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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of imidazole-derived steroidal hybrids as potent aromatase inhibitors

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Abstract Imidazolyl substituted 16*E*-arylidenosteroidal derivatives have been synthesized and evaluated for aromatase inhibitory activity. The steroidal hybrids displayed moderate inhibition of the aromatase enzyme. The enzyme activity was monitored by measuring the tritiated H_2O released from [1β-^{3}H] androstenedione during aromatization. 16-[3-{3-(Imidazol-1-yl)propoxy}benzylidene]-4-androstene-3,17-dione (**10**, IC_{50}: 4.4 μM) was found to be seven times more potent in comparison to standard drug aminoglutethimide.

Keywords 16*E*-Arylidenosteroids · Aromatase inhibitory activity · Breast cancer

Introduction

Aromatase is a cytochrome P450 enzyme, which catalyzes the conversion of androgens into estrogens in the last step of estrogen biosynthesis (Chumsria *et al.*, 2011). Compounds that inhibit aromatase have potential applications in the treatment of advanced estrogen-dependent tumors; such as breast cancer, endometrial cancer, prostatic hyperplasia, and prostate cancer (Gasi *et al.*, 2001; Miller and Jackson, 2003). Over the past two decades, substantial efforts have been directed toward developing potent inhibitors of aromatase. Clinical studies initially demonstrated that the administration of the first generation inhibitor, aminoglutethimide, caused regression of hormone-dependent breast cancer in women. This served as the impetus for developing the second and third generation inhibitors, which resulted in the availability of compounds that are much more potent than aminoglutethimide (Brueggemeier *et al.*, 2005; Saberi *et al.*, 2006). Among steroidal agents, formestane (**1**) (Fig. 1) was used widely during the early 1990 s, but it is not used nowadays because of the need to administer it by intramuscular injection. Therefore, the orally active steroid exemestane (**2**) and non-steroidal anastrozole (**3**) (Fig. 1) are the main aromatase inhibitors of contemporary importance in these days (Lombardi 2002; Brueggemeier *et al.*, 2005).

Taking into consideration the significance of azole groupings of many specific and potent cytochrome P450 inhibitors including aromatase (Leze *et al.*, 2006), we thought it worthwhile to condense imidazole group with androstane nucleus. 16-Substituted steroids have shown diversified pharmacological activities and are of interest for a medicinal chemist to develop new molecules (Numazawa and Osawa 1981). Many medicinally active steroidal derivatives with substitution at position 16 have already been described in the literature (Vicker *et al.*, 2006; Bansal and Guleria, 2008). Recent study from our laboratory has also demonstrated the effectiveness of 16*E*-arylidenosteroids as potential antitumor agents (Bansal and Guleria, 2008; Chattopadhaya *et al.*, 2004). These observations encouraged us to prepare and study some more new 16*E*-arylidenosteroids possessing an imidazole group to obtain potent aromatase inhibitors.

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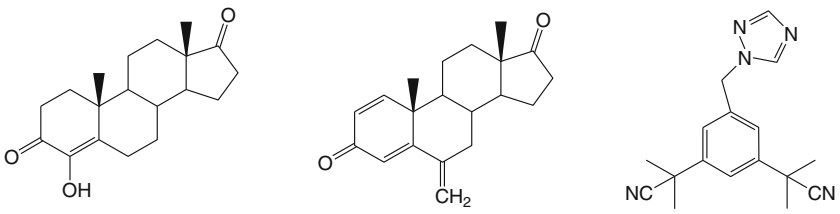


(1) (2) (3)

Fig. 1 Structures of clinically used steroidal and non-steroidal aromatase inhibitors: Formestane (**1**), Exemestane (**2**), and Anastrozole (**3**)

