Steroids elicit diverse biological actions via various functional groups located around the periphery of their rigid tetracyclic core. Nitrogen containing steroids including natural, semi-synthetic and synthetic compounds are an important type of steroids, all of which have been studied extensively. The incorporation of a heterocyclic ring or a heteroatom in the steroid backbone affects the chemical properties of a steroid and often results in useful alterations in its biological activities. The products obtained by introduction of heteroatom in the steroid nucleus are called nuclear heterosteroids. When heteroatom(s) forms a part of fused ring system, attached group or a side chain of the steroid nucleus then the products are known as extranuclear heterosteroids. Heterosteroids have the ability to regulate a variety of biological processes and thus are potential drug candidates for the treatment of a large number of diseases including breast cancer, prostate cancer, leukemia, autoimmune diseases and osteoporosis.

Development of hybrid structures, in which pharmacologically crucial structural elements from two molecules are combined to produce a non-identical twin drug, is a rational approach to obtain therapeutically useful molecules. The main focus of the current study is to design and develop synthetic strategies to produce new chemical hybrids of steroidal aromatase inhibitors such as formestane, exemestane and non-steroidal aromatase inhibitors, e.g., fadrozole. These compounds may show high specificity and increased potency as aromatase inhibitors. In view of the significance of azole moiety to inhibit P450 enzyme inhibitors including aromatase, we hypothesized that introducing imidazole moiety to the androstane nucleus might yield specific and potent P450 inhibitors. With this design, it may be possible to produce substrate like chemical entities, which not only interact with the steroid binding site of the enzyme, thus introducing high specificity, but also provide a ligand to the enzyme heme iron resulting in tight binding.

Synthesis and pharmacological activity of various new 16 and 17-substituted and D-ring modified steroidal derivatives have been reported in...
the present study. The investigations carried out have been discussed under the following heads:

1. Azole derived steroidal compounds
   1.1. C_{16}-imidazolyl substituted androstene derivatives
   1.2. C_{4}-hydroxy-C_{16}-imidazolyl substituted androstene derivatives
   1.3. C_{16}-triazolyl substituted androstene derivative
2. Formation of C_{16}, C_{17}-epoxy-C_{17}-imidazolyl androstene derivatives
3. Aldol condensation of dehydroepiandrosterone (DHA) with 4-(3-chloropropoxy)benzaldehyde and further modifications
4. Aldol condensation of dehydroepiandrosterone (DHA) with imidazole-2-carboxaldehyde and further modifications
5. Synthesis of androst-5-ene-[17,16-c] -1'H-pyrazoline-3\beta-ol derivatives
6. C_{6} and C_{16}-functionalized steroidal derivatives
7. Microwave assisted organic synthesis
8. Aromatase inhibitory activity

**1. Azole derived steroidal compounds**

   **1.1. C_{16}-imidazolyl substituted androstene derivatives**

   Literature survey indicates that structural modifications in steroidal A
   and D ring brings out noticeable changes in aromatase inhibitory potential of
   steroidal molecules and provides potent, easily obtainable and structurally
   simple aromatase inhibitors. Therefore imidazolyl-substituted D-ring
   modified steroidal derivatives containing a suitably positioned heteroatom
   capable of binding to cytochrome P450 enzymes have been synthesized.

   The synthetic route to the preparation of various new 16-substituted
   steroidal derivatives has been outlined in scheme 1. Bromination of
   dehydroepiandrosterone (DHA, 151) was carried out using cupric bromide in

   ![Scheme 1](image.png)

   dry methanol and dry toluene to afford 16\alpha-bromo-17-oxo-5-androsten-3\beta-ol
The configuration at position 16 has been assigned alpha in accordance with the earlier reports. The proton NMR spectrum exhibited a doublet at δ 5.38 (6-CH) indicating the intact C-5 olefin and a multiplet at 4.53–4.55 ppm (16β-H). Numazawa and Nagaoka have earlier observed that 16β- and 16α-protons in 16-bromosteroids are found at δ 4.46 and 4.14 ppm, respectively.

Scheme 1. Synthetic route to the formation of 16-imidazolyl substituted steroids

16α-Bromosteroid 152 was thermally fused with powdered imidazole, the obtained product processed and crystallized from acetone to afford 16β-(imidazol-1-yl)-17-oxo-5-androsten-3β-ol (153). Prominent vibrational bands at...
3303, 1744 cm\(^{-1}\) and singlets for imidazolyl protons at \(\delta\) 6.95 (5-CH), 7.12 (4-CH) and 7.70 ppm (2-CH) were observed in the infrared and nuclear magnetic resonance spectra of 16-imidazolyl substituted steroid 153. 16\(\alpha\)-H resonated as a multiplet at \(\delta\) 4.46-4.48 ppm. The downfield shift of 16\(\alpha\)-H proton may be attributed to substitution of bromo group with imidazole moiety, which produces more pronounced deshielding effects. The configuration of imidazolyl moiety at C\(_{16}\) has been assigned \(\beta\) on the basis of NMR spectral data.

In view of the literature reports that 3,17-diketosteroid with high degree of unsaturation in A-ring exhibit potent aromatase inhibitory activity,\(^{255}\) Oppenauer oxidation\(^{252}\) of 16\(\alpha\)-bromosteroid 152 was carried out using aluminium isopropoxide-cyclohexanone-toluene system. This resulted into formation of a mixture of 16\(\alpha\)-bromo and 16\(\beta\)-bromo isomers of \(\alpha,\beta\)-unsaturated ketosteroid 154. This \(\alpha,\beta\)-unsaturated steroid exhibited 16\(\alpha\)-proton triplet at \(\delta\) 4.11 and 16\(\beta\)-proton doublet at 4.56 in 6:4 area ratio, which together integrated for one proton and a 4-CH singlet at 5.76 ppm in the proton resonance spectrum. Such epimerization of 16\(\alpha\)-bromo-17-oxosteroids to the 16\(\beta\)-isomer in alkaline medium has previously been reported.\(^{253}\) Infrared bands for C=O stretching vibrations appeared at 1750 and 1669 cm\(^{-1}\).

