

IR (KBr): 3459, 3293, 2951, 1743, 1658, 1243, and 1177 cm$^{-1}$

$^1$H NMR (CDCl$_3$):  
\[ \delta 0.81 \text{ (3H, 18-CH$_3$)}, 0.92 \text{ (s, 3H 19-CH$_3$)}, 0.96 \text{ (d, 3H, 21-CH$_3$)}, 1.35 \text{ s, 3H, 21-CH$_3$)}, 2.33 \text{ (m, 2H, 6-CH$_2$)}, 3.72 \text{ (s, 3H, OCOCH$_3$)}, 4.39 \text{ (m, 1H, 16-CH)}, \text{ and 5.78 (s, 1H, NH disappeared on deuterium exchange) ppm} \]
**VI. 25(R)-5a-Oxo-5-Aza-B-Homo-3,5-seco-4-Nor-Spirostan-3-Oic Acid (560)**

Methyl 25(R)-5a-oxo-5-aza-B-homo-3,5-seco-4-nor-spirostan-3-oate (559, 1 g, 2.1 mmol) was dissolved in methanol (100 ml) and potassium hydroxide (0.4 g) was added and refluxed for 2-3 hrs. Reaction mixture was acidified with hydrochloric acid (10% w/v), poured into ice-cold water (200 ml), precipitates were filtered at pump, dried and crystallized from methanol to obtain 25(R)-5a-oxo-5-aza-B-homo-3,5-seco-4-nor-spirostan-3-oic acid (560, 0.95 g, 91%), m.p. 195-196 °C.

**Analysis:**

- R\textsubscript{f} value: (CHCl\textsubscript{3}: MeOH :: 9: 1) 0.80
- IR (KBr): 3479, 3372, 2951, 1728, 1672, 1257, and 1174 cm\textsuperscript{-1}

\textsuperscript{1}H NMR (CDCl\textsubscript{3}):

\( \delta \) 0.71 (d, 3H, 27-CH\textsubscript{3}), 0.78 (s, 3H, 18-CH\textsubscript{3}), 1.07 (s, 3H, 21-CH\textsubscript{3}), 1.18 (s, 3H, 19-CH\textsubscript{3}), 4.30 (m, 1H, 16-CH), 5.41 (s, 1H, NH, disappeared on deuterium exchange), and 8.7 (s, 1H, COOH, disappeared on deuterium exchange) ppm

\textsuperscript{13}C NMR (CDCl\textsubscript{3}):

\( \delta \) 13.9 (C-18), 18.4 (C-27), 19.8 (C-21), 21.6 (C-19), 119.2 (C-22), 177.38 (C-5a), and 180.56 (C-3) ppm.

**VII. Ethyl 25(R)-5a-Oxo-5-Aza-B-Homo-3,5-seco-4-Nor-Spirostan-3-oate (561, MK-234)**

Sulphuric acid (0.5 ml) was added to a solution 25(R)-6-oxo-5-aza-B-homo-3,5-seco-4-nor-spirostan-3-oic acid (560, 0.1 g, 0.22 mmol) in methanol (25 ml). The reaction mixture was subjected to reflux for 3 hrs. The reaction mixture was then poured into ice-cold water (20 ml), extracted with chloroform, chloroform layer was washed with sodium carbonate (5% w/v), dried, and solvent removed to get the residue which on
Experimental crystallization from methanol gave ethyl 25(R)-5a-oxo-5-aza-B-homo-3,5-seco-4-nor-spirostan-3-oate (561, 0.05 g, 53%), m.p. 183-185 °C.

