Synthesis and Biological Evaluation of 16*E*-Arylidenosteroids as Cytotoxic and Anti-aromatase Agents

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Taking into consideration the structural requirements for cytotoxicity and aromatase inhibition, several new 16*E*-arylidenosteroidal derivatives have been prepared and evaluated for their cytotoxic and aromatase inhibitory activity. The new steroidal analogues 3, 5—8 and 11 exhibited significant cytotoxic effects when screened against three cancer cell lines, MCF-7 (breast), NCl-H460 (lung) and SF-268 central nervous system (CNS) at 100 μM and sensible cytotoxic effects subsequently in sixty cancer cell lines derived from nine cancers types (leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancers). The imidazolyl substituted steroidal derivatives 5 and 7 exhibited strong inhibition of the aromatase enzyme with 16-[4-{3-(imidazol-1-yl)propoxy}-3-methoxybenzylidene]-5-androstene-3β,17β-diol (7) displaying 13 times more potency in comparison to aminoglutethimide.

Key words aromatase inhibitory activity; 16*E*-arylidenosteroid; dehyroepiandrosterone; cytotoxic activity; cancer cell line; breast cancer

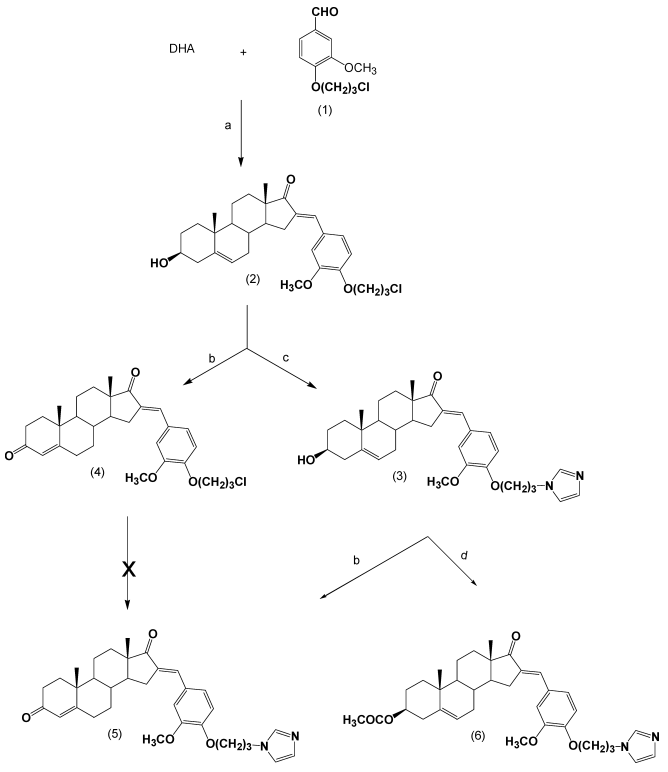
New targets are being focused by medical chemist with the aim to provide new specific and potent drugs for the treatment of cancer. Mammary tumors represent the most widespread type of malignant neoplasm and most common cause of cancer in women between the ages of 30—54[1)] and is the second leading cause of cancer deaths in women today (after lung cancer). About half of these malignancies require a source of estrogens for their growth and development. Estrogens are biosynthesized from androgens by the microsomal cytochrome P-450 enzyme system termed aromatase.[2,3)] Inhibition of aromatase is an important approach to reducing growth stimulatory effects of estrogens in estrogen-dependent breast cancer.[4)] Effective aromatase inhibitors have been developed as therapeutic agents for controlling estrogen-dependent breast cancer. Aromatase inhibitors (AIs) can reduce estrogen production by more than 90% and in addition AIs lack estrogen-agonist activity unlike tamoxifen, the most widely used antiestrogen for the management of breast cancer.[5)] Recent clinical data have also shown that these inhibitors have greater efficacy than tamoxifen in late-stage disease and preliminary data indicate that this efficacy extends to early disease. Aromatase inhibitors therefore almost certainly replace tamoxifen as the hormonal agents of choice for the treatment of breast cancer.[5,6)]

Despite the success of the third-generation steroidal and nonsteroidal AIs, they still have some major side effects, such as the increase of bone loss. For this reason, it is important to search for other potent and specific molecules with lower side effects. Taking into consideration the significance of azole groupings of many specific and potent P450 inhibitors including aromatase,[7)] we have introduced imidazole group in androstane nucleus in the present study.

A large number of potent steroidal derivatives with substitution at position 16 have been described in the literature as potent cytotoxic agents.[8—10)] Recent work from our laboratory has also demonstrated the effectiveness of 16*E*-arylidenosteroids as potential antitumour agents.[11—13)] These observations prompted us to prepare and study some more new 16*E*-arylidenosteroids possessing an imidazole group to obtain dual cytotoxic as well as aromatase inhibitory effects.

Results and Discussion

The synthetic routes to the preparation of various new steroidal derivatives have been outlined in Charts 1—3. Base catalyzed aldol condensation of dehydroepiandrosterone (DHA) with 4-(3-chloropropoxy)-3-methoxybenzaldehyde



Reagents and reaction conditions: (a) MeOH, KOH, RT; (b) Al(*t*-BuO)$_3$, cyclohexanone, reflux, 5 h; (c) imidazole, fusion 110—120 °C, 5 h; (d) (CH$_3$CO)O/dry pyridine, steam bath, 2 h.