16-Imidazolyl steroid 155 with 3-keto-4-ene system was synthesized by thermal fusion of 16\(\alpha\)-bromo analogue 154 with imidazole and the product was characterized from the appearance of proton resonance singlets of imidazolyl protons at \(\delta\) 6.95 (5-CH), 7.09 (4-CH), 7.66 ppm (2-CH) and a multiplet at \(\delta\) 4.46 ppm for the 16\(\alpha\)-H. The prominent IR vibrational bands for carbonyl stretch were found at 1748 and 1667 cm\(^{-1}\).

To allow irreversible binding of steroid to the aromatase enzyme, double bond was inserted between C\(_1\) and C\(_2\) by oxidation of compound 154 using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in refluxing dry dioxane. This afforded a mixture of 16\(\alpha\)-bromoandrosta-1,4-diene-3,17-dione (156a, 30%) and 16\(\beta\)-bromoandrosta-1,4-diene-3,17-dione (156b, 70%), which were separated using fractional crystallization. IR vibrational bands were observed...
at 1749 and 1655 cm\(^{-1}\) in case of both the isomers. Proton NMR spectra of 156a and 156b exhibited 16\(\beta\)-\(H\) and 16\(\alpha\)-\(H\) at \(\delta\) 4.5 and 4.1 ppm, respectively. The protons of 1-\(CH\), 2-\(CH\) and 4-\(CH\) were found at \(\delta\) 6.2, 7.04 and 6 ppm, respectively, in the \(^1\)H NMR spectra of both the isomers. The synthesis of compound 156 could also be achieved by direct DDQ oxidation of 3-hydroxy derivative 152, but it took 4 days to complete the reaction.

The isomeric mixture of 16-bromosteroid 156 was thermally fused with powdered imidazole, the product processed and crystallized from a mixture of ether and ethyl acetate to afford the target compound 16\(\beta\)-(imidazol-1-yl)-androsta-1,4-diene-3,17-dione (157). Vibrational bands at 1750 and 1657 cm\(^{-1}\) and a \(^1\)H NMR triplet at \(\delta\) 4.58 ppm (16\(\alpha\)-\(H\)) were observed. Protons of 1-\(CH\), 2-\(CH\) and 4-\(CH\) of A-ring of steroid nucleus were found at about \(\delta\) 6.14, 7.03 and 5.99, respectively and singlets for imidazolyl protons resonated at 6.92 (5-\(CH\)), 6.97 (4-\(CH\)) and 7.54 ppm (2-\(CH\)). \(^{13}\)C-NMR spectral analysis indicated the presence of two carbonyl carbons in the molecule due to the occurrence of downfield signals at \(\delta\) 180.98 and 207.17 ppm. Three tertiary C-1, C-2, C-4 and one quaternary olefinic carbons appeared in \(\delta\) 113-162 ppm region of \(^{13}\)C NMR spectrum. The configuration of imidazolyl moiety at C\(_{16}\) was again assigned \(\beta\) from the \(^1\)H NMR spectral data.

Repeated efforts to carry out Oppenauer oxidation of 3-hydroxy-5-ene derivative 153 for the synthesis of imidazolyl substituted 3-keto-4-ene steroid 155, as well as DDQ oxidation of 3-keto-4-ene steroid 155 to prepare target compound 157 remained unsuccessful. Therefore synthetic route, where fusion with imidazole is the last step, was adopted for the synthesis of target compounds as shown in scheme 1.
To study structure activity relationship in this particular series of compounds, further modifications of steroidal nucleus were also carried out. Treatment of 16β-imidazolyl steroid 153 with sodium borohydride in methanol at room temperature afforded 3β,17β-diol 158, which upon subsequent acetylation with acetic anhydride and dry pyridine in a steam bath yielded 3β,17β-diacetoxy derivative 159. 16β-(1imidazol-1-yl)-5-androstene-3β,17β-diol (158) revealed characteristic broad vibrational bands of intramolecular hydrogen bonded O-H at relatively lower frequency near 3259 cm⁻¹ and NMR signals as multiplets at δ 3.42-3.44 (3α-H) and 3.78-3.80 ppm (17α-H). Appearance of ¹H NMR singlets at δ 1.74 (17β-OCOCH₃) and 2.04 ppm (3β-OCOCH₃) and strong vibrational band for C=O stretch at a higher frequency 1732 cm⁻¹ confirmed the acetylation of the hydroxyl groups in compound 159.

1.2. C₄-hydroxy C₁₆-imidazolyl substituted androstene derivatives

Formestane (6) is a synthetic steroidal derivative with potent aromatase inhibitory activity. It binds irreversibly and inhibits the enzyme aromatase, thereby blocking the peripheral aromatization of androgenic precursors into estrogens. It is observed from the literature survey that the incorporation of the polar hydroxyl group at C-4 enhances aromatase inhibitory activity. Therefore investigator thought it worthwhile to synthesize some new 16-imidazolyl steroids having structural components of formestane in A ring of steroid skeleton. Epoxidation of 16-imidazolyl steroid 155 by treating with a mixture of hydrogen peroxide (30%) and sodium hydroxide (4M) in methanol at 0°C for 24 h produced a mixture of 4α,5α-epoxide 160a and 4β,5β-epoxide 160b as shown in scheme 2. The completion of reaction was monitored by thin...
layer chromatography (TLC). The oily residue obtained after processing the reaction mixture was used as such for further reaction.