Materials and methods

Chemistry

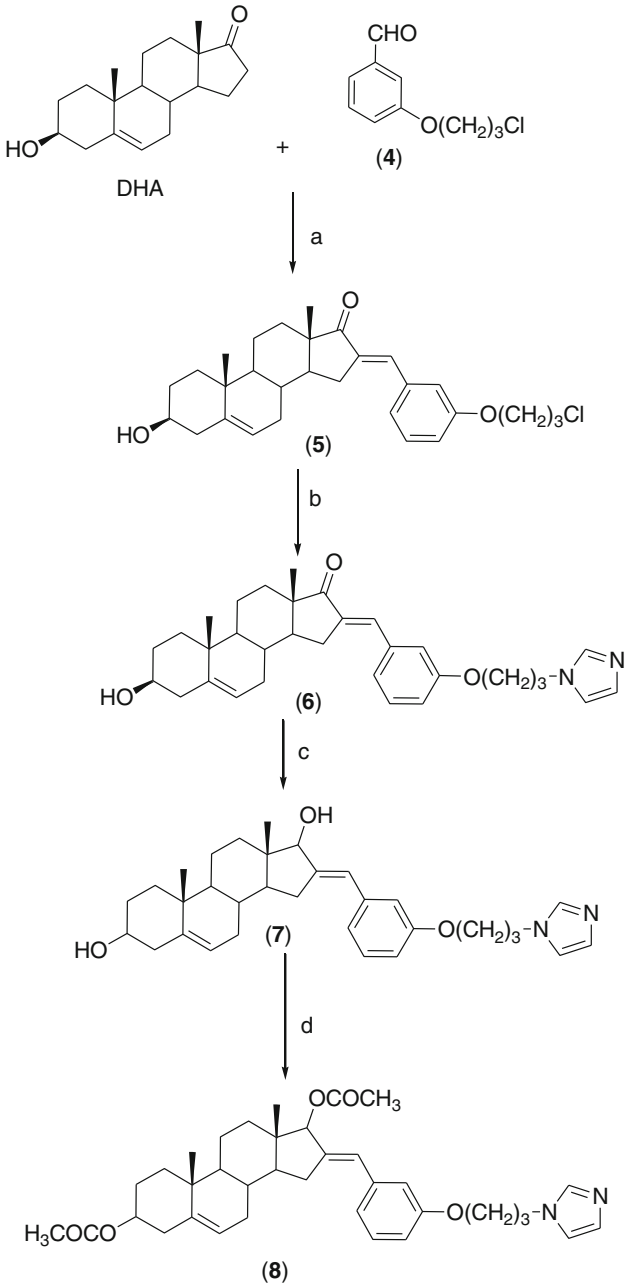
Melting points were determined on a Veego melting point apparatus and are uncorrected. UV (wavelengths in nm) was recorded on Lambda 15, IR (wavenumbers in cm^{-1}) spectra on Perkin-Elmer spectrum RX 1 FT-IR spectrophotometer models using KBr pellets. ^1H NMR spectra were recorded on Bruker AC-300F, 300 MHz using deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) containing tetramethylsilane as internal standard (chemical shifts in ppm). Elemental analyses were carried out on a Perkin-Elmer-2400 model CHN analyzer. Plates for TLC were prepared according to Stahl (E. Merck) using EtOAc as solvent (activated at 110 °C for 30 min) and were visualized by exposure to iodine vapors. Anhydrous sodium sulfate was used as drying agent. All the solvents were distilled before use according to standard procedures.

The synthetic routes to the preparation of various new steroidal derivatives have been outlined in Schemes 1, 2, 3 in accordance with the literature method (Bansal *et al.*, 2011b). Aldol condensation of DHA with substituted benzaldehydes **4** and **11** afforded 16-benzylidene steroidal derivatives **5** and **12**, respectively. 16-Arylideno steroids **5** and **12** were then thermally fused with powdered imidazole to afford the corresponding imidazolyl substituted products **6** and **13**, which on Oppenauer oxidation in aluminum isopropoxide–cyclohexanone–toluene system yielded the corresponding 4-ene-3,17-dione derivatives **10** and **14**. Reduction of compound **6** afforded 3β,17β-diol derivative **7**. Acetylation of the **7** and **6** using acetic anhydride afforded **8** and **9**, respectively.

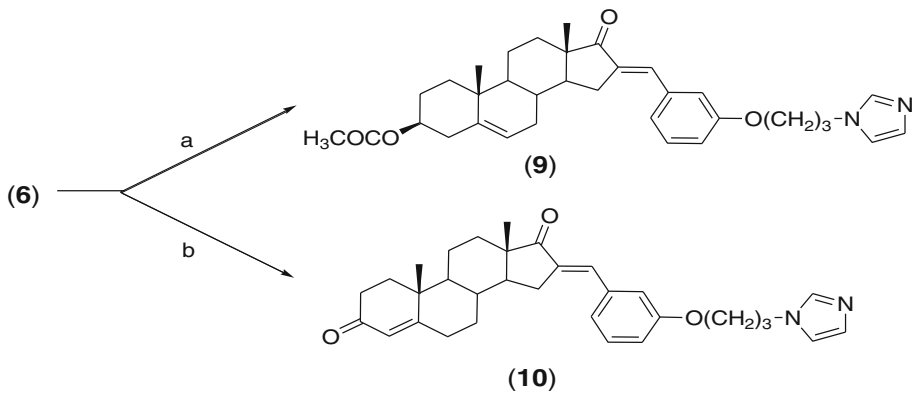
The synthesis of substituted aldehydes **4** and **11** was performed according to reported method (Bansal *et al.*, 2011a).

