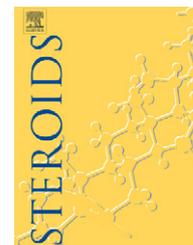


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Synthesis of 16E-[3-methoxy-4-(2-aminoethoxy)-benzylidene]androstene derivatives as potent cytotoxic agents

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ABSTRACT

The synthesis and cytotoxic studies of a new series of 16E-arylidene androstene derivatives are reported herein. The impact of incorporating bis-tertiary amino functionalities in the steroid skeleton on cytotoxicity has also been observed. The compounds have been evaluated at National cancer Institute, Bethesda, Maryland, USA for their antineoplastic activity against various tumor cell lines. The synthesized 16E-arylidenosteroids exhibited significant cytotoxicity. Bis-tertiary amino steroid **29** possessing a diethylaminoalkoxy functionality was the most promising compound of the series with a total IP and SC score of 20 in *in vivo* hollow fiber assay and was selected for further detailed *in vivo* xenograft testing.

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1. Introduction

Steroidal alkylating agents have received sporadic attention as potential antitumor agents for the past few years [1]. The research efforts have mainly been focused towards reducing systemic toxicity and improving specificity of cancer therapy using lipophilic steroid nuclei as carriers to deliver the alkylating agents such as nitrogen and sulphur mustards to a specific target tissue with ease [2,3]. Further, as expected the synergistic activity out of these hybrid steroidal structures was also obtained.

Interference with neuromuscular transmission has been reported to be a side effect of potent antitumor agent vincristine [4]. Comparison of its molecular structure with potent neuromuscular blockers indicated drug-receptor similarities owing to presence of common structural features like well-spaced two tertiary amino groups in lipophilic

steroid nucleus [5]. Investigation of a homologous series of amino steroids for antitumor activity indicated the essential requirement of two piperidine substituents. Among these, 2,16-bis(amino) steroid; 2 β ,16 β -dipiperidino-5 α -androstane-3,17-diol dipivalate hydrochloride (**1**) (DAP) (Fig. 1), showed significant antitumor activity against transplantable tumors. The acute toxicity of DAP was similar to that of cyclophosphamide and considerably lower than vinblastine [6].

A large number of potent steroidal derivatives with substitution at position 16 have been described in the literature as potent cytotoxic agents [7–9]. Recently some interesting 16E-arylidene androstene derivatives (**2**) (Fig. 1) have also been reported from our laboratory as strong *in vitro* inhibitors of the growth of many types of human tumor cells [10,11]. In view of the potent cytotoxic activity of bis-tertiary amino steroids and that of new 16E-arylidenosteroids, we decided to further explore the anticancer properties of 16-substituted

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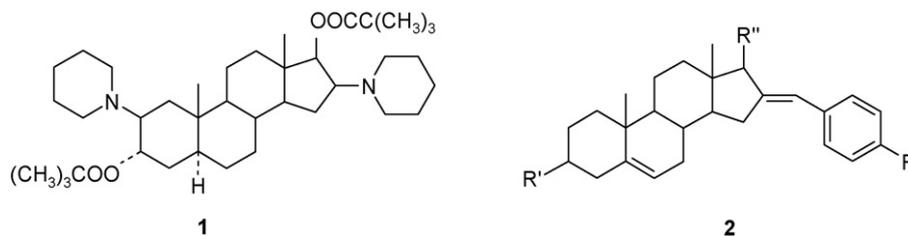


Fig. 1 – Structures of DAP and 16-benzylidene steroids.

heterosteroidal derivatives by synthesizing new analogues with suitable structural modifications and also to observe the impact of incorporating bis-tertiary amino functionalities in these steroidal products.

2. Experimental

2.1. General

Melting points were determined on a Veego melting point apparatus and are uncorrected. UV (wavelengths in nm) were recorded on Lambda 15 and IR (wavenumbers in cm^{-1}) spectra on PerkinElmer 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 dimethylsulphoxide ($\text{DMSO}-d_6$) containing tetramethylsilane as internal standard (chemical shifts in δ , ppm). Elemental analyses were carried out on a PerkinElmer-2400 model CHN analyzer. All solvents were distilled prior to use according to standard procedures.

2.2. General method for the preparation of compounds 3–5

Hydrochloride of requisite dialkylaminoethyl chloride (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 until the reaction was completed (monitored by TLC). The reaction mixture was cooled, filtered and solvent was removed under reduced pressure to obtain the corresponding oily residue (3–5), which was used as such for subsequent reaction.

2.3. General method for the preparation of compounds 6–8

A mixture of dehydroepiandrosterone (2.60 mmol) appropriate aldehyde (oily residues, 3–5) and sodium hydroxide (1 g) in methanol (20 ml) was stirred at room temperature for 2 h until the reaction was completed (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 6–8.