Scheme 2. Synthesis of compound 160

Treatment of the intermediate epoxides mixture 160 with formic acid afforded the target 4-hydroxy-16α/β-(imidazol-1-yl)-4-androstene-3,17-dione (161), which was crystallized from a mixture of acetone and n-hexane. Prominent vibrational bands appeared at 3423 (O-H), 1739 (C=O) and 1668 cm⁻¹ (C=O) in the infrared spectrum of compound 161.

The ¹H-NMR spectrum of imidazolyl substituted α,β-unsaturated 4-hydroxy steroid displayed 16α-proton triplet at δ 4.38 and 16β-proton doublet at 4.89 ppm in 3:1 area ratio and both together integrated for one proton. A singlet appeared at 5.70 (OH), which disappeared on deuterium exchange. Split singlets appeared for imidazolyl protons at δ 6.72 (s) and 6.89 (s) (1:3 area ratio, 1H, 5-CH), 7.03 (4-CH) and 7.43 (s) and 7.53 ppm (s) (1:3 area ratio, 1H, 2-CH) confirming the presence of isomeric mixture in compound 161. Repeated efforts to separate the two isomers by fractional crystallization remained unsuccessful.

1.3. C₁₅-triazolyl substituted androstene derivative

Several nonsteroidal aromatase inhibitors such as anastrazole (8) and
letrozole (9) containing a triazole ring have been successfully developed as potent aromatase inhibitors.257 A higher degree of enzyme specificity has been reached with the new generation of triazole derivatives. By combining the structural features of anastrozole with steroidal structure, triazolyl substituted androstene derivative was synthesized to enhance the specificity.

16β-(1,2,4-Triazol-1-yl)-17-oxo-5-androsten-3β-ol (162) was prepared by alkylation of 1,2,4-triazole with 16α-bromosteroid 152 in refluxing ethyl methyl ketone in the presence of anhydrous potassium carbonate. To improve upon the yield, thermal fusion method was avoided and less drastic reaction conditions were adopted. This resulted into the formation of desired 16β-triazolyl substituted steroid 162 with slightly improved product yield. A downfield triplet at δ 5.07 was observed for 16α-H. Triazolyl protons resonated as singlets at δ 7.92 (5-CH) and 8.49 ppm (3-CH). Highly deshielded 17-carbonyl carbon due to presence of adjacent triazole ring appeared at δ 211.81 ppm in 13C-NMR spectrum. Triazolyl carbons exhibited signals at δ 141.22 and 151.56 ppm. The configuration of triazolyl moiety at C16 was assigned β from the 1H NMR spectral data.

2. Formation of C16,C17-epoxy-17β-imidazolyl androstene derivatives

It was observed that thermal fusion method for the preparation of 16-imidazolyl derivatives results in lower yields of the products. Therefore an alternative method, in which 16α-bromosteroid 152 was treated with imidazole in refluxing ethyl methyl ketone in the presence of anhydrous potassium carbonate was tried. Interestingly, this led to the formation of 16α,17α-epoxy-17β-imidazolyl androstene derivative 163 as outlined in scheme 3. The product exhibited characteristic IR vibrational bands for O-H stretchings at 3235 cm⁻¹. Singlets at δ 4.05 (16β-H) and for imidazolyl protons at 7.01 (5-
7.15 (4-CH) and 7.96 ppm (2-CH) were observed in the ¹H-NMR spectrum of 163. Imidazolyl carbons exhibited downfield signals at δ 118.73, 128.63 and 137.22 ppm in ¹³C-NMR spectrum.

Similar treatment of 16α/β-bromosteroid 154 with imidazole in refluxing ethyl methyl ketone in the presence of anhydrous potassium carbonate afforded 17β-imidazolyl steroidal derivative 164. Infrared vibrational bands at 1648 cm⁻¹ for α,β-unsaturated ketone were observed.

A slight upfield shift was observed for 16β-H, which appeared as a singlet at δ 3.93 ppm. Singlets for imidazolyl protons at δ 7.05 (5-CH), 7.08 (4-CH) and 7.60 ppm (2-CH) were also observed. The structure of compound 164 was further confirmed from the X-ray crystallographic studies (figure 8).

Scheme 3. Synthesis of 16α,17α-epoxy-17β-imidazolyl androstenes 163 and 164

Figure 8. A view of the crystal structure of compound 164
Mechanism

The proposed mechanism involved in the formation of 16α,17α-epoxy-17β-(imidazol-1-yl)-4-androstene-3-one (164) is depicted in figure 9. The nucleophilic attack of imidazole to carbonyl carbon at 17 position resulted in the formation of tetrahedral intermediate A. The formation of A is in equilibrium with reactants. Oxy anion of A attacks carbon bearing bromo group to form an epoxide with a loss of bromide ion. Ultimately loss of one proton from the intermediate B results in to the formation of compound 164. The loss of the bromide and proton forms hydrogen bromide, which probably acts as a driving force of the reaction.
3. Aldol condensation of dehydroepiandrosterone (DHA) with 4-(3-chloropropoxy)benzaldehyde and further modifications

16-Substituted steroids have shown diversified pharmacological activities and are of interest for a medicinal chemist to develop new molecules. Many potent steroidal derivatives with substitution at position 16 have been described in the literature. Some interesting 16E-arylidene steroids have recently been reported from our laboratory as strong in vitro inhibitors of the growth of many types of human tumor cells and also as aromatase inhibitors.

Synthesis and aromatase inhibitory activity of a new series of 2-benzylidene indanones is also recently reported by our research group. The vanilloid-based derivative exhibited maximum inhibition of human placental aromatase and was found to be 54 times more potent as compared to aminoglutethimide.

These observations motivated us to continue the exploration of biological properties of 16E-arylidene steroidal derivatives by designing and synthesizing some new steroidal analogues.

Alkylation of 4-hydroxybenzaldehyde with 3-bromo-1-chloropropane in refluxing ethyl methyl ketone in the presence of anhydrous potassium carbonate afforded 4-(3-chloropropoxy)benzaldehyde (165). The completion of reaction was monitored by thin layer chromatography. The oily residue obtained after processing the reaction mixture was used as such for further reaction.