*General method for the preparation of compounds **5**, **12***

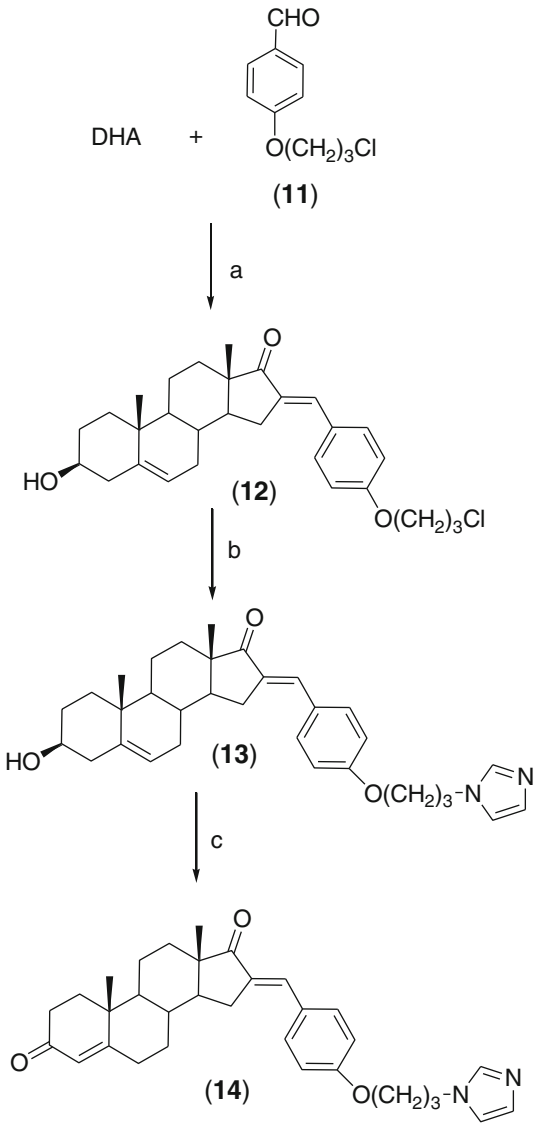
A mixture of dehydroepiandrosterone (0.75 g, 2.60 mmol), appropriate substituted aldehyde (**4, 11**; oily residues



Scheme 1 Synthesis of the compounds **4**–**8**. Reagents and reaction conditions: **a** MeOH, KOH, RT; **b** Imidazole, fusion 110–120 °C, 5 h; **c** $NaBH_4$; **d** $(CH_3CO)_2O$/dry pyridine, steam bath, 2 h.



Scheme 2 Synthesis of compounds **9** and **10**. Reagents and reaction conditions: **a** $(CH_3CO)_2O$/dry pyridine, steam bath, 2 h; **b** Al(*t*-BuO)$_3$, cyclohexanone, reflux, 5 h.



Scheme 3 Synthesis of compounds **11**–**14**. Reagents and reaction conditions: **a** MeOH, KOH, RT; **b** Imidazole, fusion 110–120 °C, 5 h; **c** Al(*t*-BuO)$_3$, cyclohexanone, reflux, 5 h.

obtained from 1 g of respective aldehyde), and sodium hydroxide (1 g) in methanol (20 ml) was stirred at room temperature for 8 h, the reaction being monitored by TLC. Cold water was added to the reaction mixture, and the precipitate obtained was filtered, washed with water, dried, and crystallized from methanol to yield corresponding 16-arylideno steroids **5** and **12**.