2.3.1. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}-benzylidene]-17-oxo-5-androsten-3 β -ol (6) (DPJ-RG-1071)

Yield: 97.3%, m.p., 212–214 °C. UV_{max} (MeOH): 242.0 ($\log \epsilon$ 3.99) and 330.6 ($\log \epsilon$ 4.31). IR: 3240 (O–H), 1711 (C=O), 1622 (C=C), 1094 (C–O). ^1H NMR (CDCl_3): 0.98 (s, 3H, H-18); 1.07 (s, 3H, H-19); 2.52 (m, 4H, $-\text{N}(\text{CH}_2)_2-$); 2.83 (t, 2H, $-\text{CH}_2\text{N}<$); 3.53 (m, 1H, H-3 α); 3.89 (s, 3H, $-\text{OCH}_3$); 4.19 (t, 2H, $-\text{OCH}_2-$); 5.40 (d, 1H, H-6); 6.93 (d, 1H, $J_{5',6'} = 8.25$ Hz, H-5'); 7.06 (s, 1H, H-2'); 7.16 (dd, 1H, $J_{2',6'} = 1.51$ Hz, $J_{5',6'} = 8.37$ Hz, H-6'); 7.38 (s, 1H, H-vinyl). Anal. calcd for $\text{C}_{34}\text{H}_{47}\text{NO}_4$: C, 76.50; H, 8.87; N, 2.62; found: C, 76.22; H, 9.07; N, 2.71.

2.3.2. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}-benzylidene]-17-oxo-5-androsten-3 β -ol (7) (DPJ-RG-1147)

Yield: 92.1%, m.p., 210–212 °C. UV_{max} (MeOH): 246.6 ($\log \epsilon$ 3.83) and 331.0 ($\log \epsilon$ 4.21). IR: 3238 (O–H), 1710 (C=O), 1624 (C=C), 1095 (C–O). ^1H NMR (CDCl_3): 0.97 (s, 3H, H-18); 1.07 (s, 3H, H-19); 2.65 (m, 4H, $-\text{N}(\text{CH}_2)_2-$); 2.97 (t, 2H, $-\text{CH}_2\text{N}<$); 3.50 (m, 1H, H-3 α); 3.88 (s, 3H, $-\text{OCH}_3$); 4.20 (t, 2H, $-\text{OCH}_2-$); 5.39 (d, 1H, H-6); 6.93 (d, 1H, $J_{5',6'} = 8.39$ Hz, H-5'); 7.05 (d, 1H, $J_{2',6'} = 1.84$ Hz, H-2'); 7.15 (dd, 1H, $J_{2',6'} = 1.73$ Hz, $J_{5',6'} = 8.39$ Hz, H-6'); 7.38 (s, 1H, H-vinyl). Anal. calcd for $\text{C}_{33}\text{H}_{45}\text{NO}_4$: C, 76.26; H, 8.73; N, 2.70; found: C, 76.01; H, 9.02; N, 2.55.

2.3.3. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-17-oxo-5-androsten-3 β -ol (8) (DPJ-RG-1097)

Yield: 93.4%, m.p., 176–178 °C. UV_{max} (MeOH): 242.8 ($\log \epsilon$ 4.02) and 330.8 ($\log \epsilon$ 4.35). IR: 3251 (O–H), 1711 (C=O), 1622 (C=C). ^1H NMR (CDCl_3): 0.97 (s, 3H, H-18); 1.07 (t, 6H, $-\text{N}(\text{CH}_2\text{CH}_3)_2$ and s (merged), 3H, H-19); 2.65 (q, 4H, $-\text{N}(\text{CH}_2)_2$); 2.90 (t, 2H, $-\text{CH}_2\text{N}<$); 3.51 (m, 1H, H-3 α); 3.89 (s, 3H, $-\text{OCH}_3$); 4.13 (t, 2H, $-\text{OCH}_2-$); 5.38 (d, 1H, H-6); 6.93 (d, 1H, $J_{5',6'} = 8.29$ Hz, H-5'); 7.05 (d, 1H, $J_{2',6'} = 1.51$ Hz, H-2'); 7.15 (dd, 1H, $J_{2',6'} = 1.59$ Hz, $J_{5',6'} = 8.43$ Hz, H-6'); 7.38 (s, 1H, H-vinyl). Anal. calcd for $\text{C}_{33}\text{H}_{47}\text{NO}_4$: C, 75.97; H, 9.08; N, 2.68; found: C, 75.99; H, 9.24; N, 2.71.