Analysis:

R_f value: (CHCl_3: MeOH :: 9: 1) 0.70
IR (KBr): 3432, 2926, 1735, 1656, 1208, and 1175 cm\(^{-1}\)
\(^1\)H NMR (CDCl_3): \(\delta\) 0.72 (d, 3H, 27-CH_3), 0.77 (s, 3H, 18-CH_3), 0.91 (s, 3H, 21-CH_3), 1.1 (s, 3H, 19-CH_3), 1.17 (t, 3H, OCH_2CH_3), 2.7 (m, 2H, CH_2), 4.18 (q, 2H, OCH_2CH_3), and 6.12 (s, 1H, NH, disappeared on deuterium exchange) ppm
\(^13\)C NMR (CDCl_3): \(\delta\) 13.4 (C-18), 15.9 (OCOCH_2CH_3), 18.5 (C-27), 19.3 (C-21), 21.7 (C-19), 60.5 (OCOCH_2CH_3), 119.6 (C-22), 173.95 (C-3), and 177.84 (C-5a) ppm

VIII. ISOPROPYL 25(R)-5a-OXO-5-AZA-B-HOMO-3,5-SECO-4-NOR- 
SPIROSTAN-3-OATE (562, MK-235)

Sulphuric acid (0.5 ml) was added to a solution of (25(R)-5a-oxo-5-aza-B-homo-3,5-seco-4-nor-spirostan-3-oic acid (560) (0.1 g, 0.22 mmol) in methanol (5 ml). The reaction mixture was subjected to reflux for 3 hrs. The reaction mixture was then poured into ice-cold water (20 ml), extracted with chloroform, chloroform layer was washed with sodium carbonate (5% w/v), dried, solvent removed to get the residue which on crystallization gave isopropyl 25(R)-5a-oxo-5-aza-B-homo-3,5-seco-4-nor-spirostan-3-oate (562, 0.64 g, 60%), m.p. 186-187 °C.

Analysis:

R_f value: (CHCl_3: MeOH :: 9: 1) 0.76
IR (KBr): 3439, 2926, 1738, 1660, and 1175 cm\(^{-1}\)
IX. 25(R)-5-AZA-B-HOMO-3,5-SECO-4-NOR-SPIROSTAN-3-OL (563, MK-236)

A suspension of methyl 25(R)-5α-oxo-5-aza-B-homo-3,5-seco-4-nor-spirostan-3-oate (559, 0.5 g, 2.2 mmol) and lithium aluminium hydride (1 g) in dry diethyl ether (50 ml) was stirred under nitrogen for 72 hrs. The reaction flask was cooled with an ice-water bath and water (10 ml) was added carefully. The resulting mixture was refluxed for 20 min and the warm suspension was filtered through a pad of celite. The celite was washed with hot chloroform, the filtrates were combined, and solvents removed to give a residue which on crystallization gave 25(R)-5-aza-B-homo-3,5-seco-4-nor-spirostan-3-ol (560, 0.13 g, 29%), m.p. 232-234 °C.

Analysis:

Rf value: (CHCl3: MeOH :: 9: 1) 0.78
IR (KBr): 3358, 3427, 2951, 1518, and 1244 cm⁻¹

δ 0.73 (s, 3H, 18-CH₃), 0.93 (s, 3H, 19-CH₃), 1.57 (s, 3H, 21-CH₃), 2.56 (m, 2H, 5α-CH₂) 4.31 (s, 1H, OH disappeared on deuterium exchange), and 6.12 (s, 1H, NH disappeared on deuterium exchange) ppm