Chart 1. Synthetic Protocol of the Compounds **1**—**6**

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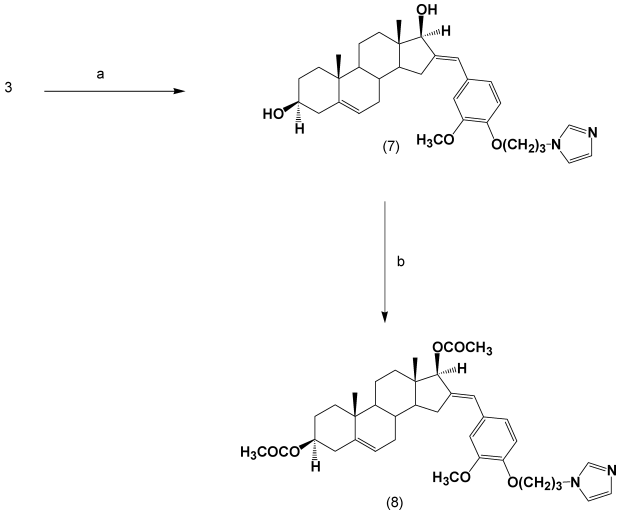
(**1**) was carried out at room temperature to afford **2**. The aldol product **2** showed prominent infrared vibrational bands at 3220.0 (O–H) and 1709.1 (C=O) cm^{-1} and ^{1}H-NMR signals at δ 3.77 (t, 2H, –C<u>H</u>$_2$Cl) and 7.38 ppm (s, 1H, vinylic-<u>H</u>, 16-arylidene). The configuration at C_{16} with respect to the carbonyl at C_{17} has been assigned *E* in analogy with earlier reports.[11] Oppenauer oxidation of 5-ene-3-hydroxy chloro product **2** resulted in the formation of 4-ene-3-keto analogue **4**. Repeated efforts to prepare imidazolyl substituted steroid **5** from **4** remained unsuccessful. Therefore an alternative route was followed to prepare **5**, 16-arylidene steroid **2** was first thermally fused with powdered imidazole to obtain the imidazolyl substituted product **3**, which on Oppenauer oxidation gave target 4-ene-3-keto steroid **5** (Chart 1). Presence of imidazolyl protons at δ 6.93 (5-C<u>H</u>), 7.06 (4-C<u>H</u>), 7.48 (2-C<u>H</u>) and a triplet at 3.97 ppm for C<u>H</u>$_2$N< in the ^{1}H-NMR spectrum confirmed the formation of **3**. A down field shift of 4-C<u>H</u> proton (δ 5.76 ppm) was observed for 3-keto steroid **5** in comparison to 5-C<u>H</u> of 3-hydroxy derivative **3**, which resonated at δ 5.39 ppm. Acetylation of **3** afforded corresponding acetoxy compound **6**.

Reduction of compound **3** using sodium borohydride in methanol at room temperature afforded 3β,17β-diol derivative **7** (Chart 2).

The broad band for O–H stretching vibration at 3235.9 cm^{-1} and ^{1}H-NMR signals for 3α-<u>H</u> and 17α-<u>H</u> were observed at δ 3.53 and 4.06 ppm, respectively. The proton of methine bridge was found at an upfield position in **7** (δ 6.45 ppm) in comparison to its parent 17-keto derivative **3** (δ 7.38 ppm). Acetylation of 3β,17β-diol derivative **7** afforded corresponding acetoxy compound **8**. The methyl proton of acetoxy group resonated at δ 2.00 ppm and C=O stretching absorption was seen at 1733.7 cm^{-1} in IR spectra. The methine-bridged proton was found further at an upfield value (δ 6.15 ppm) as compared to its 17β-diol counterpart **7** in case of compound **8**.

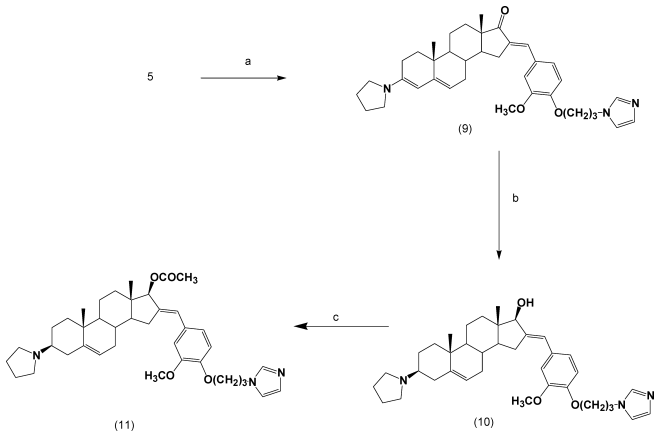
For the preparation 3-pyrrolidinyl substituted 16-arylidene steroidal derivatives, **5** was heated under reflux with pyrrolidine in methanol to yield an unstable dienamine **9** as shown in Chart 3, which upon sodium borohydride reduction at room temperature afforded 3β-pyrrolidino-5-androsten-17β-ol derivative **10**. ^{1}H-NMR spectrum displayed a broad singlet for *N*-methylenes of pyrrolidino functionality at δ 2.61 ppm. Further, acetylation of compound **10** with acetic anhydride using dry pyridine as catalyst afforded the 17β-acetoxy derivative **11**. The characteristic IR band for acetoxy group was present at 1725.6 cm^{-1}.