2.4. General Method for the preparation of compounds 9–11

A mixture of aldol products 6–8 (0.94 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 cold water and basified with liquor ammonia. The precipitate obtained was filtered, washed with water, dried and crystallized from n-hexane to yield 9–11.

2.4.1. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}-benzylidene]-17-oxo-5-androsten-3 β -yl acetate (9) (DPJ-RG-1111)

Yield: 70.4%, m.p., 126–128 °C. UV_{max} (MeOH): 242.8 (log ϵ 3.82) and 332.0 (log ϵ 4.36). IR: 1722 (C=O), 1617 (C=C) 1098 (C–O). ¹H NMR (CDCl₃): 0.97 (s, 3H, H-18); 1.08 (s, 3H, H-19); 2.03 (s, 3H, –OCOCH₃); 2.47–2.52 (m, 4H, –N(CH₂)₂–); 2.84 (t, 2H, –CH₂N<); 3.89 (s, 3H, –OCH₃); 4.18 (t, 2H, –OCH₂–); 4.60 (m, 1H, H-3 α); 5.42 (d, 1H, H-6); 6.92 (d, 1H, $J_{5',6'} = 8.27$ Hz, H-5'); 7.05 (d, 1H, $J_{2',6'} = 1.40$ Hz, H-2'); 7.16 (dd, 1H, $J_{2',6'} = 1.29$ Hz, $J_{5',6'} = 8.32$ Hz, H-6'); 7.37 (s, 1H, H-vinyl). Anal. calcd for C₃₆H₄₉NO₅: C, 75.09; H, 8.58; N, 2.43; found: C, 75.10; H, 8.51; N, 2.22.

2.4.2. 16-(3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}-benzylidene)-17-oxo-5-androsten-3 β -yl acetate (10) (DPJ-RG-1175)

Yield: 66.6%, m.p., 114–115 °C. UV_{max} (MeOH): 246.8 (log ϵ 3.74) and 331.0 (log ϵ 4.15). IR: 1724 (C=O), 1625 (C=C) 1249 (C(=O)–O), 1097 (C–O). ¹H NMR (CDCl₃): 0.94 (s, 3H, H-18); 1.03 (s, 3H, H-19); 2.08 (s, 3H, –OCOCH₃); 2.59 (m, 4H, –N(CH₂)₂–); 2.90 (t, 2H, –CH₂N<); 3.85 (s, 3H, –OCH₃); 4.10 (t, 2H, –OCH₂–); 4.52 (m, 1H, H-3 α); 5.40 (d, 1H, H-6); 6.84 (d, 1H, $J_{5',6'} = 8.43$ Hz, H-5'); 6.95 (d, 1H, $J_{2',6'} = 1.35$ Hz, H-2'); 7.06 (dd, 1H, $J_{2',6'} = 1.36$ Hz, $J_{5',6'} = 8.47$ Hz, H-6'); 7.26 (s, 1H, H-vinyl). Anal. calcd for C₃₅H₄₇NO₅: C, 74.83; H, 8.43; N, 2.49; found: C, 74.69; H, 8.71; N, 2.38.

2.4.3. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-17-oxo-5-androsten-3 β -yl acetate (11) (DPJ-RG-1146)

Yield: 66.7%, m.p., 118–120 °C. UV_{max} (MeOH): 242.8 (log ϵ 4.03) and 330.8 (log ϵ 4.36). IR: 1731 (C=O), 1627 (C=C) 1247 (C(=O)–O), 1095 (C–O). ¹H NMR (CDCl₃): 0.97 (s, 3H, H-18); 1.07 (t, 6H, –N(CH₂CH₃)₂); 1.09 (s, 3H, H-19); 2.03 (s, 3H, –OCOCH₃); 2.64 (q, 2H, –N(CH₂CH₃)₂); 2.94 (t, 2H, –CH₂N<); 3.88 (s, 3H, –OCH₃); 4.11 (t, 2H, –OCH₂–); 4.59 (m, 1H, H-3 α); 5.42 (d, 1H, H-6); 6.91 (d, 1H, $J_{5',6'} = 8.49$ Hz, H-5'); 7.03 (d, 1H, $J_{2',6'} = 1.78$ Hz, H-2'); 7.13 (dd, 1H, $J_{2',6'} = 1.57$ Hz, $J_{5',6'} = 8.47$ Hz, H-6'); 7.39 (s, 1H, H-vinyl). Anal. calcd for C₃₅H₄₉NO₅: C, 74.56; H, 8.76; N, 2.48; found: C, 74.55; H, 8.92; N, 2.51.

2.5. General method for the preparation of compounds 12–14

To the stirred suspension of aldol products 6–8 (1.9 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 4 h. Solvent was removed under reduced pressure and ice water was added to it. The precipitate formed was filtered, washed with water, dried and crystallized from method to yield 12–14.