3.2.7. SYNTHESIS OF MISCELLANEOUS ANDROSTENE DERIVATIVES

I. 17a-AZA-D-HOMO-ANDROST-4-EN-3,6-DIONE (564, MK-237)

Jones' reagent (0.5 ml, 0.267 mol/l) was added dropwise into a stirred solution of 17a-aza-D-homo-androst-4-ene-3β-ol (508, 0.3 g, 1 mmol) in acetone (20 ml). The mixture was stirred at room temperature for 1 hr and then neutralized with potassium carbonate solution (10% w/v). The suspension was filtered through a pad of celite, solvent removed, and residue crystallized using methanol to obtain 17a-aza-D-homo-androst-4-ene-3,6-dione (564, 0.2 g, 67%), m.p. 232-234 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9: 1) 0.68
IR (KBr): 3476, 3182, 2953, 1687, 1654, 1323, and 1053 cm⁻¹
¹H NMR (CDCl₃): δ 1.17 (s, 3H, 19-C₃H₃), 1.18 (s, 3H, 18-C₃H₃), 2.93 (d, 1H, C₇-CH), 6.72 (s, 1H, C₄-vinylic), and 7.01 (1H, s, NH, disappeared on deuterium exchange) ppm
¹³C NMR (CDCl₃): δ 17.24 (C-19), 21.62 (C-18), 46.53 (C-7), 125.29 (C-4), 160.21 (C-5), 199.80 (C-3), and 202.08 (C-6) ppm

II. 17a-AZA-D-HOMO-ANDROST-4-EN-3,6-DITHIOSEMICARBAZIDE (565, MK-238)

A mixture of 17a-aza-D-homo-androst-4-ene-3,6-dione (564, 0.3 g, 1 mmol), thiosemicarbazide (0.07 g, 2 mmol), and a few drops of glacial acetic acid in ethanol (20 ml, 95% v/v) was stirred at 60-70 °C for 2 hrs. After completion of the reaction, the majority of solvent was evaporated and some water was added to this solution. The mixture was extracted with DCM and the extract was washed with saturated brine, dried, and solvent evaporated. The resulting residue was crystallized from methanol to give 17a-aza-D-homo-androst-4-en-3,6-dithiosemicarbazide (565, 0.2 g, 45%), m.p. 265-266 °C.

Analysis:
Experimental

Rf value: (CHCl₃: MeOH :: 9: 1) 0.72

IR (KBr): 3436, 3342, 2939, 2860, 1629, 1582, 1472, 1371, 1224, and 1154 cm⁻¹

¹H NMR (CDCl₃): δ 0.02 (1H, bs, NNH), 0.9 (3H, s, 18-CH₃), 1.21 (3H, s, 19-CH₃), 6.65 (1H, s, 4-vinylc), 8.62 (1H, s, NH, disappeared on deuterium exchange), and 8.63 (1H, br s, NH₂, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃): δ 18.91 (C-18), 19.82 (C-19), 133.24 (C-4), 138.83 (C-5), 146.82 (C-6), 150.48 (C-3), 180.63 (CS), and 181.78 (CS) ppm

Mass (ESI): m/z 448.03 [M+1]⁺.

III. 3,16-DIOXIMINO-17a-AZA-D-HOMO-ANDROST-4-ENE (566, MK-239)

17a-Aza-D-homo-androst-4-ene-3,6-dione (564, 0.3 g, 1 mmol) was dissolved in 40 ml of ethanol (95% v/v). The mixture was heated to 60 °C, sodium acetate trihydrate (0.16 g, 1.2 mmol) and hydroxylamine hydrochloride (0.09 g, 1.3 mmol) added into the solution in 10 min. The mixture was stirred for 1 hr at 60 °C. The reaction was terminated and the majority of solvent was evaporated under reduced pressure. Water was added into the reaction mixture, and the product was extracted with ethyl acetate. The combined extract was washed with saturated brine, dried, and solvent evaporated under reduced pressure. The residue was crystallized from methanol to produce 3,6-dioximino-17a-aza-D-homo-androst-4-ene (566, 0.26 g, 80%), m.p. 220-222 °C.