Biological Activity All newly synthesized 16-arylideno steroidal derivatives, selected for screening by NCI, exhibited significant cytotoxic effects in all the three cell lines at 100 mM and sensible cytotoxic effects in sixty cancer cell lines as shown in Tables 1 and 2. In general, it is observed that 16-arylidene group in steroids represents a good pharmacophore for cytotoxic activity. Although introduction of a pyrrolidine group at 3 position of 16-arylidene steroid skeleton again resulted in loss of cytotoxicity as is shown by data of **10** and which is also in agreement with earlier reports from our laboratory,[11] its 17-acetoxy 3-pyrrolidinyl counterpart **11** displayed good cytotoxic effects. It implies that presence of acetoxy group at 17 position is favorable for activity



Reagents and reaction conditions: (a) $NaBH_4$; (b) $(CH_3CO)O$/dry pyridine, steam bath, 2 h.

Chart 2. Synthetic Protocol of the Compounds **7** and **8**



Reagents and reaction conditions: (a) pyrrolidine/MeOH; (b) $NaBH_4$; (c) $(CH_3CO)O$/dry pyridine, steam bath, 2 h.

Chart 3. Synthetic Protocol of the Compounds **9**—**11**

Table 1. Growth Percentage at 10^{-4} M Concentration in 3-Cell Line *in Vitro* Cytotoxocity Screening

S. No.	Compound (code)	NSC No.	Growth percentage		
			Breast (MCF-7)	Non-small cell lung (NCI-H460)	CNS (SF-268)
1	**3**[a] (DPJ-RG-1151)	728325	9	0	1
2	**5**[a] (DPJ-RG-1177)	728324	8	0	1
3	**6**[a] (DPJ-RG-1196)	730461	4	1	7
4	**7**[a] (DPJ-RG-1219)	730478	0	0	16
5	**8**[a] (DPJ-RG-1227)	730479	1	0	11
6	**10** (DPJ-RG-1179)	728326	83	76	108
7	**11**[a] (DPJ-RG-1195)	730460	0	0	0

a) 3-Cell line actives.

Table 2. Mean Log Dose Response Parameters such as GI_{50}, TGI and LC_{50} of the 60-Cell Line Assay

S. No.	Compound No. (code)	Mean $\log_{10}$ GI_{50} (M)	Mean $\log_{10}$ TGI (M)	Mean $\log_{10}$ LC_{50} (M)
1	**3** (DPJ-RG-1151)	−4.74	−4.36	−4.09
2	**5** (DPJ-RG-1177)	−5.31	−4.73	−4.26
3	**6** (DPJ-RG-1196)	−5.02	−4.62	−4.38
4	**7** (DPJ-RG-1219)	−5.53	−4.90	−4.47
5	**8** (DPJ-RG-1227)	−5.10	−4.70	−4.42
6	**11** (DPJ-RG-1195)	−6.49	−5.40	−4.69

Table 3. Aromatase Inhibitory Data of Various Compounds

S. No.	Compound (code)	Inhibition on CYP 19[a)]	RP[b)]
1	**3** (DPJ-RG-1151)	48% inhibition at 36 μM	
2	**4** (DPJ-RG-1176)	26% inhibition at 36 μM	
3	**5** (DPJ-RG-1177)	IC_{50}=4.4 μM	6.8
4	**6** (DPJ-RG-1196)	46% inhibition at 36 μM	
5	**7** (DPJ-RG-1219)	IC_{50}=2.4 μM	12.4

a) [1β,2β-^{3}H]testosterone. *b*) Relative potency=relative to aminoglutethimide (RP=1; IC_{50}=28.5 μM).

and may lead to maintenance of cytotoxic effects even if unfavorable 3-pyrrolidinyl group is present as is depicted by cytotoxic effects of **11** and also the earlier studies.

Four imidazolyl substituted 16-arylideno steroids were screened for aromatase inhibitory activity and the data is presented in Table 3. It is anticipated that imidazole 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. Of these compounds, 4-ene-3-keto **5** (IC_{50}=4.4 μM) and diol derivative **7** (IC_{50}=2.4 μM) exhibited strong inhibition of the enzyme in comparison to 3-hydroxy (**3**) and 3-acetoxy (**6**) substituted steroids. 16-[4-{3-(Imidazol-1-yl)propoxy}-3-methoxybenzylidene]-5-androstene-3β,17β-diol (**7**) was found to be 13 times more potent in comparison to aminoglutethimide. It is observed that structural modifications of steroids lead to changes in three dimensional attachments of the compounds with the enzyme site.

Conclusion

It is concluded that 16-arylidene steroids represent an important pharmacophore for anticancer activity. Suitable structural modifications in steroid skeleton may lead to compounds with dual cytotoxic and antiaromatase properties. Further, the study provides new evidence showing the relationship between the chemical structure and biological function.