16-[4-(3-Chloropropoxy)benzylidene]-7-oxo-5-androsten-3β-ol (166) was prepared by base catalyzed aldol condensation of dehydroepiandrosterone (DHA, 151) with substituted aromatic aldehyde 165 at room temperature as shown in scheme 4. Infrared spectrum showed vibrational bands at 3419 and 1710 cm\(^{-1}\) for hydroxyl and carbonyl stretching vibrations. \(^1\)H NMR spectrum exhibited triplets, integrating for two protons, at \(\delta\) 3.78 \(-\text{CH}_2\text{Cl}\) and at 4.19 ppm for \(-\text{OCH}_2\). The vinylic proton of 16-arylidene appeared at \(\delta\) 7.42 ppm as a singlet. Aromatic protons appeared as doublets with those ortho to alkoxy slightly upfield in comparison to meta protons. The
configuration at C\textsubscript{16} is assigned \textit{E} with respect to the keto group at C\textsubscript{17} in analogy with the earlier reports.\textsuperscript{261,262}

\[
\begin{align*}
\text{(151)} & + \\
& \text{(165)}
\end{align*}
\]

\[
\begin{align*}
\text{CHO} \\
\text{OCH}_2\text{CH}_2\text{CH}_2\text{Cl} \\
\text{OCH}_2\text{CH}_2\text{CH}_2\text{Cl}
\end{align*}
\]

\[
\begin{align*}
\text{(166)}
\end{align*}
\]

\textbf{Scheme 4. Synthesis of 16-benzylidene derivative 166}

Thermal fusion of aldol product 166 with powdered imidazole and recrystallization using ethyl acetate and petroleum ether afforded the desired compound 16-\{4-\text{[3-(imidazol-1-yl)propoxy]benzylidene]-17-oxo-5-androsten-3\}-ol (167). A triplet at $\delta$ 3.94 for -$\text{CH}_2\text{N}<$ and imidazolyl protons at 6.92 (5-$\text{CH}$), 7.06 (2-$\text{CH}$) and 7.49 ppm (4-$\text{CH}$) appeared in the $^1\text{H}$ NMR spectrum. The infrared spectrum exhibited characteristic vibrational bands at 3219 (O-H) and 1710 cm$^{-1}$ (C=O).
Oppenauer oxidation\textsuperscript{252} of the chloropropoxy benzylidene 166 in aluminium isopropoxide-cyclohexanone-toluene system afforded the 4-ene-3,17-dione derivative 168.

![Figure 1](https://example.com/image1.png)

The methine-bridged proton at C\textsubscript{16} appeared at $\delta$ 7.41 and 4-CH proton singlet appeared downfield at $\delta$ 5.76 ppm in the proton NMR spectrum of compound 168. Methylene protons attached to chloro and oxygen were present as triplets at $\delta$ 3.76 and 4.16 ppm, respectively. IR spectrum exhibited prominent peaks at 1712 and 1671 cm\textsuperscript{-1} for carbonyl stretching vibrations.

Thermal fusion of Oppenauer product 168 with powdered imidazole and recrystallization using ethyl acetate and diethyl ether afforded the desired compound 16-[4-{3-(imidazol-1-yl)propoxy}benzylidene]-4-androstene-3,17-dione (169). Signals for protons of imidazole ring were observed at $\delta$ 6.95 (5-CH), 7.11 (4-CH) and 7.65 ppm (2-CH) in the proton nuclear magnetic resonance spectrum. Attempts were also made to prepare the target imidazolyl steroid 169 by Oppenauer oxidation of the 3-hydroxy analogue 167, however impure product was obtained every time.

4. Aldol condensation of dehydroepiandrosterone (DHA) with imidazole-2-carboxaldehyde and further modifications

A series of 2-(4-pyridylmethylene) and 2-(4-pyridylmethyl)-1-indanones with hydroxy or methoxy substituents at 4 and 5 positions have been
synthesized and evaluated for aromatase inhibitory activity. It was found that most of the azolyl substituted indanone derivatives were more potent as compared to aminogluthethimide and were also more selective towards aromatase.

Figure 10 - Structural features of steroidal and non-steroidal aromatase inhibitors

Therefore introduction of these structural features (figure 10) in steroidal moiety was considered as a rational approach to synthesize potent aromatase inhibitors. Aldol condensation of dehydroepiandrosterone (151) with imidazole-2-carboxaldehyde (170) in methanol at room temperature gave

Scheme 5. Synthesis of 16-imidazolylidene steroids 171 and 172
16-[(1H-imidazol-2-yl)methylene]-17-oxo-5-androsten-3β-ol (171) as depicted in scheme 5. The formation of aldol product 171 was confirmed by various spectral analysis. The $^1$H-NMR spectrum exhibited characteristic signals at $\delta$ 7.22 ppm for vinylic proton of imidazolylidene ring. The configuration at C$_{16}$ is assigned E with respect to the keto group at C$_{17}$ in analogy with the earlier reports.$^{261,262}$ Oppenauer oxidation$^{252}$ of compound 171 using cyclohexanone-toluene-aluminium isopropoxide system afforded α,β-unsaturated ketone 172 (scheme 5), which displayed C=O stretchings at 1712 cm$^{-1}$ in infrared spectrum. The $^1$H-NMR signal for 4-CH appeared as a clear downfield singlet at $\delta$ 5.75 ppm.