2.5.1. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}-benzylidene]-5-androstene-3 β ,17 β -diol (12) (DPJ-RG-1214)

Yield: 85%, m.p., 174–176 °C. UV_{max} (MeOH): 263.8 (log ϵ 3.95). IR: 3363 (O–H), 1660 (C=C). ¹H NMR (CDCl₃): 0.71 (s, 3H, H-18); 1.04 (s, 3H, H-19); 2.61 (m, 4H, –N(CH₂)₂–); 2.80 (t, 2H, –CH₂N<); 3.49 (m, 1H, H-3 α); 3.85 (s, 3H, –OCH₃); 3.99 (s, 1H, H-17 α); 4.12 (t, 2H, –OCH₂–); 5.33 (d, 1H, H-6); 6.39 (d, 1H, H-vinyl); 6.80–6.89

(m, 3H, H-2', H-5' and H-6'). Anal. calcd for C₃₄H₄₉NO₄: C, 76.22; H, 9.22; N, 2.61; found: C, 76.35; H, 9.29; N, 2.67.

2.5.2. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}-benzylidene]-5-androstene-3 β ,17 β -diol (13) (DPJ-RG-1217)

Yield: 89.6%, m.p., 113–115 °C. UV_{max} (MeOH): 248.8 (log ϵ 4.0). IR: 3368 (O–H), 1659 (C=C). ¹H NMR (CDCl₃): 0.72 (s, 3H, H-18); 1.04 (s, 3H, H-19); 2.66 (m, 4H, –N(CH₂)₂–); 2.96 (t, 2H, –CH₂N<); 3.53 (m, 1H, H-3 α); 3.86 (s, 3H, –OCH₃); 4.05 (s, 1H, H-17 α); 4.15 (t, 2H, –OCH₂–); 5.37 (d, 1H, H-6); 6.44 (d, 1H, H-vinyl); 6.86–6.96 (m, 3H, H-2', H-5' and H-6'). Anal. calcd for C₃₃H₄₇NO₄: C, 75.97; H, 9.08; N, 2.68; found: C, 75.99; H, 8.91; N, 2.61.

2.5.3. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-5-androstene-3 β ,17 β -diol (14) (DPJ-RG-1216)

Yield: 79.7%, m.p., 97–99 °C. UV_{max} (MeOH): 264.2 (log ϵ 4.39). IR: 3403 (O–H), 1595 (C=C). ¹H NMR (CDCl₃): 0.72 (s, 3H, H-18); 1.07 (t, 6H, –N(CH₂CH₃)₂ and s (merged), 3H, H-19); 2.65 (q, 4H, –N(CH₂CH₃)₂); 2.93 (t, 2H, –CH₂N<); 3.54 (m, 1H, H-3 α); 3.86 (s, 3H, –OCH₃); 4.05 (s, 1H, H-17 α); 4.10 (t, 2H, –OCH₂–); 5.38 (d, 1H, H-6); 6.44 (d, 1H, H-vinyl); 6.87 (d, 1H, $J_{5',6'} = 8.25$ Hz, H-5'); 6.91 (d, 1H, $J_{2',6'} = 1.73$ Hz, H-2'); 6.95 (dd, 1H, $J_{2',6'} = 1.71$ Hz, $J_{5',6'} = 8.35$ Hz, H-6'). Anal. calcd for C₃₃H₄₉NO₄: C, 75.68; H, 9.43; N, 2.67; found: C, 75.91; H, 9.68; N, 2.56.

2.6. General procedure for the preparation of compounds 15–17

A mixture of diol 12–14 (0.93 mmol), acetic anhydride (1 ml) and dry pyridine (2 ml) was heated in a steam bath for 2 h. The contents of the reaction mixture were then poured into cold water and basified with liquor ammonia. The precipitate obtained was collected by filtration, washed with water, dried and crystallized from *n*-hexane to yield 15–17.

2.6.1. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}-benzylidene]-5-androstene-3 β ,17 β -diol diacetate (15) (DPJ-RG-1215)

Yield: 69.2%, m.p., 152–153 °C. UV_{max} (MeOH): 265.8 (log ϵ 4.25). IR: 1735 (C=O), 1600 (C=C), 1237 (C(=O)–O). ¹H NMR (CDCl₃): 0.79 (s, 3H, H-18); 1.07 (s, 3H, H-19); 2.03 (s, 3H, OCOCH₃-3 β); 2.22 (s, 3H, OCOCH₃-17 β); 2.51 (t, 4H, –N(CH₂)₂–); 2.80 (t, 2H, –CH₂N<); 3.86 (s, 3H, –OCH₃); 4.15 (t, 2H, –OCH₂–); 4.63 (m, 1H, H-3 α); 5.36 (s, 1H, H-17 α); 5.40 (d, 1H, H-6); 6.14 (d, 1H, H-vinyl); 6.84–6.93 (m, 3H, H-2', H-5' and H-6'). Anal. calcd for C₃₈H₅₃NO₆: C, 73.63; H, 8.62; N, 2.26; found: C, 73.66; H, 8.96; N, 2.30.