*16-[3-(3-Chloropropoxy)benzylidene]-17-oxo-5-androsten-3β-ol (**5**)*

Yield: 40.98 %; m.p. 145–147 °C; UV_{max} (MeOH): 292.0 nm (Log ε 4.24); IR: 3419, 2932, 1715, 1628, 1580, 1445, 1375, 1257, 1215, 1163, 1054, 1008, 918, 873 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90 (s, 3H, 18-C*H*$_3$), 1.00 (s, 3H, 19-C*H*$_3$), 2.19 (m, 2H, –OCH$_2$C*H*$_2$CH$_2$Cl), 3.40 (m, 1H, 3α-*H*), 3.67 (t, 2H, –C*H*$_2$Cl), 4.06 (t, 2H, –OC*H*$_2$–), 5.27 (d, 1H, 6-C*H*), 6.79 (d, 1H, $J_o = 8.01$ Hz, 4-C*H*, aromatic), 6.93 (s, 1H, 2-C*H*, aromatic), 7.02 (d, 1H, $J_o = 7.55$ Hz, 6-C*H*, aromatic), and 7.18–7.24 ppm (m, 2H, 5-C*H*, aromatic and vinylic-*H*, 16-arylidene). Anal. Calc. for $C_{29}H_{37}O_3Cl$: C: 74.26, H: 7.95; found; C: 74.64, H: 8.12.

*16-[4-(3-Chloropropoxy)benzylidene]-17-oxo-5-androsten-3β-ol (**12**)*

Yield: 55.37 %; m.p.111–113 °C; UV_{max} (MeOH): 317.2 nm (log ε 5.45); IR: 3419, 2934, 1710, 1604, 1459, 1373, 1253, 1174, 1047, 830, 717 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.99 (s, 3H, 18-C*H*$_3$), 1.10 (s, 3H, 19-C*H*$_3$), 2.28 (m, 2H, –OCH$_2$C*H*$_2$CH$_2$Cl), 3.60 (m, 1H, 3α-*H*), 3.78 (t, 2H, –C*H*$_2$Cl), 4.19 (t, 2H, –OC*H*$_2$–), 5.42 (d, 1H, 6-C*H*), 6.97 (d, 2H, $J_o = 8.8$ Hz, 3-C*H* and 5-C*H*, aromatic), 7.42 (s, 1H, vinylic-*H*, 16-arylidene), and 7.54 ppm (d, 2H, $J_o = 8.8$ Hz, 2-C*H* and 6-C*H*, aromatic). Anal. Calc. for $C_{29}H_{37}O_3$ Cl: C: 74.26, H: 7.95; found: C: 74.35, H: 8.08 %.

*General method for the preparation of compounds **6**, **13***

A mixture of benzylidene **5** or **12** (0.47 g, 1 mmol) and powdered imidazole (0.68 g, 10 mmol, in excess) was fused at 110–120 °C for 5 h. The completion of reaction was monitored by TLC. The reaction was quenched with cold water, and the solid obtained was filtered, washed with water, dried, and crystallized from ethyl acetate to yield **6** and **13**, respectively.

*16-[3-{3-(Imidazol-1-yl)propoxy}benzylidene]-17-oxo-5-androsten-3β-ol (**6**)*

Yield: 45.87 %; m.p. 211–212 °C. UV_{max} (MeOH): 290.4 nm (log ε 4.25); IR: 3253, 2932, 2837, 1713, 1629, 1576, 1448, 1385, 1269, 1160, 1067, 919, 872 and 779 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.98 (s, 3H, 18-C*H*$_3$), 1.07 (s, 3H, 19-C*H*$_3$), 2.22 (m, 2H, $OCH_2CH_2CH_2N<$), 3.49 (m, 1H, 3α-*H*), 3.92 (t, 2H, –C*H*$_2$N<), 4.21 (t, 2H, OC*H*$_2$–), 5.35 (s, 1H, 6-C*H*), 6.84 (dd, 1H, $J_m = 2.17$ Hz, $J_o = 8.17$ Hz, 4-C*H*, aromatic) 6.88 (s, 1H, 5-C*H*, imidazole), 7.02 (m, 2H, 2-C*H*, aromatic and 4-C*H*, imidazole), 7.13 (d, 1H, $J_o = 7.64$ Hz, 6-C*H*, aromatic), 7.26–7.34 (m, 2H, 5-C*H*, aromatic and vinylic-*H*, 16-arylidene), and 7.44 ppm (s, 1H, 2-C*H*, imidazole). Anal. Calc. for $C_{32}H_{40}N_2O_3$: C: 76.77, H: 8.05, N: 5.59; found; C: 76.85, H: 8.19, N: 5.65.