5. Synthesis of androst-5-ene[17,16-c]-1H-pyrazoline-3β-ol derivatives

Pyrazolines form an interesting group of compounds, many of which possess wide-spread pharmacological properties such as antitumor, analgesic, antipyretic and antidepressant actions.$^{263}$ In view of these observations, efforts were made to synthesize some new steroidal derivatives containing a pyrazoline ring fused at 16 and 17 position of steroidal structure (Scheme 6). Base catalysed aldol condensation$^{260}$ of dehydropiandrosterone (151) with 4-methoxybenzaldehyde (173) in methanol at room temperature gave 16E-[(4-methoxybenzylidene)-17-oxo-5-androsten-3β-ol (176). Pyrazoline derivative 179 was obtained by direct condensation of benzylidene 176 with hydrazine hydrate in refluxing methanol. The structure of compound 179 was confirmed by using various spectral analysis. Vibrational bands were present at 3429 (O-H), 1652 (C=N) and 1610 cm$^{-1}$ (C=C) in infrared spectrum. A triplet of doublets was observed at $\delta$ 3.37 for 16-CH due to its coupling with adjacent pyrazoline proton and 15-CH$_2$ protons. Pyrazoline proton appeared downfield at $\delta$ 4.38 ppm in the $^1$H NMR spectrum.

16E-Pyridylmethylene steroidal derivatives 177 and 178 were obtained by aldol condensation of dehydropiandrosterone with pyridine-3-carboxaldehyde (174) and pyridine-4-carboxaldehyde (175), respectively, in accordance with the previous reports.$^{260}$ Pyridyl substituted pyrazoline derivatives 180 and 181 were obtained by condensation of 177 and 178 with hydrazine hydrate. IR
spectra exhibited prominent peaks at 3348 (O-H) and 1647 (C=N) in case of 3-picolyl derivative 180, whereas O-H vibrational band was observed at a lower frequency at 3285 and C=N band at 1655 cm$^{-1}$ for 4-picolyl derivative 181. 16-CH proton was observed as a triplet of doublets at $\delta$ 3.87 and as a triplet of doublets at an upfield value of 3.34 ppm for pyridyl derivatives 180 and 181, respectively. Pyrazoline proton resonated as a doublet at $\delta$ 4.05 for compound 180 but as a downfield doublet at 4.42 ppm in $^1$H-NMR spectrum of its 4-substituted counterpart 181. Protons of pyridine ring appeared separately at $\sim \delta$ 7.4 (5-CH), 8.1 (4-CH), 8.5 (6-CH) and 8.7 ppm (2-CH) for pyrazoline derivative 180 and as double doublets at $\delta$ 7.41 (3-CH, 5-CH) and 8.56 (2-CH, 6-CH) for steroidal derivative 181 in the proton nuclear magnetic resonance spectra.

Repeated efforts to carry out cyclization of 16-imidazolylidene derivative 171 using hydrazine hydrate to prepare the pyrazoline derivative
remained unsuccessful. This may be due to steric hindrance caused by imidazolyl ring for the nucleophilic attack.

**Mechanism**

The proposed mechanism involved in the formation of androst-5-ene-[17,16-c]-1'H-pyrazoline-3β-ol derivatives 179-181 has been outlined in figure 11.

*Figure 11. Mechanism involved in the synthesis of pyrazoline derivatives 179-181*
Hydrazine hydrate reacts with 17-carbonyl group of steroidal derivatives 176-178 to form 17-hydrazone with the loss of two molecules of water. The nucleophilic attack of NH$_2$ group due to presence of lone pair of electrons on the electron deficient double bond from $\alpha$-face and further rearrangement led to the formation of pyrazoline derivatives 179-181.

6. C$_6$ and C$_{16}$-functionalized steroidal derivatives

Steroidal oximes exhibit valuable biological activities such as cytotoxicity and aromatase inhibitory activities.\textsuperscript{264} Interestingly, the analysis of the chemical structure and biofunctions has shown that the cytotoxicity and aromatase inhibitory activity of these compounds is dependent on the location of the hydroximino group on the steroidal nucleus. The parental steroids with a hydroximino group located at different positions display a remarkable difference in their activities.\textsuperscript{264} Taking these observations in consideration it was thought to synthesize C$_6$ and C$_{16}$ functionalized steroidal oximes to obtain therapeutically useful molecules.

The synthetic route to the preparation of various new 6 and 16-functionalized steroidal derivatives have been shown in scheme 7. Treatment of 16$\alpha$-bromo steroid 152 with sodium borohydride in methanol at room temperature afforded 3$\beta$,17$\beta$-diol 183, which upon subsequent acetylation with acetic anhydride and dry pyridine in a steam bath yielded 3$\beta$,17$\beta$-diacetoxy derivative 184. 16$\alpha$-Bromo-5-androstene-3$\beta$,17$\beta$-diol (183) revealed characteristic broad vibrational band for O-H stretchings at 3358 cm$^{-1}$. 3$\alpha$-H and 17$\alpha$-H resonated together as a multiplet at $\delta$ 3.52-3.60 in $^1$H-NMR spectrum. Both 16$\beta$-H and -OH were present as a multiplet at 4.58-4.65 ppm, which integrated for two protons. In case of 3$\beta$,17$\beta$-diacetoxy derivative 184, a characteristic downfield shift of 16$\beta$-H ($\delta$ 4.99) was observed due to the presence of 17$\beta$-acetoxy group. Sharp vibrational band for C=O stretchings was also observed at higher frequency 1735 cm$^{-1}$ in the infrared spectrum. It was observed that configuration at 16 position of steroid was retained as alpha in both the compounds 183 and 184.
Further epoxidation\textsuperscript{264} of compound 184 with \textit{m}-chloroperbenzoic acid (MCPBA) in chloroform resulted in the formation of a mixture of \textalpha- and \textbeta-
epoxides 185. 16\textalpha-\textit{H} and 16\textbeta-\textit{H} proton appeared at $\delta$ 4.54 (t) and 5.10 ppm (d) in 3:7 area ratio and integrating for one proton in the proton NMR spectrum of 185. A 30\% conversion of configuration at 16 position of steroid from alpha to beta was observed as is evident from $^1$H-NMR spectral data.