Analysis:

Rf value: (CHCl₃: MeOH :: 9: 1) 0.68

IR (KBr): 3407, 3326, 2925, 2847, 1719, 1634, 1446, 1401, 1352, 1220, 1286, and 1061 cm⁻¹

¹H NMR (CDCl₃): δ 1.10 (3H, s, 19-CH₃), 1.3 (3H, s, 18-CH₃), 6.58 (1H, s, 3-NOH, disappeared on deuterium exchange), 6.84 (1H, s, 6-NOH, disappeared on deuterium exchange), 6.65
Experimental

(1H, s, 4-vinylic), and 8.24 (1H, s, NH, disappeared on deuterium exchange) ppm

$^{13}$C NMR (CDCl$_3$): δ 21.27 (C-18), 21.28 (C-19), 118.5 (C-4), 146.21 (C-5), 146.75 (C-6), and 154.89 (C-3) ppm


IV. **17 AZA-D-HOMO-ANDROST-4-ENE-3,6,16,17a-TETRAONE (567, MK-240)**

Jones’ reagent (0.5 ml, 0.267 mol/l) was added dropwise to the solution of 17-aza-D-homo-androst-4-ene-16,17a-dione (527, 0.3 g, 1 mmol) in acetone (20 ml). The mixture was stirred at room temperature for 1 hr and then neutralized with potassium carbonate solution (10% w/v). The suspension was filtered through a pad of celite, solvent removed, and residue crystallized using methanol to obtain 17-aza-D-homo-androst-4-ene 3,6,16,17a-tetraone (567, 0.2 g, 66%), m.p. 273-275 °C.

Analysis:

R$_f$ value: (CHCl$_3$: MeOH :: 9: 1) 0.62

IR (KBr): 3483, 3179, 2962, 1725, 1693, 1684, 1654, 1464, 1256, 1331, and 1057 cm$^{-1}$

$^1$H NMR (CDCl$_3$): δ 01.02 (3H, s, 19-CH$_3$), 1.3 (3H, s, 18-CH$_3$), 6.36 (1H, s, 4-vinylic), and 7.21 (1H, s, NH, disappeared on deuterium exchange) ppm

$^{13}$C NMR (CDCl$_3$): δ 19.74 (C-19), 19.82 (C-18), 158.21 (C-17a), 123.23 (C-4), 162.01 (C-5), 170.25 (C-16), 199.89 (C-3), and 206.29 (C-6) ppm

Mass (ESI): m/z 330.08 [M+H]$^+$.  

V. **17-aza-D-HOMO-ANDROST-4-EN-3,6-DITHIOSEMICARBAZIDE (568, MK-241)**

A mixture of 17-aza-D-homo-androst-4-en-3,6,16,17a-tetraone (567, 0.3 g, 1 mmol), thiosemicarbazide (0.07 g, 2 mmol), and a few drops of glacial acetic acid in ethanol (20 ml, 95% v/v) was stirred at 60-70 °C for 2 hrs. After completion of the
reaction, the majority of solvent was evaporated and some water was added to this
solution. The mixture was extracted with DCM and the extract was washed with saturated
brine, dried, and solvent evaporated. The resulting residue was crystallized from methanol
to give 17-aza-D-homo-androst-4-en-3,6-dithiosemicarbazide (568, 0.2 g, 46%), m.p. 223-225 °C.

Analysis:

Rf value: (CHCl3: MeOH :: 9: 1) 0.60

IR (KBr): 3425, 3342, 2939, 2860, 1727, 1638, 1463, 1225, and 1165 cm⁻¹

¹H NMR (CDCl₃):
δ 0.91 (3H, s, 19-CH₃), 1.19 (3H, s, 18-CH₃), 6.93 (1H, s, 4-vinyl), 8.48 (1H, s, NH, disappeared on deuterium exchange), 8.51 (1H, bs, NH₂, disappeared on deuterium exchange), and 9.12 (1H, br s, NNHC, disappeared on deuterium exchange) ppm

¹³C NMR (CDCl₃):
δ 19.89 (C-18), 19.92 (C-19), 134.12 (C-4), 137.83 (C-5), 145.21 (C-6), 154.04 (C-3), 155.68 (C-17a), 169.32 (C-16), 180.27 (CS), and 181.06 (CS) ppm