Experimental

Chemistry Melting points were determined on a Veego melting point apparatus and are uncorrected. IR (wavenumber in cm^{-1}) spectra were taken on a Perkin-Elmer spectrum RX 1 FT-IR spectrophotometer model using KBr pellets. ^{1}H-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 shift in δ, ppm). Elemental analyses were carried out on a Perkin-Elmer-2400 CHN elemental analyzer. Plates for thin layer chromatography (TLC) were prepared with silica gel G according to method of Stahl (E. Merck) using ethyl acetate as solvent and activated at 110 °C for 30 min. Iodine was used to develop the TLC plates. Anhydrous sodium sulphate was utilized as drying agent. All solvents were freshly distilled and dried prior to use according to standard procedures. All solvents were freshly distilled and dried prior to use according to standard procedures.

Synthesis of 16-[4-(3-Chloropropoxy)-3-methoxybenzylidene]-17-oxo-5-androsten-3β-ol (2) (DPJ-RG-1150) 1-Bromo-3-chloropropane (6.57 mmol) was added to a stirred and refluxing suspension of vanillin (6.57 mmol) and anhydrous potassium carbonate (2 g) in ethyl methyl ketone (100 ml). The reaction mixture was further refluxed for 6 h with continuous stirring. 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)-3-methoxybenzaldehyde (**1**),[14] which was used as such for further reaction.

A mixture of dehydroepiandrosterone (DHA) (2.60 mmol), above obtained oily residue **1** and sodium hydroxide (1 g) in methanol (10 ml) was stirred at room temperature for 2 h and the completion of reaction was monitored by TLC. The product was precipitated by addition of cold water and the precipitate obtained was filtered, washed with water, dried and crystallized from methanol to yield **2**.

Yield: 76.96%. mp: 210—212 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, s, 18-C$\underline{H}_3$), 1.08 (3H, s, 19-C$\underline{H}_3$), 2.31 (2H, m, –OCH$_2$C$\underline{H}_2$CH$_2$Cl), 3.53 (1H, m, 3α-$\underline{H}$), 3.77 (2H, t, –C$\underline{H}_2$Cl), 3.89 (3H, s, –OC$\underline{H}_3$), 4.21 (2H, t, –OC$\underline{H}_2$–), 5.40 (1H, d, 6-C$\underline{H}$), 6.95 (1H, d, J_o=8.27 Hz, 5-C$\underline{H}$, aromatic), 7.06 (1H, d, J_m=1.73 Hz, 2-C$\underline{H}$, aromatic), 7.16 (1H, dd, J_m=1.73 Hz, J_o=8.45 Hz, 6-C$\underline{H}$, aromatic), 7.38 (1H, s, vinylic-$\underline{H}$, 16-arylidene). FT-IR ν_{max} (KBr) cm^{-1}: 3220, 2922, 2829, 1709, 1623, 1593, 1515, 1447, 1325, 1260, 1140, 1094, 1057, 1023, 916, 806. *Anal.* Calcd for $C_{30}H_{39}O_4Cl$: C, 72.20; H, 7.88. Found: C, 72.40; H, 8.02.

16-[4-{3-(Imidazol-1-yl)propoxy}-3-methoxybenzylidene]-17-oxo-5-androsten-3β-ol (3) (DPJ-RG-1151) A mixture of **2** (1 mmol) and powdered imidazole (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 solid obtained was filtered, washed with water, dried and crystallized from ethyl acetate to yield **3**.

Yield: 71.47%. mp: 199—201 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, s, 18-C$\underline{H}_3$), 1.07 (3H, s, 19-C$\underline{H}_3$), 2.30 (2H, m, –OCH$_2$C$\underline{H}_2$CH$_2$N<), 3.54 (1H, m, 3α-$\underline{H}$), 3.91 (3H, s, –OC$\underline{H}_3$), 3.97 (2H, t, –C$\underline{H}_2$N<), 4.23 (2H, t, –OC$\underline{H}_2$–), 5.39 (1H, d, 6-C$\underline{H}$), 6.85 (1H, d, J_o=8.33 Hz, 5-C$\underline{H}$, aromatic), 6.93 (1H, s, 5-C$\underline{H}$, imidazole), 7.06 (2H, d, 2-C$\underline{H}$, aromatic and 4-C$\underline{H}$, imidazole), 7.13 (1H, d, J_o=8.26 Hz, 6-C$\underline{H}$, aromatic), 7.38 (1H, s, vinylic-$\underline{H}$, 16-arylidene) and 7.48 (1H, s, 2-C$\underline{H}$, imidazole). FT-IR ν_{max} (KBr) cm^{-1}: 3215, 2934, 1700, 1593, 1514, 1463, 1329, 1260, 1143, 1066, 917, 832. *Anal.* Calcd for $C_{33}H_{42}N_2O_4$: C, 74.68; H, 7.98; N, 5.28. Found: C, 74.62; H, 7.82; N, 5.31.

General Procedure for the Synthesis of Compounds 4 and 5 Compounds **2** and **3** (2 mmol) were 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 aluminium isopropoxide (1 g) in dry toluene (15 ml) drop wise. 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 solvents 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 **4** and **5**.