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{Scheme_7.png}
\caption{Synthetic route to the preparation of C\textsubscript{6} and C\textsubscript{16} functionalized derivatives}
\end{scheme}

Oxidation\textsuperscript{264} of epoxide 185 using chromium trioxide gave the compound 16\textalpha-bromo-5\textalpha-hydroxy-6-oxo-5\textalpha-androstane-3\textbeta,17\textbeta-diyl diacetate (186).
Infrared bands at 3493, 1745 and 1704 were observed for O-H and C=O stretching vibrations of ester and ketone, respectively. \(^1\)H-NMR spectrum exhibited multiplets at \(\delta\) 4.55-4.60 (3\(\alpha\)-H) and 4.97-5.07 ppm (m, 17\(\alpha\)-H and 16\(\beta\)-H). The configuration at position 16 was observed as alpha from the \(^1\)H-NMR spectral data.

Treatment of 16\(\alpha\)-bromo steroid 186 with thionyl chloride in pyridine afforded the diacetoxy-\(\alpha,\beta\)-unsaturated 6-oxo steroid 187. Highly deshielded 4-CH proton appeared downfield at \(\delta\) 6.10 ppm and proton of 16\(\beta\)-H was observed as a doublet at \(\delta\) 5.17 ppm in the proton nuclear magnetic resonance spectrum. IR spectrum exhibited prominent peaks at 1738 and 1690 cm\(^{-1}\) for carbonyl stretching vibrations of ester and ketone.

The target 16\(\alpha\)-bromo-6\(E\)-hydroximino-androst-4-ene-3\(\beta\),17\(\beta\)-diyl diacetate (188) was obtained by the reaction of 187 with hydroxylamine hydrochloride. The infrared spectrum exhibited vibrational bands at 3405 (O-H), 1736 (C=O) and 1658 cm\(^{-1}\). A doublet of doublets was observed at \(\delta\) 3.29 for 7\(\beta\)-H, whereas 16\(\beta\)-H resonated as a doublet at 5.0 ppm.

To synthesize target compound 16-(imidazol-1-yl)-6\(E\)-hydroximino-androst-4-ene-3\(\beta\),17\(\beta\)-diyl diacetate (189), steroidal oxime 188 was treated with imidazole using various methods like thermal fusion and microwave synthetic techniques, but the target compound could not be obtained.

7. Microwave assisted organic synthesis

High speed microwave assisted chemistry has been applied successfully in various fields of synthetic organic chemistry as it allows faster work, generate
higher yields, and increase product purity by reducing unwanted side reactions compared to conventional heating methods. A large range and wide variety of industrially important compounds and intermediates, which, obtained otherwise by conventional procedures, contribute to the burden of chemical pollution, have been successfully synthesized using microwave-assisted solvent-free protocols.\textsuperscript{265,266}

Microwave assisted organic synthesis is a technique, which can be used to rapidly explore ‘chemistry space’ and increase the successfully diversity of the compounds produced. Nowadays, its considered that all of the conventionally synthesized compounds can also be obtained using this technique with improved yield and reduced reaction time.\textsuperscript{267}

\textbf{Alkylation reaction}

\textit{Synthesis of 4-(3-chloropropoxy)benzaldehyde (165) from 4-hydroxybenzaldehyde}

The microwave assisted chemical synthesis of 4-(3-chloropropoxy) benzaldehyde (165) was carried out by alkylation of 4-hydroxybenzaldehyde with 3-bromo-1-chloropropane in ethyl methyl ketone in the presence of anhydrous potassium carbonate. The microwave vial (10-15 ml) containing reaction mixture of 4-hydroxybenzaldehyde (0.5 g), 1-bromo-3-chloropropane (0.4 ml) and anhydrous potassium carbonate (1 g) in ethyl methyl ketone (10 ml) was irradiated in microwave synthesizer (Initiator 2.5, Biotage) at 100° C for 30 min. The completion of the reaction was monitored by TLC. The reaction mixture was cooled, filtered and the excess of solvent was removed under reduced pressure to obtain an oily residue of 4-(3-chloropropoxy)benzaldehyde (165), which was sufficiently pure and was used as such for further reaction. Microwave synthesis reduced reaction time from 6 h (conventional) to 30 min (microwave technique) and the amount of the solvent used was also considerably less than the conventional method as shown in table 3.

\textbf{Thermal fusion}

\textit{Synthesis of 16β-(imidazol-1-yl)-androst-1,4-diene-3,17-dione (157) from 16α/β-bromoandrost-1,4-diene-3,17-dione (156)}
The microwave vial (10-15 ml) containing triturated reaction mixture of 16β-bromoandrosta-1,4-diene-3,17-dione (156, 0.3 g) and imidazole (0.6 g) was irradiated in microwave synthesizer (Initiator 2.5, Biotage) at 80° C for 15 min. The reaction mixture was cooled at room temperature and cold water was added to it. The solid obtained was filtered, washed with water, dried and crystallized from a mixture of ethylacetate and diethyl ether. The reaction time was considerably shortened from 45 minutes by conventional method to 15 minutes by microwave method but no significant change in yield was observed as shown in table 3.

Table 3. Comparison of conventional synthesis vs microwave synthesis

<table>
<thead>
<tr>
<th>Reaction type</th>
<th>Conventional synthesis</th>
<th>Microwave synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>Yield</td>
</tr>
<tr>
<td>Alkylation</td>
<td>6 h</td>
<td>50%</td>
</tr>
<tr>
<td>Thermal fusion</td>
<td>45 min</td>
<td>45%</td>
</tr>
</tbody>
</table>

8. AROMATASE INHIBITORY ACTIVITY

The in vitro aromatase inhibitory activity of 153-159, 161-164, 166-169, 171, 172, 179-181, 185, 187 and 188 using human placental microsomes and [1β,2β-3H] androstenedione was carried out in collaboration with Prof. Rolf W. Hartmann, University of Saarland, Germany. The enzyme was obtained from the microsomal fraction of freshly delivered human term placental tissue according to the procedure of Thompson and Siiteri. The aromatase inhibitory activity data of the newly synthesized steroids is presented in table 4.