*16-[4-{3-(Imidazol-1-yl)propoxy}benzylidene]-17-oxo-5-androsten-3β-ol (**13**)*

Yield: 35.85 %; m.p. 201–203 °C. UV_{max} (MeOH): 317.6 nm (log ε 4.47); IR: 3219, 2932, 1710, 1601, 1510, 1464, 1371, 1248, 1176, 1060, 830, and 742 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.98 (s, 3H, 18-C*H*$_3$), 1.08 (s, 3H, 19-C*H*$_3$), 2.24 (p, 2H, $–OCH_2CH_2CH_2N<$), 3.51 (m, 1H, 3α-*H*), 3.94 (t, 2H, –C*H*$_2$N<), 4.21 (t, 2H,–OC*H*$_2$–), 5.40 (s, 1H, 6-C*H*), 6.92 (m, 3H, 5-C*H*, imidazole, 3-C*H* and 5-C*H*, aromatic), 7.06 (s, 1H, 2-C*H*, imidazole), 7.40 (s, 1H, vinylic-*H*, 16-arylidene), and 7.49 ppm (m, 3H, 4-C*H*, imidazole, 2-C*H* and 6-C*H*, aromatic).

*General procedure for the synthesis of compounds **10**, **14***

Compounds **6** or **13** (1 g, 2 mmol) was dissolved in a mixture of cyclohexanone (10 ml) and dry toluene (150 ml). Traces of moisture were removed by azeotropic distillation. The distillation was continued at a slow rate while adding a solution of aluminum isopropoxide (1 g) in dry toluene (15 ml) dropwise. The reaction mixture was refluxed for 5 h, and allowed to stand at room temperature overnight. The slurry was filtered, and the residue was washed thoroughly with dry toluene. The combined filtrate and the washings were steam distilled until the removal of organic solvent was affected. The solid obtained was filtered, washed with water, dried, and treated with diethyl ether and *n*-hexane to furnish the corresponding 4-ene-3-keto steroids **10** and **14**, respectively.

*16-[3-{3-(Imidazol-1-yl)propoxy}benzylidene]-4-androstene-3,17-dione (**10**)*

Yield: 50.25 %; m.p. 81–83 °C; UV_{max} (MeOH): 239.6 nm (log ε 4.34), 291.0 nm (log ε 4.25); IR: 2937, 1717, 1663, 1622, 1576, 1448, 1256, 1226, 1179, 1162, 1080, 1026, 915, 864 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.02 (s, 3H, 18-C*H*$_3$), 1.25 (s, 3H, 19-C*H*$_3$), 2.26 (m, 2H, $–OCH_2CH_2CH_2N<$), 3.94 (t, 2H, –C*H*$_2$N<), 4.23 (t, 2H, –OC*H*$_2$–), 5.76 (d, 1H, 4-C*H*), 6.90 (dd, 1H, $J_m = 1.90$ Hz, $J_o = 8.12$ Hz, 4-C*H*, aromatic) 6.95 (s, 1H, 5-C*H*, imidazole), 7.02 (s, 1H, 2-C*H*, aromatic), 7.08 (s, 1H, 4-C*H*, imidazole), 7.16 (d, 1H, $J_o = 7.68$ Hz, 6-C*H*, aromatic), 7.33 (t, 1H, $J_o = 7.91$ Hz, 5-C*H*, aromatic), 7.40 (s, 1H, vinylic-*H*, 16-arylidene), and 7.57 ppm (s, 1H, 2-C*H*, imidazole). Anal. Calc. for $C_{32}H_{38}N_2O_3$: C: 77.08, H: 7.68, N: 5.62; found; C: 77.22, H: 7.49, N: 5.88.