2.6.2. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}-benzylidene]-5-androstene-3 β ,17 β -diol diacetate (16) (DPJ-RG-1218)

Yield: 68.9%, m.p., 157–158 °C. UV_{max} (MeOH): 250.4 (log ϵ 4.14). IR: 1736 (C=O), 1601 (C=C). ¹H NMR (CDCl₃): 0.79 (s, 3H, H-18); 1.05 (s, 3H, H-19); 2.03 (s, 3H, OCOCH₃-3 β); 2.20 (s, 3H, OCOCH₃-17 β); 2.63 (t, 4H, –N(CH₂)₂–); 2.95 (t, 2H, –CH₂N<); 3.86 (s, 3H, –OCH₃); 4.17 (t, 2H, –OCH₂–); 4.61 (m, 1H, H-3 α); 5.36 (s, 1H, H-17 α); 5.40 (d, 1H, H-6); 6.14 (d, 1H, H-vinyl); 6.85–6.93 (m, 3H, H-2', H-5' and H-6'). Anal. calcd for C₃₇H₅₁NO₆: C, 73.35; H, 8.49; N, 2.31; found: C, 73.66; H, 8.29; N, 2.01.

2.6.3. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-5-androstene-3 β ,17 β -diol diacetate (17) (DPJ-RG-1226)

Yield: 69%, m.p., 87–88 °C. UV_{max} (MeOH): 265.6 (log ϵ 4.14). IR: 1736 (C=O), 1600 (C=C), 1238 (C(=O)–O). ¹H NMR (CDCl₃): 0.79 (s, 3H, H-18); 1.05 (s, 3H, H-19); 1.12 (t, 6H, –N(CH₂CH₃)₂); 2.03 (s, 3H, OCOCH₃-3 β); 2.20 (s, 3H, OCOCH₃-17 β); 2.72 (t, 4H, –N(CH₂)₂–); 3.01 (t, 2H, –CH₂N<); 3.85 (s, 3H, –OCH₃); 4.15 (t, 2H, –OCH₂–); 4.61 (m, 1H, H-3 α); 5.36 (s, 1H, H-17 α); 5.40 (d, 1H, H-6); 6.15 (d, 1H, H-vinyl); 6.85–6.93 (m, 3H, H-2', H-5' and H-6'). Anal. calcd for C₃₇H₅₃NO₆: C, 73.11; H, 8.79; N, 2.30; found: C, 73.12; H, 9.01; N, 2.41.

2.7. General method for the preparation of compounds 18–20

The aldol products 6–8 (1.92 mmol) were dissolved in a mixture of cyclohexanone (10 ml) and dry toluene (150 ml). Azeotropic distillation was continued at a slow rate while adding a solution of aluminium isopropoxide (1 g) in dry toluene (15 ml) dropwise. The reaction mixture was then 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 solid product obtained was filtered, washed with water, dried and treated with diethyl ether and *n*-hexane to yield crystals of 18–20.

2.7.1. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}-benzylidene]-4-androstene-3,17-dione (18) (DPJ-RG-1072)

Yield: 100%, m.p., 88–90 °C. UV_{max} (MeOH): 240.8 (log ϵ 4.36) and 331.2 (log ϵ 4.30). IR: 1713 (C=O), 1623 (C=C), 1093 (C–O). ¹H NMR (CDCl₃): 1.01 (s, 3H, H-18); 1.25 (s, 3H, H-19); 2.50 (m, 4H, –N(CH₂)₂–); 2.84 (t, 2H, –CH₂N<); 3.90 (s, 3H, –OCH₃); 4.18 (t, 2H, –OCH₂–); 5.76 (s, 1H, H-4); 6.93 (d, 1H, *J*_{5',6'} = 8.34 Hz, H-5'); 7.05 (s, 1H, H-2'); 7.15 (d, 1H, *J*_{5',6'} = 8.66 Hz, H-6'); 7.39 (s, 1H, H-vinyl). Anal. calcd for C₃₄H₄₅NO₄: C, 76.79; H, 8.53; N, 2.63; found: C, 76.49; H, 8.72; N, 2.59.