In case of 16-imidazolyl steroids, 16-bromo compounds 154 (IC50 = 2.65 μM) and 156 (IC50 = 13.2 μM) exhibited moderate inhibition of the enzyme. 17-Keto-3-hydroxy imidazolyl substituted derivative 153 with IC50 = 3.3 μM displayed substantial binding with aromatase enzyme as compared to...
Table 4. Aromatase inhibitory data of various compounds

<table>
<thead>
<tr>
<th>Compd. No. (Code)</th>
<th>CHEMICAL STRUCTURE</th>
<th>Inhibition of CYP 19&lt;sup&gt;a&lt;/sup&gt; IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>RP</th>
</tr>
</thead>
<tbody>
<tr>
<td>153 (RB-1240)</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>3.30</td>
<td>9&lt;sup&gt;b&lt;/sup&gt; 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>154</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>2.65</td>
<td>11&lt;sup&gt;b&lt;/sup&gt; 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>155 (RB-1241)</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>0.18</td>
<td>160&lt;sup&gt;b&lt;/sup&gt; 1.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>156 (RB-408)</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>13.20</td>
<td>2&lt;sup&gt;b&lt;/sup&gt; 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>157 (RB-401)</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>0.168</td>
<td>170&lt;sup&gt;b&lt;/sup&gt; 1.43&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Compd. No. (Code)</td>
<td>CHEMICAL STRUCTURE</td>
<td>Inhibition of CYP 19&lt;sup&gt;a&lt;/sup&gt; IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>RP</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------</td>
<td>-----------------------------------------------</td>
<td>----</td>
</tr>
<tr>
<td>158 (RB-1317)</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>No inhibition at 36 µM</td>
<td></td>
</tr>
<tr>
<td>159 (RB-1318)</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>53% inhibition at 36 µM</td>
<td></td>
</tr>
<tr>
<td>161 (RB-492)</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>5.4% inhibition at 5 µM</td>
<td></td>
</tr>
<tr>
<td>162 (RB-458)</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>1.3% inhibition at 5 µM</td>
<td></td>
</tr>
<tr>
<td>163 (RB-457)</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>15% inhibition at 5 µM</td>
<td></td>
</tr>
<tr>
<td>Compd. No. (Code)</td>
<td>CHEMICAL STRUCTURE</td>
<td>Inhibition of CYP 19&lt;sup&gt;a&lt;/sup&gt; IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>RP</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------</td>
<td>---------------------------------</td>
<td>----</td>
</tr>
<tr>
<td>164 (RB-456)</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>11.34</td>
<td>2.7&lt;sup&gt;b&lt;/sup&gt; 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>166 (RB-329)</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>0.2% inhibition at 5 µM</td>
<td></td>
</tr>
<tr>
<td>167 (RB-330)</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>4.1% inhibition at 5 µM</td>
<td></td>
</tr>
<tr>
<td>168 (RB-404)</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>1.5% inhibition at 5 µM</td>
<td></td>
</tr>
<tr>
<td>169 (RB-405)</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>11.9</td>
<td>2.6&lt;sup&gt;b&lt;/sup&gt; 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Compd. No. (Code)</td>
<td>CHEMICAL STRUCTURE</td>
<td>Inhibition of CYP 19α IC_{50} (µM)</td>
<td>RP</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------</td>
<td>----------------------------------</td>
<td>----</td>
</tr>
<tr>
<td>171 (RB-402)</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>Not active</td>
<td></td>
</tr>
<tr>
<td>172 (RB-403)</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>22.7</td>
<td>1.3²</td>
</tr>
<tr>
<td>179 (RB-406)</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>Not active</td>
<td></td>
</tr>
<tr>
<td>180 (RB-407)</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>12.9</td>
<td>2.5²</td>
</tr>
<tr>
<td>181 (RB-459)</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>11.8</td>
<td>2.6²</td>
</tr>
</tbody>
</table>


RESUME AND DISCUSSION

<table>
<thead>
<tr>
<th>Compd. No. (Code)</th>
<th>CHEMICAL STRUCTURE</th>
<th>Inhibition of CYP 19 * IC_{50} (\mu M)</th>
<th>RP</th>
</tr>
</thead>
<tbody>
<tr>
<td>185 (RB-462)</td>
<td><img src="image1.png" alt="Chemical Structure" /></td>
<td>1.4% inhibition at 5 \mu M</td>
<td></td>
</tr>
<tr>
<td>187 (RB-464)</td>
<td><img src="image2.png" alt="Chemical Structure" /></td>
<td>1.1% inhibition at 5 \mu M</td>
<td></td>
</tr>
<tr>
<td>188 (RB-465)</td>
<td><img src="image3.png" alt="Chemical Structure" /></td>
<td>3.5% inhibition at 5 \mu M</td>
<td></td>
</tr>
</tbody>
</table>

\* [1\beta-\textsuperscript{3}H] androstenedione
\textsuperscript{\textdagger} Relative Potency = Relative to aminoglutethimide (RP = 1; IC_{50} = 28.5 \mu M).
\textsuperscript{\textdagger} Relative Potency = Relative to exemestane (RP = 1; IC_{50} = 0.23 \mu M)

... to dihydroxy 158 and diacetoxy steroidal analogue 159, which produced only 53\% inhibition at 36 \mu M indicating the importance of oxidation in D ring for enzyme binding. 16\beta-(Imidazol-1-yl)-4-androstene-3,17-dione (155) (IC_{50} = 0.180 \mu M) and 16\beta-(imidazol-1-yl)-androsta-1,4-diene-3,17-dione (157) (IC_{50} = 0.168 \mu M) exhibited strong inhibition of the enzyme. The compounds were found to be approximately 160 and 170 times more potent in comparison to aminoglutethimide and 1.2 and 1.4 times more potent in assessment to standard drug exemestane (IC_{50} = 230 nM), respectively. The substitution of...
the bromine by an imidazole ring resulted into potent inhibition of the aromatase enzyme. It is assumed that the observed variation in aromatase inhibitory profile of these derivatives might have resulted due to changes in three dimensional attachments of the compounds with the enzyme. To allow