*16-[4-{3-(Imidazol-1-yl)propoxy}benzylidene]-4-androstene-3,17-dione (**14**)*

Yield: 23.2 %; m.p. 171–173 °C; UV_{max} (MeOH): 316.4 nm (log ε 5.66); IR: 2933, 1713, 1665, 1606, 1508, 1458, 1251, 1179, 1091, 1027, 923, 828, 748 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.01 (s, 3H, 18-C*H*$_3$), 1.24 (s, 3H, 19-C*H*$_3$), 2.25 (p, 2H, $–OCH_2CH_2CH_2N<$), 3.94 (t, 2H, –C*H*$_2$N<), 4.21 (t, 2H, –OC*H*$_2$–), 5.76 (s, 1H, 4-C*H*), 6.90 (d, 3H, $J_o = 8.76$ Hz, 3-C*H*, 5-C*H*, aromatic and 5-C*H*, imidazole), 7.07 (s, 1H, 4-C*H*, imidazole), 7.41 (s, 1H, vinylic-*H*, 16-arylidene), and 7.49 ppm (m, 3H, 2-C*H*, 6-C*H*, aromatic and 2-C*H*, imidazole). Anal. Calc. for $C_{32}H_{38}N_2O_3$: C: 77.08, H: 7.68, N: 5.62; found; C: 77.24, H: 7.66, N: 5.82 %.

*16-[3-{3-(Imidazol-1-yl)propoxy}benzylidene]-5-androstene-3β,17β-diol (**7**)*

To a stirred suspension of **6** (1 g, 2 mmol) in methanol (100 ml) at room temperature, sodium borohydride (1.5 g) was added in small fractions over a period of 2 h. The reaction mixture was further stirred for 6 h. Solvent was removed under reduced pressure, and cold water was added. The precipitate obtained was filtered, washed with water, dried, and crystallized from acetone to afford **7**.

Yield: 50.02 %; m.p. 133–135 °C; UV_{max} (MeOH): 256.6 nm (log ε 4.02); IR: 3373, 2936, 1601, 1438, 1366, 1252, 1161, 1050, 955 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.72 (s,

3H, 18-CH_3), 1.04 (s, 3H, 19-CH_3), 2.25 (m, 2H, –OCH$_2$CH_2CH$_2$N<), 3.54 (m, 1H, 3α-*H*), 3.91 (t, 2H, –CH_2N<), 4.06 (s, 1H, 17α-*H*), 4.21 (t, 2H, –OCH_2–), 5.38 (d, 1H, 6-C*H*), 6.48 (d, 1H, vinylic-*H*, 16-arylidene), 6.73 (dd, 1H, $J_m = 2.29$ Hz, $J_o = 8.03$ Hz, 4-C*H*, aromatic), 6.87 (m, 4H, 2-C*H*, 6-C*H*, aromatic and 4-C*H*, 5-C*H*, imidazole), 7.26 (m, 1H, 5-C*H*, aromatic) and 7.48 ppm (s, 1H, 2-C*H*, imidazole). Anal. Calc. for $C_{32}H_{42}N_2O_3$: C: 76.46, H: 8.42, N: 5.57; found; C: 76.42, H: 8.59, N: 5.41.

*16-[3-{3-(Imidazol-1-yl)propoxy}benzylidene]-5-androstene-3β,17β-diol diacetate (**8**)*

A mixture of **7** (0.52 g, 1 mmol), acetic anhydride (1 ml), and dry pyridine (2 ml) was heated in a steam bath for 2 h. The reaction mixture was then poured into cold water and basified with liquid ammonia. The precipitate formed was collected by filtration, washed with water, dried, and crystallized from *n*-hexane to get the compound **8**.

Yield: 34.48 %; m.p. 97–99 °C; UV_{max} (MeOH): 255.8 nm (log ε 4.47); IR: 2939, 1732, 1600, 1440, 1372, 1241, 1162, 1034, 907.6 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.79 (s, 3H, 18-CH_3), 1.05 (s, 3H, 19-CH_3), 2.04 (s, 3H, 3β-OCOCH_3), 2.21 (s, 3H, 17β-OCOCH_3), 2.32 (m, 2H, –OCH$_2$CH_2CH$_2$N<), 3.91 (t, 2H, –CH_2N<), 4.20 (t, 2H, –OCH_2–), 4.59 (m, 1H, 3α-*H*), 5.37 (s, 1H, 17α-*H*), 5.41 (d, 1H, 6-C*H*), 6.17 (s, 1H, vinylic-*H*, 16-arylidene), 6.73 (d, 1H, $J_o = 7.96$ Hz, 4-C*H*, aromatic), 6.84 (s, 1H, 2-C*H*, aromatic), 6.93–7.06 (m, 3H, 6-C*H*, aromatic and 4-C*H*, 5-C*H*, imidazole), 7.26 (m, 1H, 5-C*H*, aromatic), and 7.49 ppm (s, 1H, 2-C*H*, imidazole); Anal. Calc. for $C_{36}H_{46}N_2O_5$: C: 73.69, H: 7.90, N: 4.77; found; C: 73.51, H: 7.99, N: 4.94.