16-[4-{3-(Chloropropoxy)}-3-methoxybenzylidene]-4-androstene-3,17-dione (4) (DPJ-RG-1176) Yield: 70.28%. mp: 165—167 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, s, 18-C$\underline{H}_3$), 1.24 (3H, s, 19-C$\underline{H}_3$), 2.32 (2H, m, –OCH$_2$C$\underline{H}_2$CH$_2$Cl), 3.76 (2H, t, –C$\underline{H}_2$Cl), 3.85 (3H, s, –OC$\underline{H}_3$), 4.14 (2H, t, –OC$\underline{H}_2$–), 5.65 (1H, s, 4-C$\underline{H}$), 6.84 (1H, d, J_o=8.39 Hz, 5-C$\underline{H}$, aromatic), 6.95 (1H, d, J_m=1.14 Hz, 2-C$\underline{H}$, aromatic), 7.04 (1H, d, J_o=8.51 Hz, 6-C$\underline{H}$, aromatic), 7.26 (1H, s, vinylic-$\underline{H}$, 16-arylidene). FT-IR ν_{max} (KBr) cm^{-1}: 2939, 1709, 1665, 1614, 1594, 1511, 1464, 1421, 1328, 1262, 1141, 1095,

1030, 934, 864, 807. *Anal.* Calcd for $C_{30}H_{37}O_4Cl$: C, 72.49; H, 7.50. Found: C, 72.35; H, 7.62.

16-[4-{3-(Imidazol-1-yl)propoxy}-3-methoxybenzylidene]-4-androstene-3,17-dione (5) (DPJ-RG-1177) Yield: 75.28%. mp: 107—109 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.01 (3H, s, 18-C$\underline{H}_3$), 1.25 (3H, s, 19-C$\underline{H}_3$), 2.26 (2H, m, –OCH$_2$C$\underline{H}_2$CH$_2$N<), 3.92 (3H, s, –OC$\underline{H}_3$), 3.97 (2H, t, –C$\underline{H}_2$N<), 4.24 (2H, t, –OC$\underline{H}_2$–), 5.76 (1H, s, 4-C$\underline{H}$), 6.85 (1H, d, J_o=8.30 Hz, 5-C$\underline{H}$, aromatic), 6.94 (1H, s, 5-C$\underline{H}$, imidazole), 7.06 (2H, m, 2-C$\underline{H}$, aromatic and 4-C$\underline{H}$, imidazole), 7.13 (1H, dd, J_m=1.27 Hz, J_o=8.20 Hz, 6-C$\underline{H}$, aromatic), 7.39 (1H, s, vinylic-$\underline{H}$, 16-arylidene) and 7.50 (1H, s, 2-C$\underline{H}$, imidazole). FT-IR ν_{max} (KBr) cm^{-1}: 2936, 1712, 1666, 1622, 1595, 1512, 1463, 1328, 1260, 1230, 1141, 1094, 1027, 917, 810.6. *Anal.* Calcd for $C_{33}H_{40}N_2O_4$: C, 74.97; H, 7.63; N, 5.30. Found: C, 74.82; H, 7.66; N, 5.49.

16-[4-{3-(Imidazol-1-yl)propoxy}-3-methoxybenzylidene]-3-pyrrolidino-3,5-androsta-dien-17-one (9) (DPJ-RG-1178) Pyrrolidine (1 ml) was added to a refluxing solution of **5** (1.89 mmol) in methanol (10 ml). The reaction mixture was further refluxed for 1 h and chilled on ice. The crystalline material obtained was filtered, washed with methanol and dried to afford **9**.

Yield: 45.45%. mp: 148—150 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.07 (3H, s, 18-C$\underline{H}_3$), 1.14 (3H, s, 19-C$\underline{H}_3$), 2.30 (2H, m, –OCH$_2$C$\underline{H}_2$CH$_2$N<), 2.77 (4H, br s, –N-(C$\underline{H}_2$)$_2$–, pyrrolidine), 3.60 (3H, s, –OC$\underline{H}_3$), 3.97 (2H, s, –C$\underline{H}_2$N<), 4.27 (2H, t, –OC$\underline{H}_2$–), 4.97 (2H, m, 4-C$\underline{H}$ and 6-C$\underline{H}$) and 6.97—7.54 (7H, m, 2-C$\underline{H}$, 5-C$\underline{H}$, 6-C$\underline{H}$, aromatic; 2-C$\underline{H}$, 4-C$\underline{H}$, 5-C$\underline{H}$, imidazole and vinylic-$\underline{H}$,16-arylidene). FT-IR ν_{max} (KBr) cm^{-1}: 2938, 1711, 1656, 1623, 1595, 1513, 1460, 1419, 1376, 1328, 1260, 1143, 1096, 1027, 914, 852, 809.4.

General Procedure for the Synthesis of Compounds 7 and 10 To a stirred suspension of requisite keto steroid **3** and **9** (1.88 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 iced water was added to it. The precipitate obtained was filtered, washed with water, dried and crystallized from methanol to yield **7** and **10**, respectively.