VI. 3,6-DIOXIMINO-17-AZA-D-HOMO-ANDROST-4-EN-16,17a-DIONE (569, MK-242)

17-Aza-D-homo-androst-4-en-3,6,16,17a-tetraone (567, 0.3 g, 1 mmol) was
dissolved in 40 ml ethanol (95% v/v). The mixture was heated to 60 °C, sodium acetate
trihydrate (0.16 g, 1.2 mmol) and hydroxylamine hydrochloride (0.09 g, 1.3 mmol) added
into the solution in 10 min. The mixture was stirred for 1 hr at 60 °C. The reaction was
terminated and the majority of solvent was evaporated under reduced pressure. Water was
added into the reaction mixture, and the product was extracted with ethyl acetate. The
combined extract was washed with saturated brine, dried, and evaporated under reduce
pressure. The residue was crystallized from methanol to obtain 3,6-dioximino-17-aza-D-
homo-androst-4-en-16,17a-dione (569, 0.25 g, 80%), m.p. 256-257 °C.
Experimental Analysis:

R_f value: (CHCl_3: MeOH :: 9: 1) 0.68

IR (KBr): 3418, 3182, 2967, 1715, 1698, 1681, 1459, 1261, 1329, and 1059 cm^{-1}

^1H NMR (CDC_3):  δ 1.02 (3H, s, 19-CH_3), 1.23 (3H, s, 18-CH_3), 6.41 (1H, s, 6-NOH, disappeared on deuterium exchange), 6.61 (1H, s, 3-NOH, disappeared on deuterium exchange), and 8.24 (1H, s, NH, disappeared on deuterium exchange) ppm

^1^C NMR (CDC_3):  δ 19.24 (C-18), 20.81 (C-19), 125.72 (C-4), 142.92 (C-5), 149.67 (C-6), 157.41 (C-3), 157.92 (C-17a), and 169.01 (C-16) ppm

Mass (ESI): m/z 360.09 [M+1]^+. 

3.3. ADME predictive studies

In order to get better correlation of in silico hits with actual biological activity, it is important to study the drug like behaviour of the active ligand by analyzing various pharmacokinetic parameters. Various physicochemically significant descriptors and pharmacokinetically relevant properties were predicted using QikProp, version 3.5, Schrödinger, LLC, New York, NY, 2012 of Schrödinger software. All the compounds were neutralized and minimized before being used by Qikprop. Using rational drug design approach, it would be extremely advantageous if information about the ADME properties of the studied molecules could be produced in the early stages of the drug discovery process. Once obtained, this information could be expected to help chemists to ameliorate the pharmacokinetic profile of the compounds.

3.4. Biological evaluation

3.4.1. In vitro inhibitory activity

HEK-I and HEK-II served cell lines were used as a source of 5AR isozymes. HEK-293 cells are human embryonic kidney cells which were made cultivatable for 5AR-1 and 5AR-2 when cDNAs encoding 5AR-1 and 5AR-2 were inserted into pRcCMV vector (Cytomegalovirus promoter of the eukaryotic expression vector) and expressed in
them. The work was performed at the laboratory of Prof R. W. Hartmann at Saarland University, Germany as collaboration.

I. Cell Culture

The adherent fibroblastoid HEK293 cell line was obtained from DSMZ, Braunschweig, Germany (DSM ACC 305) and maintained in Dulbecco's modified Eagle medium with 10% fetal calf serum, 0.25% sodium hydrogen carbonate, 100 units penicillin/ml, and 100 μg streptomycin/ml. The cells were grown in a humidified 95% O₂-5% CO₂ atmosphere at 37°C in 175 cm² tissue culture flasks. Every 3-4 days they were split at a ratio of 1:6. For transfection experiments cells were used at passage number 8-10. Tissue culture reagents were from c.c.pro (Neustadt/W., Germany), except G418 sulfate, which was from Calbiochem (Bad Soden, Germany).²⁹⁶

II. Construction of 5AR expression

a. Plasmids

The Not I-insert of the plasmid ph5α45 is a full length human cDNA encoding the 5AR-1 isoenzyme. It was inserted downstream of the Cytomegalovirus (CMV) promoter of the eucaryotic expression vector pRcCMV (Invitrogen, Groningen, Netherlands). This vector carries an additional neomycin resistance. The new construct which encoded human 5AR-1 was named pRcCMV-I and used for transfection.