*16-[3-{3-(Imidazol-1-yl)propoxy}benzylidene]-17-oxo-5-androsten-3β-yl-acetate (**9**)*

A mixture of **6** (0.5 g, 1 mmol), acetic anhydride (1 ml), and dry pyridine (2 ml) was heated in a steam bath for 2 h. The reaction contents were then poured into iced water and basified with liquid ammonia. The precipitate obtained was filtered, washed with water, dried, and crystallized from diethyl ether to afford **9**.

Yield: 47.16 %; m.p. 148–150 °C; UV_{max} (MeOH): 291.0 nm (log ε 4.38); IR: 2940, 1728, 1631, 1575, 1508, 1443, 1374, 1254, 1163, 1030, 908, 873 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.98 (s, 3H, 18-CH_3), 1.09 (s, 3H, 19-CH_3), 2.03 (s, 3H, –OCOCH_3), 2.23 (m, 2H, –OCH$_2$CH_2CH$_2$N<), 3.91 (t, 2H, –CH_2N<), 4.21 (t, 2H, –OCH_2–), 4.58 (m, 1H, 3α-*H*), 5.41 (d, 1H, 6-C*H*), 6.83 (dd, 1H, $J_m = 2.15$ Hz, $J_o = 8.11$ Hz, 4-C*H*, aromatic) 6.87 (s, 1H, 5-C*H*, imidazole), 6.97 (s, 1H, 2-C*H*, aromatic), 7.02 (s, 1H, 4-C*H*, imidazole), 7.13 (d, 1H, $J_o = 7.73$ Hz, 6-C*H*, aromatic), 7.30 (m, 2H, 5-C*H*, aromatic and vinylic-*H*, 16-arylidene), and 7.43 ppm (s, 1H, 2-C*H*, imidazole). Anal, Calc. for $C_{33}H_{42}N_2O_4$: C: 74.69, H: 7.98, N: 5.28; found; C: 74.82, H: 7.49, N: 5.31.

Biological activity

Preparation of aromatase

The enzyme was obtained from the microsomal fraction of freshly delivered human termplacental tissue according to the procedure of Thompson and Siiteri (Thompson and Siiteri 1974). The isolated microsomes were suspended in the minimum volume of phosphate buffer (0.05 M, pH 7.4) and stored at −30 °C as described. No loss of activity was observed within 4 months.

Inhibition of aromatase in vitro

The assay was performed similar to the described methods (Foster *et al.*, 1983; Graves and Salhanick 1979; Hartmann and Batzl 1986), monitoring enzyme activity by measuring the ^3H_2O formed from [1β-^{3}H]androstenedione during aromatization. Each incubation tube contained 15 nM [1β-^{3}H]androstenedione (0.08 μCi), 485 nM unlabeled androstenedione, 2 mM NADP, 20 mM glucose-6-phosphate, 0.4 units of glucose-6-phosphate-dehydrogenase, and inhibitor (in at least three different concentrations for determining the IC_{50} value) in phosphate buffer (0.05 M, pH 7.4). The test compounds were dissolved in DMSO and diluted with buffer. The final DMSO concentration in the control and inhibitor incubation was 2 %. Each tube was preincubated for 5 min at 30 °C in a water bath. Microsomal protein was added to start the reaction (0.1 mg). The total volume for each incubation was 0.2 ml. The reaction was terminated by the addition of 200 μl of a cold 1 mM $HgCl_2$ solution. After addition of 200 μl of an aqueous dextran-coated charcoal (DCC) suspension (2 %), the vials were shaken for 20 min and centrifuged at 1,500×*g* for 5 min to separate the charcoal-absorbed steroids. The supernatant was assayed for ^3H_2O by counting in a scintillation mixture using a LKB-Wallac β-counter.