16-[4-{3-(Imidazol-1-yl)propoxy}-3-methoxybenzylidene]-5-androstene-3β,17β-diol (7) (DPJ-RG-1219) Yield: 84.68%. mp: 197—198 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s, 18-C$\underline{H}_3$), 1.05 (3H, s, 19-C$\underline{H}_3$), 2.25 (2H, m, –OCH$_2$C$\underline{H}_2$CH$_2$N<), 3.53 (1H, m, 3α-$\underline{H}$), 3.89 (3H, s, –OC$\underline{H}_3$), 3.95 (2H, t, –C$\underline{H}_2$N<), 4.06 (1H, s, 17α-$\underline{H}$), 4.23 (2H, t, –OC$\underline{H}_2$), 5.38 (1H, d, 6-C$\underline{H}$), 6.45 (1H, s, vinylic-$\underline{H}$, 16-arylidene), 6.81 (1H, m, 5-C$\underline{H}$, aromatic), 6.93 (3H, m, 2-C$\underline{H}$, 6-C$\underline{H}$, aromatic and 5-C$\underline{H}$, imidazole), 7.07 (1H, s, 4-C$\underline{H}$, imidazole), 7.54 (1H, s, 2-C$\underline{H}$, imidazole). FT-IR ν_{max} (KBr) cm^{-1}: 3235, 2928, 1599, 1514, 1463, 1410, 1323, 1258, 1231, 1167, 1140, 1079, 1052, 949, 915, 798. *Anal.* Calcd for $C_{33}H_{44}N_2O_4$: C, 74.40; H, 8.33; N, 5.26. Found: C, 74.27; H, 8.39; N, 5.39.

16-[4-{3-(Imidazol-1-yl)propoxy}-3-methoxybenzylidene]-3β-pyrrolidino-5-androsten-17β-ol (10) (DPJ-RG-1179) Yield: 54.62%. mp: 249—251 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s, 18-C$\underline{H}_3$), 1.03 (3H, s, 19-C$\underline{H}_3$), 2.28 (2H, m, –OCH$_2$C$\underline{H}_2$CH$_2$N<), 2.61 (4H, br s, –N-(C$\underline{H}_2$)$_2$–, pyrrolidine), 3.88 (3H, s, –OC$\underline{H}_3$), 3.94 (2H, t, –C$\underline{H}_2$N<), 4.04 (1H, s, 17α-$\underline{H}$), 4.22 (2H, t, –OC$\underline{H}_2$–), 5.36 (1H, d, 6-C$\underline{H}$), 6.45 (1H, s, vinylic-$\underline{H}$, 16-arylidene), 6.80 (1H, d, J_o=8.78 Hz, 5-C$\underline{H}$, aromatic), 6.93 (3H, m, 2-C$\underline{H}$, 6-C$\underline{H}$, aromatic and 5-C$\underline{H}$, imidazole), 7.05 (1H, s, 4-C$\underline{H}$, imidazole), 7.49 (1H, s, 2-C$\underline{H}$, imidazole). FT-IR ν_{max} (KBr) cm^{-1}: 3184, 2930, 2784, 1600, 1513, 1462, 1382, 1325, 1254, 1135, 1075, 1029, 949, 913, 796. *Anal.* Calcd for $C_{37}H_{51}N_3O_3$: C, 75.86; H, 8.78; N, 7.17. Found: C, 75.92; H, 8.98; N, 7.22.

General Procedure for the Synthesis of Compounds 6, 8 and 11 A mixture of respective hydroxyl derivative **3**, **7** (0.94 mmol) and **10** (0.85 mmol), acetic anhydride (1 ml) and dry pyridine (2 ml, 0.5 ml was used for **11**) was heated in a steam bath for 2 h. The reaction contents were then poured into cold water and basified with liquid ammonia. The precipitate obtained was filtered, washed with water, dried and crystallized from *n*-hexane to afford corresponding acetoxy steroids **6**, **8** and **11**.

16-[4-{3-(Imidazol-1-yl)propoxy}-3-methoxybenzylidene]-17-oxo-5-androsten-3β-yl Acetate (6) (DPJ-RG-1196) Yield: 50.04%. mp: 109—111 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, s, 18-C$\underline{H}_3$), 1.09 (3H, s, 19-C$\underline{H}_3$), 2.04 (3H, s, –OCOC$\underline{H}_3$), 2.29 (2H, m, –OCH$_2$C$\underline{H}_2$CH$_2$N<), 3.91 (3H, s, –OC$\underline{H}_3$), 3.98 (2H, t, –C$\underline{H}_2$N<), 4.23 (2H, t, –OC$\underline{H}_2$–), 4.61 (1H, m, 3α-$\underline{H}$), 5.42 (1H, d, 6-C$\underline{H}$), 6.85 (1H, d, J_o=8.48 Hz, 5-C$\underline{H}$, aromatic), 6.93 (1H, s, 5-C$\underline{H}$, imidazole), 7.07 (2H, m, 2-C$\underline{H}$, aromatic and 4-C$\underline{H}$, imidazole), 7.12 (1H, dd, J_m=1.44 Hz, J_o=8.26 Hz, 6-C$\underline{H}$, aromatic), 7.38 (1H, s, vinylic-$\underline{H}$, 16-arylidene), 7.50 (1H, s, 2-C$\underline{H}$, imidazole). FT-IR ν_{max} (KBr) cm^{-1}: 2942, 1729, 1628, 1596, 1513, 1465, 1371, 1325, 1248, 1139, 1095, 1029, 915, 812. *Anal.* Calcd for $C_{35}H_{44}N_2O_5$: C, 73.40; H, 7.74; N, 4.89. Found: C, 73.52; H, 7.95; N, 5.01.