The Sal I/Not I-insert of the plasmid pBS-76-1 correspond to the full length human cDNA encoding the 5AR-2 isoenzyme. It was first inserted by the SalI/NotI-sites into pUC21-vector and recleaved by Hind III and Xbal. By this strategy a 5'-Hind III-and a 3'-Xbal-site was added to the 5AR-2 encoding DNA fragment, by which it was inserted into the expression vector pRcCMV. The resulting plasmid (pRcCMV-II) was used for transfecting HEK293 cells.²⁹⁶

b. Transfection procedure

One day before the transfection experiment 1 x 10⁷ HEK293 cells were seeded in 100 mm culture dishes. By this procedure the culture is approximately 70% confluent on the day of transfection. The liposomal transfecting reagent Roti®-Fect was used for transfecting cells either with pRcCMV-I or pRcCMV-II following the manufacturers recommendations. The optimal DNA/reagent ratio was 10 μg plasmid and 20 μl Roti®-Fect reagent.
c. Selection of stable clones

Initially the concentration at which G418 sulfate inhibits the growth of untransfected HEK293 cells was determined. Therefore, varying concentrations of G418 sulfate (50 µg/ml-1000 µg/ml) were added to adherent HEK293 cells seeded in 24-well tissue culture plates at a density of 200,000 cells/well. After 6 days incubation at 37°C, viable cells were determined using the trypan blue exclusion test. At a dose of 400µg/ml G418 sulfate 50% of the cells were killed. Two days after transfection the growth medium was replaced by medium containing 500 µg/ml G418 sulfate. During the following 3 weeks of incubation untransfected cells subsequently died. To remove cell debris the medium was replaced every four days. Stable cell clones could be identified by phase contrast microscopy at the end of the second week. Single cell clones were picked and transferred into 60 mm culture plates for further analysis.

III. Preparation of solutions used in inhibitory assay

a. 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 x 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 20x compound solution 25µl is used in a total test volume of 500µl.

b. 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 1ml tris buffer. A 1:2:1 mixture of this regenerating system (NADP: glucose: glucose-6-P-dehydrogenase) was stored in the refrigerator and 50µl of it was used in the end assay.

c. Androstenedione

A concentrated stock solution of 1mM 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.

d. 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 labelled 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.

e. Standard

Finasteride, a clinically used drug was used as standard and was provided by Merck Sharp and Dohme (Rahway, USA).

IV. 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-C₈ was applied as stationary phase using prepacked columns (125 x 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.

V. 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 a 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.

### 3.4.2. In vivo inhibitory activity

Male wistar rats were used in the study in accordance with the protocol approved by the Institutional Animal Ethical Committee (IAEC) vide letter no IAEC/282 dated 30/08/2012 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. Finasteride was procured from Cipla, Mumbai while Dutasteride was procured from Dr Reddy’s Laboratories, Hyderabad. Testosterone was procured from Asg Biochem. Private Limited, New Delhi.

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 1 mg/kg was administered orally once daily for 14 consecutive days. All the test and reference compounds were suspended in 0.5% carboxymethylcellulose sodium salt (Himedia) solution. Rats were weighed and sacrificed by ether anesthesia on the 15th day after 24 hrs 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: Naive (animals receiving only vehicle i.e. 0.50 % CMC), others were being Finasteride (0.6 mg/kg ), Dutasteride ( 0.06 mg /kg ), testosterone (3mg/ kg ) and synthesized compounds (1mg/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 Dunnet’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.