2.7.2. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}-benzylidene]-4-androstene-3,17-dione (19) (DPJ-RG-1148)

Yield: 100%, m.p., 104–106 °C. UV_{max} (MeOH): 241.0 (log ϵ 4.40) and 330.8 (log ϵ 4.35). IR: 1713 (C=O), 1623 (C=C), 1095 (C–O). ¹H NMR (CDCl₃): 1.00 (s, 3H, H-18); 1.25 (s, 3H, H-19); 2.63 (m, 4H, –N(CH₂)₂–); 2.96 (t, 2H, –CH₂N<); 3.89 (s, 3H, –OCH₃); 4.18 (t, 2H, –OCH₂–); 5.75 (s, 1H, H-4); 6.92 (d, 1H, *J*_{5',6'} = 8.32 Hz, H-5'); 7.04 (d, 1H, *J*_{2',6'} = 1.70 Hz, H-2'); 7.14 (dd, 1H, *J*_{2',6'} = 1.67 Hz, *J*_{5',6'} = 8.25 Hz, H-6'); 7.38 (s, 1H, H-vinyl). Anal. calcd for C₃₃H₄₃NO₄: C, 76.56; H, 8.37; N, 2.71; found: C, 76.82; H, 8.10; N, 3.02.

2.7.3. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-4-androstene-3,17-dione (20) (DPJ-RG-1098)

Yield: 100%, m.p., 105–108 °C. UV_{max} (MeOH): 240.6 (log ϵ 4.37) and 331.0 (log ϵ 4.31). IR: 1714 (C=O), 1623 (C=C), 1095 (C–O). ¹H NMR (CDCl₃): 1.01 (s, 3H, H-18); 1.07 (6H, t, –N(CH₂CH₃)₂); 1.25 (s, 3H, H-19); 2.66 (q, 4H, –N(CH₂CH₃)₂); 2.93 (t, 2H, –CH₂N<); 3.89 (s, 3H, –OCH₃); 4.14 (t, 2H, –OCH₂–); 5.76 (s, 1H, H-4); 6.93 (d, 1H, *J*_{5',6'} = 8.03 Hz, H-5'); 7.06 (s, 1H, H-2'); 7.15 (d, 1H, *J*_{5',6'} = 8.82 Hz, H-6'); 7.39 (s, 1H, H-vinyl). Anal. calcd for

C₃₃H₄₅NO₄: C, 76.26; H, 8.73; N, 2.70; found: C, 76.32; H, 9.01; N, 2.68.

2.8. General procedure for the preparation of compounds 24–26

Pyrrolidine (1 ml) was added to the refluxing suspension of dione 18–20 (1.93 mmol) in methanol (10 ml). The reaction mixture was further refluxed for 15 min and chilled. The crystalline material was filtered, washed with methanol and dried to afford intermediate enamines 21–23. To the stirred suspension of the enamines 21–23 so formed (1.71 mmol) in methanol (100 ml) at room temperature, sodium borohydride (1.5 g) was added in small amounts over a period of 2 h and stirring was further continued in vacuum and ice water was added. The precipitate obtained was filtered, washed with water, dried and crystallized from methanol to yield corresponding diaminosteroids 24–26.

2.8.1. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}-benzylidene]-3-pyrrolidino-3,5-androsta dien-17-one (21) (DPJ-RG-1073)

Yield: 72.7%, m.p., 132–135 °C. UV_{max} (MeOH): 242.0 (log ϵ 4.12) and 330.8 (log ϵ 4.31). IR: 1712 (C=O), 1624 (C=C), 1025 (C–O). ¹H NMR (CDCl₃): 0.93 (s, 3H, H-18); 1.00 (s, 3H, H-19); 2.37 (brs, 4H, –N(CH₂)₂–); 2.73 (t, 2H, –CH₂N<); 3.07 (brs, 4H, –N(CH₂)₂-3); 3.80 (s, 3H, –OCH₃); 4.10 (t, 2H, –OCH₂–); 4.70 (s, 1H, H-4); 5.00 (s, 1H, H-6); 6.70–7.33 (m, 4H, H-2', H-5', H-6' and H-vinyl).

2.8.2. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}-benzylidene]-3-pyrrolidino-3,5-andros-tadien-17-one (22) (DPJ-RG-1149)

Yield: 63.5%, m.p., 125–127 °C. UV_{max} (MeOH): 241.0 (log ϵ 3.95) and 330.8 (log ϵ 4.10). IR: 1713 (C=O), 1622 (C=C), 1095 (C–O). ¹H NMR (CDCl₃): 1.00 (s, 3H, H-18); 1.07 (s, 3H, H-19); 2.63 (br, 4H, –N(CH₂)₂–); 2.96 (t, 2H, –CH₂N<); 3.12 (t, 4H, –N(CH₂)₂-3); 3.90 (s, 3H, –OCH₃); 4.18 (t, 2H, –OCH₂–); 4.79 (s, 1H, H-4); 5.08 (s, 1H, H-6); 6.92 (d, 1H, *J*_{5',6'} = 8.30 Hz, H-5'); 7.07 (d, 1H, *J*_{2',6'} = 1.20 Hz, H-2'); 7.15 (d, 1H, *J*_{5',6'} = 8.30 Hz, H-6'); 7.38 (s, 1H, H-vinyl).