16-[4-{3-(Imidazol-1-yl)propoxy}-3-methoxybenzylidene]-5-androstene-3β,17β-diol Diacetate (8) (DPJ-RG-1227) Yield: 65.64%. mp: 163—165 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.80 (3H, s, 18-C$\underline{H}_3$), 1.05 (3H, s, 19-C$\underline{H}_3$), 2.04 (3H, s, 3β-OCOC$\underline{H}_3$), 2.22 (3H, s, 17β-OCOC$\underline{H}_3$), 2.26 (2H, m, –OCH$_2$C$\underline{H}_2$CH$_2$N<), 3.89 (3H, s, –OC$\underline{H}_3$), 3.95 (2H, t, –C$\underline{H}_2$N<), 4.23 (2H, t, –OC$\underline{H}_2$–), 4.61 (1H, m, 3α-$\underline{H}$), 5.37 (1H, s, 17α-$\underline{H}$), 5.40 (1H, d, 6-C$\underline{H}$), 6.15 (1H, s, vinylic-$\underline{H}$, 16-arylidene), 6.80 (1H, d, J_o=8.08 Hz, 5-C$\underline{H}$, aromatic), 6.89 (2H, s, 2-C$\underline{H}$, aromatic and 5-C$\underline{H}$, imidazole), 6.93 (1H, dd, J_m=1.78 Hz, J_o=8.86 Hz, 6-C$\underline{H}$, aromatic), 7.06 (1H, s, 4-C$\underline{H}$, imidazole), 7.54 (1H, s, 2-C$\underline{H}$, imidazole). FT-IR ν_{max} (KBr) cm^{-1}: 2938, 1733, 1595, 1512, 1443, 1371, 1239, 1141, 1034, 804. *Anal.* Calcd for $C_{37}H_{48}N_2O_6$: C, 72.05; H, 7.84; N, 4.54. Found: C, 72.14; H, 7.72; N, 4.66.

16-[4-{3-(Imidazol-1-yl)propoxy}-3-methoxybenzylidene]-3β-pyrrolidino-5-androsten-17β-yl Acetate (11) (DPJ-RG-1195) Yield: 59.71%. mp: 95—97 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.77 (3H, s, 18-C$\underline{H}_3$), 1.03 (3H, s, 19-C$\underline{H}_3$), 2.21 (1H, s, –OCOC$\underline{H}_3$), 2.28 (2H, m, –OCH$_2$C$\underline{H}_2$CH$_2$N<), 2.62 (4H, br s, –N-(C$\underline{H}_2$)$_2$–, pyrrolidine), 3.88 (3H, s, –OC$\underline{H}_3$), 3.95 (2H, t, –C$\underline{H}_2$N<), 4.22 (2H, t, –OC$\underline{H}_2$–), 5.37 (2H, s, 6-C$\underline{H}$ and 17α-$\underline{H}$), 6.15 (1H, s, vinylic-$\underline{H}$, 16-arylidene), 6.80 (1H, d, J_o=8.09 Hz, 5-C$\underline{H}$, aromatic), 6.89 (2H, s, 2-C$\underline{H}$, aromatic and 5-C$\underline{H}$, imidazole), 6.92 (1H, m, 6-C$\underline{H}$, aromatic), 7.05 (1H, s, 4-C$\underline{H}$, imidazole), 7.49 (1H, s, 2-C$\underline{H}$, imidazole). FT-IR ν_{max} (KBr) cm^{-1}: 2937, 1725, 1599, 1512, 1463, 1374, 1240, 1142, 1040, 964, 807. *Anal.* Calcd for $C_{39}H_{53}N_3O_4$: C, 74.60; H, 8.51; N, 6.69. Found: C, 74.65; H, 8.42; N, 6.74.

Antineoplastic Activity The synthesized compounds were screened at National Cancer Institute, Bethesda, U.S.A. for *in vitro* and *in vivo* antineoplastic activity.

3-Cell Line Assay The compounds **3**, **5**—**8**, **10**, **11** were selected by Drug Synthesis and Chemistry Branch, National Cancer Institute, based in general, on the basis of degree of novelty of the structure and computer modeling techniques for anticancer screening. Firstly, they were assayed using one dose (10^{-4} M) primary anticancer *in vitro* assay against tumor in the 3-cell line panel consisting of MCF-7 (breast), NCI-H460 (lung) and SF-268 central nervous system (CNS) (Table 1) and then were passed on for evaluation in the full panel of 60-cell lines over a 5-log dose range.