Table 5. Irreversible aromatase inhibition of various compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound No. (Code)</th>
<th>Inhibition of aromatase after incubation for irreversible binding(^a)</th>
<th>Inhibitor concentration [(\mu\text{M})]</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>153 (RB-1240)</td>
<td></td>
<td>3</td>
<td>n. i.(^b)</td>
</tr>
<tr>
<td>2</td>
<td>154</td>
<td></td>
<td>2.5</td>
<td>n. i.(^b)</td>
</tr>
<tr>
<td>3</td>
<td>155 (RB-1241)</td>
<td></td>
<td>0.2</td>
<td>n. i.(^b)</td>
</tr>
<tr>
<td>4</td>
<td>156 (RB-408)</td>
<td></td>
<td>13</td>
<td>27.6 ± 3.6</td>
</tr>
<tr>
<td>5</td>
<td>157 (RB-401)</td>
<td></td>
<td>0.2</td>
<td>n. i.(^b)</td>
</tr>
<tr>
<td></td>
<td>Exemestane</td>
<td></td>
<td>0.2</td>
<td>45.9 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>Aminogluthethimide</td>
<td></td>
<td>30</td>
<td>n. i.(^b)</td>
</tr>
</tbody>
</table>

\(^a\)[\(3\text{-}^3\text{H}\) androstenedione; 
\(^b\) n. i.: inhibition ≤ 10 %

irreversible binding of steroid to the aromatase enzyme, double bond was inserted between C\(_1\) and C\(_2\) by oxidation with DDQ as in compound 157. As is evident from irreversible binding studies (table 5), steroidal derivatives in this series except bromo substituted 1,4-diene 156 are acting as competitive inhibitors that compete with substrate androstenedione for noncovalent binding to the active site of the enzyme. It is also anticipated that imidazole group containing steroids interfere with steroid hydroxylations by the binding of the sterically available N with the heme Fe (III) iron of cytochrome P\(_{450}\). Of all only compound 156 is behaving as an irreversible inhibitor although to a lesser extent in comparison to exemestane (table 5). Despite the structural
similarity of compounds 156, 157 and exemestane with respect to ring A, it seems that the nitrogen of the imidazolyl-substituted compound 157 is able to complex the heme-iron of the enzyme leading to a different binding mode than the bromo-substituted compound 156, which is probably binding like exemestane (Fig 12). The present findings are potentially useful for understanding the spatial and electronic nature of the binding site of aromatase as well as for developing effective steroidal aromatase inhibitors.

Surprisingly the steroidal hybrid 4-hydroxy-16-imidazolyl derivative 161 exhibited only 5.4% inhibition at 5 μM, probably due to the formation of isomeric mixture. 16-Triazolyl derivative 162 also displayed mild inhibitory activity at 5 μM. In another series of 16,17-epoxy-17-imidazolyl substituted compounds, 163 exhibited only 15.7% inhibition at 5 μM but its oxidative derivative 164 (IC$_{50} = 11.34$ μM) exhibited moderate inhibition of the enzyme.

Steroidal derivative 16-[4-{3-(imidazol-1-yl)propoxy}benzylidene]-17-oxo-5-androsten-3β-ol (167) displayed 4.1% inhibition at 5 μM, while its A-ring oxidized counterpart 4-ene-3-keto steroid 169 (IC$_{50} = 11.9$ μM) exhibited good aromatase inhibitory activity. 16-Imidazolymethylene steroid 172 (IC$_{50} = 22.7$ μM) exhibited moderate inhibition of aromatase, while saturation of its A-ring lead to generation of inactive compound 171. It indicates that increasing A-
Pyridyl substituted pyrazoline derivatives 180 (IC\textsubscript{50} = 12.9 µM) and 181 (IC\textsubscript{50} = 11.8 µM) displayed moderate inhibition of the aromatase enzyme compared to the methoxy benzylidene analogue 179. It is anticipated that pyridine group possessing a sterically available N will be able to interact with the active site of aromatase by complexing the Fe (III) iron of cytochrome P450.

In case of C\textsubscript{6} and C\textsubscript{16}-functionalized steroidal derivatives, oxime 188 exhibited only 3.5% inhibition at 5 µM and decreased inhibition was also observed in case of 6-oxo derivative 187.

In summary, a new series of 16β-azolyl androstene derivatives has been synthesized and evaluated for aromatase inhibitory activity. Various synthetic routes were adopted to synthesize 16-substituted steroids. Some of the synthesized compounds exhibited potent anti-aromatase activity \textit{in vitro}. The imidazolyl substituted steroidal derivatives 155 and 157 were obtained as the powerful inhibitors of aromatase with IC\textsubscript{50} = 0.18 µM and IC\textsubscript{50} = 0.168 µM, respectively, approximately 1.2 and 1.4 times more potent in comparison to standard drug exemestane. The presence of an azolyl group at C-16 and an elevated degree of unsaturation in ring A of steroid skeleton in newly synthesizing molecules have resulted in higher aromatase inhibitory activity for breast cancer therapy than existing structural moieties.

In an overall view, azoles at the C-16 position, A-ring unsaturation and presence of 3, 17 carbonyl groups appears to play a critical role for binding to the aromatase enzyme. Preliminary structure activity relationships have been put forward based on the biological results. The outcome of the current work is the generation of an important pool of new steroidal derivatives, which gives a deeper insight into the universe of structure variability significantly affecting the biological properties.