2.8.3. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-3-pyrrolidino-3,5-androsta dien-17-one (23) (DPJ-RG-1099)

Yield: 72.6%, m.p., 130–133 °C. UV_{max} (MeOH): 239.4 (log ϵ 4.08) and 330.0 (log ϵ 4.27). IR: 1713 (C=O), 1624 (C=C), 1026 (C–O). ¹H NMR (CDCl₃): 0.93 (s, 3H, H-18); 1.03 (t, 6H, –N(CH₂CH₃)₂); 1.13 (s, 3H, H-19); 2.66 (q, 4H, –N(CH₂CH₃)₂); 3.10 (m, 6H, –CH₂N< and –N(CH₂)₂-3); 3.81 (s, 3H, –OCH₃); 4.00 (t, 2H, –OCH₂–); 4.57 (s, 1H, H-4); 5.27 (1H, s, H-6); 6.90–7.15 (m, 3H, H-2', H-5' and H-6'); 7.50 (s, 1H, H-vinyl).

2.8.4. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}-benzylidene]-3 β -pyrrolidino-5-androsten-17 β -ol (24) (DPJ-RG-1074)

Yield: 77.3%, m.p., 200–203 °C. UV_{max} (MeOH): 264.8 (log ϵ 4.44). IR: 3366 (O–H), 1511 (C=C). ¹H NMR (CDCl₃): 0.72 (s, 3H, H-18); 1.04 (s, 3H, H-19); 2.51 (brs, 4H, –N(CH₂)₂–); 2.62 (brs, 4H, –N(CH₂)₂-3); 2.82 (t, 2H, –CH₂N<); 3.87 (s, 3H, –OCH₃); 4.05 (s, 1H, H-17 α); 4.15 (t, 2H, –OCH₂–); 5.37 (d, 1H, H-6); 6.44 (s, 1H,

H-vinyl); 6.85–6.96 (m, 3H, H-2', H-5' and H-6'). Anal. calcd for $C_{38}H_{56}N_2O_3$: C, 77.50; H, 9.58; N, 4.75; found: C, 77.69; H, 9.71; N, 4.71.

2.8.5. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}-benzylidene]-3 β -pyrrolidino-5-androsten-17 β -ol (25) (DPJ-RG-1122)

Yield: 69.5%, m.p., 181–183 °C. UV_{max} (MeOH): 265.0 ($\log \epsilon$ 4.32). IR: 3351 (O–H), 1512 (C=C). 1H NMR ($CDCl_3$): 0.71 (s, 3H, H-18); 1.03 (s, 3H, H-19); 2.61 (m, 8H, $2 \times -N(CH_2)_2-$); 2.94 (t, 2H, $-CH_2N<$); 3.86 (s, 3H, $-OCH_3$); 4.03 (s, 1H, H-17 α); 4.15 (t, 2H, $-OCH_2-$); 5.35 (d, 1H, H-6); 6.44 (s, 1H, H-vinyl); 6.86–6.96 (m, 3H, H-2', H-5' and H-6'). Anal. calcd for $C_{37}H_{54}N_2O_3$: C, 77.31; H, 9.47; N, 4.87; found: C, 77.39; H, 9.09; N, 5.01.

2.8.6. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-3 β -pyrrolidino-5-androsten-17 β -ol (26) (DPJ-RG-1100)

Yield: 67.5%, m.p., 179–181 °C. UV_{max} (MeOH): 264.8 ($\log \epsilon$ 4.32); IR: 3375 (O–H), 1512 (C=C). 1H NMR ($CDCl_3$): 0.71 (s, 3H, H-18); 1.07 (t, 6H, $-N(CH_2CH_3)_2$) and s (merged), 3H, H-19); 2.64 (m, 8H, $-N(CH_2CH_3)_2$ and $-N(CH_2)_2-$); 2.90 (t, 2H, $-CH_2N<$); 3.85 (s, 3H, $-OCH_3$); 4.02 (s, 1H, H-17 α); 4.08 (t, 2H, $-OCH_2-$); 5.34 (d, 1H, H-6), 6.43 (d, 1H, H-vinyl); 6.83–6.94 (m, 3H, H-2', H-5' and H-6'). Anal. calcd for $C_{37}H_{56}N_2O_3$: C, 77.04; H, 9.79; N, 4.86; found: C, 77.41; H, 10.01; N, 4.92.