60-Cell Line Assay The 3-cell line actives meaning the compounds, which reduced the growth of any one of the cell lines to approximately 32% or less, were assayed *in vitro* against a panel consisting of 60 human tumor cell lines, derived from nine cancer types (leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancers), using five concentrations at 10-fold dilutions, the highest being 10^{-4} M. A 48 h continuous drug protocol was used and a sulforhodamine B (SRB) protein assay was used to estimate the cell viability or growth.[15,16] Mean log dose response parameters such as GI_{50} (drug concentration resulting in a 50% reduction in the net protein increase), TGI (drug concentration of total growth inhibition) and LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) are summarized in Table 2. Two standard drugs, meaning that their activities against the cell lines are well documented, were tested against each cell line: NSC 19893 (5-Fluorouracil) and NSC 123127 (Adriamycin).

In general, a compound is selected for *in vivo* studies if its mean $\log_{10} GI_{50} \leq -6$ in 60-cell line assay. The total pattern of activity of the compounds is also taken into consideration by the Biological Evaluation Committee for Cancer Drugs to select the compound for further *in vivo* evaluation.

Aromatase Inhibitory Activity. Preparation of Aromatase The enzyme was obtained from the microsomal fraction of freshly delivered human term placental tissue according to the procedure of Thompson and Siiteri.[17] 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 four months.

Inhibition of Aromatase *in Vitro* The assay was performed similar to the described methods[18,19] monitoring the enzyme activity by measuring the ^3H_2O formed from [1β, 2β-3H] testosterone during aromatization. Each incubation tube contained 0.225 μCi of [1β, 2β-3H] testosterone, 5 μM unlabeled testosterone, 2 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 20 mM glucose-6-phosphate, 1EU glucose-6-phosphate dehydrogenase and inhibitor (0—250 μM) in phosphate buffer (0.05 M, pH 7.4). The test compound had been dissolved in EtOH and diluted with buffer. The final ethanol concentration of the control and inhibitor incubation was 2%. Each tube was preincubated for 5 min at 30 °C in a shaking water bath. Microsomal protein (0.5 mg) was added to start the reaction. The total volume of each incubation was 0.5 ml. The reaction was terminated by withdrawing

100 μl aliquots at 0, 7, 14 and 21 min and pipetting them into 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 1500 ***g*** for 5 min to separate the charcoal-adsorbed steroids. Aliquots of the supernatant were assayed for ^3H_2O by counting in a scintillation mixture in a 1209 Rackbeta Wallac liquid scintillation spectrometer (Pharmacia LKB, Freiburg, Germany).

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References

1) Sharma S. K., Weizhong Z., Alummoottil V., Douglas N., Abrams A. J. B., McEwan A., *Bioorg. Med. Chem. Lett.*, **18**, 5563—5566 (2008).
2) Thompson E. A., Siiteri P. K., *J. Biol. Chem.*, **249**, 5373—5378 (1974).
3) Simpson E. R., Mahendroo M. S., Means G. D., Kilgore M. W., Hinshelwood M. M., Graham-Lorence S., *Endocr. Rev.*, **15**, 342—355 (1994).
4) Djurendi E., Daljeva J., Sakac M., Canadi J., Santa S. J., Andric S., Klisuric O., Kojic V., Bogdanovic G., Djurendic-Brenesela M., Novakovic S., Gasi K. P., *Steroids*, **73**, 129—138 (2008).
5) Baum M., Budzar A. U., Cuzick J., Forbes J., Houghton J. H., Klijn J. G., Sahmoud T., *Lancet*, **359**, 2131—2139 (2002).
6) Smith I. E., Dowsett M., *New Engl. J. Med.*, **348**, 2431—2442 (2003).
7) Plourde P. V., Dyroff M., Dukes M., *Breast Cancer Res. Treat.*, **30**, 103—111 (1994).
8) Christiansen R. G., Clinton R. O., Greenbush E., Dean J. W., U.S. Patent 3163641 (1965).
9) Schenider F., Hamsher J., Beyler R. E., *Steroids*, **8**, 553—563 (1966).
10) Numazawa M., Osawa Y., *Steroids*, **38**, 149—159 (1981).
11) Bansal R., Guleria S., *Steroids*, **73**, 1391—1399 (2008).
12) Chattopadhaya R., Jindal D. P., Minu M., Gupta R., *Arzneim-Forsch/Drug Res.*, **54**, 551—556 (2004).
13) Dubey S., Piplani P., Jindal D. P., *Chem. Biodivers.*, **1**, 1529—1536 (2004).
14) Bansal R., Kumar R. S., Kumar G., Thota S., Thamotharan S., Parthasarathi V., Linden A., *J. Heterocycle. Chem.*, **45**, 1789—1795 (2008).
15) Grever M. R., Schepartz S. A., Chabner, B. A., *Semin. Oncol.*, **19**, 622—638 (1992).
16) Boyd M. R., Paul K. D., *Drug Dev. Res.*, **34**, 91—109 (1995).
17) Thompson E. A., Siiteri P. K., *J. Biol. Chem.*, **249**, 5364—5372 (1974).
18) Foster A. B., Jarman M., Leung S., Rowlands M. G., Taylor G. N., *J. Med. Chem.*, **26**, 50—54 (1983).
19) Graves P. E., Salhanick H. A., *Endocrinology*, **105**, 52—57 (1979).