Results and discussion

Chemistry

Base catalyzed aldol condensation of DHA with substituted benzaldehydes **4** and **11** afforded 16-benzylidene steroidal derivatives **5** and **12**, respectively. The methine-bridged proton of 16-arylidene resonated downfield at δ 7.3 ppm in

the ^{1}H NMR spectra. The configuration at C_{16} is assigned *E* with respect to the keto group at C_{17} in analogy with earlier reports (Thamotharan *et al.*, 2004). 16-Arylideno steroids **5** and **12** were then thermally fused with powdered imidazole to afford the corresponding imidazolyl substituted products **6** and **13**, which on Oppenauer oxidation in aluminum isopropoxide-cyclohexanone-toluene system yielded the corresponding 4-ene-3,17-dione derivatives **10** and **14**. The appearance of a downfield 4-C*H* proton at δ 5.76 ppm (in the proton NMR spectra), and the vibrational bands at 1,713 and 1,665 cm^{-1} confirmed the formation of **10** and **14**.

To study structure activity relationship in this particular type of compounds, further modifications of steroidal nucleus substituted with 3-hydroxybenzaldehyde derivatives were carried out. Reduction of compound **6** using sodium borohydride in methanol at room temperature afforded 3β,17β-diol derivative **7**. The broad vibrational band for O–H stretching absorption at 3373.7 cm^{-1}, and ^{1}H NMR signals for 3α-*H* and 17α-*H* were observed at δ 3.54 and 4.06 ppm, respectively. The vinylic proton at C_{16} was found at an upfield position (δ 6.48 ppm), as compared with its parent compound **6** (δ 7.3 ppm). Acetylation of the dihydroxy derivative **7** using acetic anhydride afforded diacetoxy compound, 16-[3-{3-(imidazol-1-yl)propoxy}-benzylidene]-5-androsten-3β,17β-diol diacetate (**8**). The methine-bridged proton was found further at an upfield value at δ 6.17 ppm, as compared with its 3,17-diol counterpart. Acetylation of **6** with acetic anhydride yielded monoacetylated 3β-acetoxy derivative **9**.

Aromatase inhibitory activity

The newly synthesized imidazolyl substituted 16-arylideno steroids were screened for aromatase inhibitory activity by measuring the tritiated water released during aromatization. Table 1 displays the IC_{50} values for aromatase inhibition and relative potency of these compounds in comparison to standard drug aminoglutethimide. Despite the presence of an imidazole group, the 3-hydroxy-17-keto aldol products **6** and **13** displayed insignificant inhibition of the enzyme irrespective of the *meta* or *para* substitution of the side chain. Although hydroxylation at 3 and 17 positions in case of derivative **7** resulted in moderate inhibition of the enzyme with IC_{50} = 9.1 μM, the presence of acetoxy groups in compounds **8** and **9** resulted in significant loss of aromatase inhibitory activity. Increase in oxidation of A ring as in case of 4-ene-3-keto steroids **10** (IC_{50}: 4.4 μM) and **14** (IC_{50} = 11.9 μM) improved upon the binding affinity of steroids with aromatase enzyme. Imidazolyl substituted benzylidene derivative **10** with side chain at *meta* position exhibited ~2.5 times more binding affinity for aromatase enzyme, as compared to its *para* substituted analogue **14**. Although it is expected that the presence of imidazole group possessing a sterically available N will be able to enhance the interaction of steroid with the active site of aromatase by complexing the Fe(III) iron of cytochrome P_{450}, it is observed from the biological data that structural modifications of steroids might lead to changes in 3D attachments of the compounds with the enzyme site. This may result in significant loss in binding affinity. Although shift in position of phenyl substituent from *meta* to *para* position brought marginal changes in aromatase inhibitory properties of the steroidal derivatives, *meta* seems more promising. The 'not so potent' aromatase inhibitory activity of the synthesized compounds discouraged us to further explore the structural modifications in steroidal series with *para* phenyl substituents.

Table 1 Aromatase inhibitory data of various compounds

S. no.	Compound no.	Inhibition on CYP 19[a] IC_{50} (μM)	RP[b]
1	**6**	34 % Inhibition at 36 μM	
2	**7**	9.1	3.3
3	**8**	34 % Inhibition at 36 μM	
4	**9**	38 % Inhibition at 36 μM	
5	**10**	4.4	6.8
6	**12**	0.2 % Inhibition at 5 μM	
7	**13**	4.1 % Inhibition at 5 μM	
8	**14**	11.9	2.6

[a] [1β-^{3}H] androstenedione

[b] Relative potency = relative to aminoglutethimide (RP = 1; IC_{50} = 28.5 μM)

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