2.9. General procedure for the preparation of compounds 27–29

A mixture containing respective diol 24–26 (0.85 mmol), acetic anhydride (1 ml) and dry pyridine (0.5 ml) was heated in a steam bath for 1.5 h. The reaction contents were poured in a mixture of crushed ice and dichloromethane. The resultant turbid solution was basified with liquor ammonia and extracted with dichloromethane (3×50 ml). The combined organic extract was washed with water and dried. Solvent was

then removed under reduced pressure to obtain a white solid, which was crystallized from diethyl ether to afford 27–29.

2.9.1. 16-[3-Methoxy-4-{2-(piperidin-1-yl)ethoxy}-benzylidene]-3 β -pyrrolidino-5-androsten-17 β -yl acetate (27) (DPJ-RG-1087)

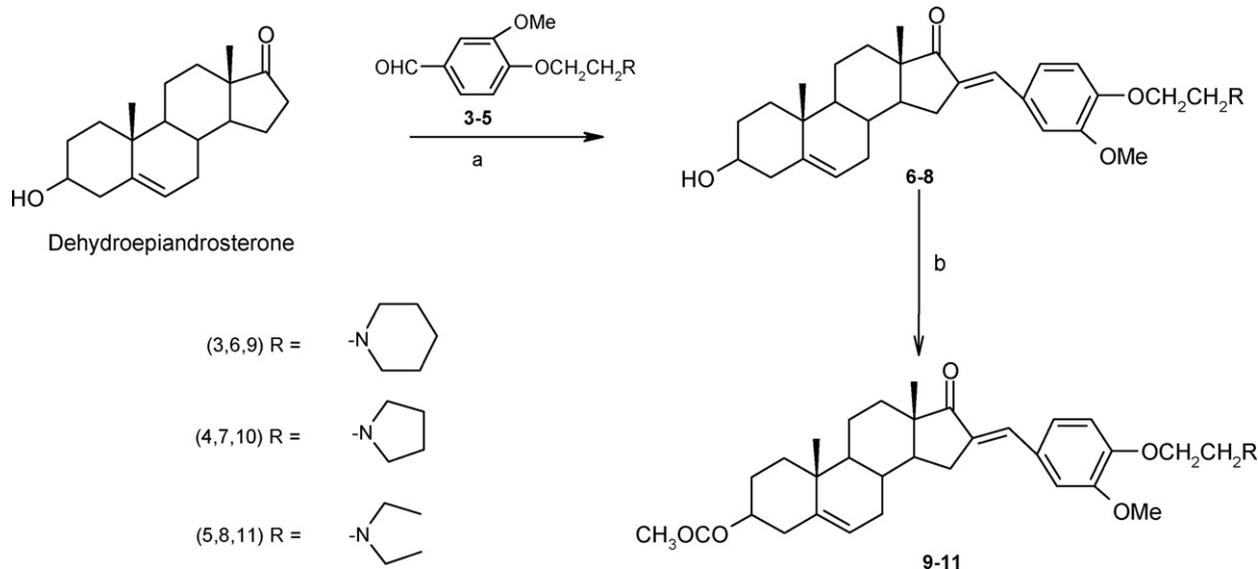
Yield: 84%, m.p., 110–113 °C. UV_{max} (MeOH): 265.2 ($\log \epsilon$ 4.33). IR: 1720 (C=O), 1514 (C=C). 1H NMR ($CDCl_3$): 0.79 (s, 3H, H-18); 1.02 (s, 3H, H-19); 2.20 (s, 3H, $-OCOCH_3$); 2.50 (m, 4H, $-N(CH_2)_2-$); 2.58 (brs, 4H, $-N(CH_2)_2-3$); 2.80 (t, 2H, $-CH_2N<$); 3.87 (s, 3H, $-OCH_3$); 4.14 (t, 2H, $-OCH_2-$); 5.36 (s, 2H, H-17 α and H-6); 6.14 (s, 1H, H-vinyl); 6.84–6.94 (m, 3H, H-2', H-5' and H-6'). Anal. calcd for $C_{40}H_{58}N_2O_4$: C, 76.14; H, 9.26; N, 4.44; found: C, 76.49; H, 9.66; N, 4.49.

2.9.2. 16-[3-Methoxy-4-{2-(pyrrolidin-1-yl)ethoxy}-benzylidene]-3 β -pyrrolidino-5-androsten-17 β -yl acetate (28) (DPJ-RG-1170)

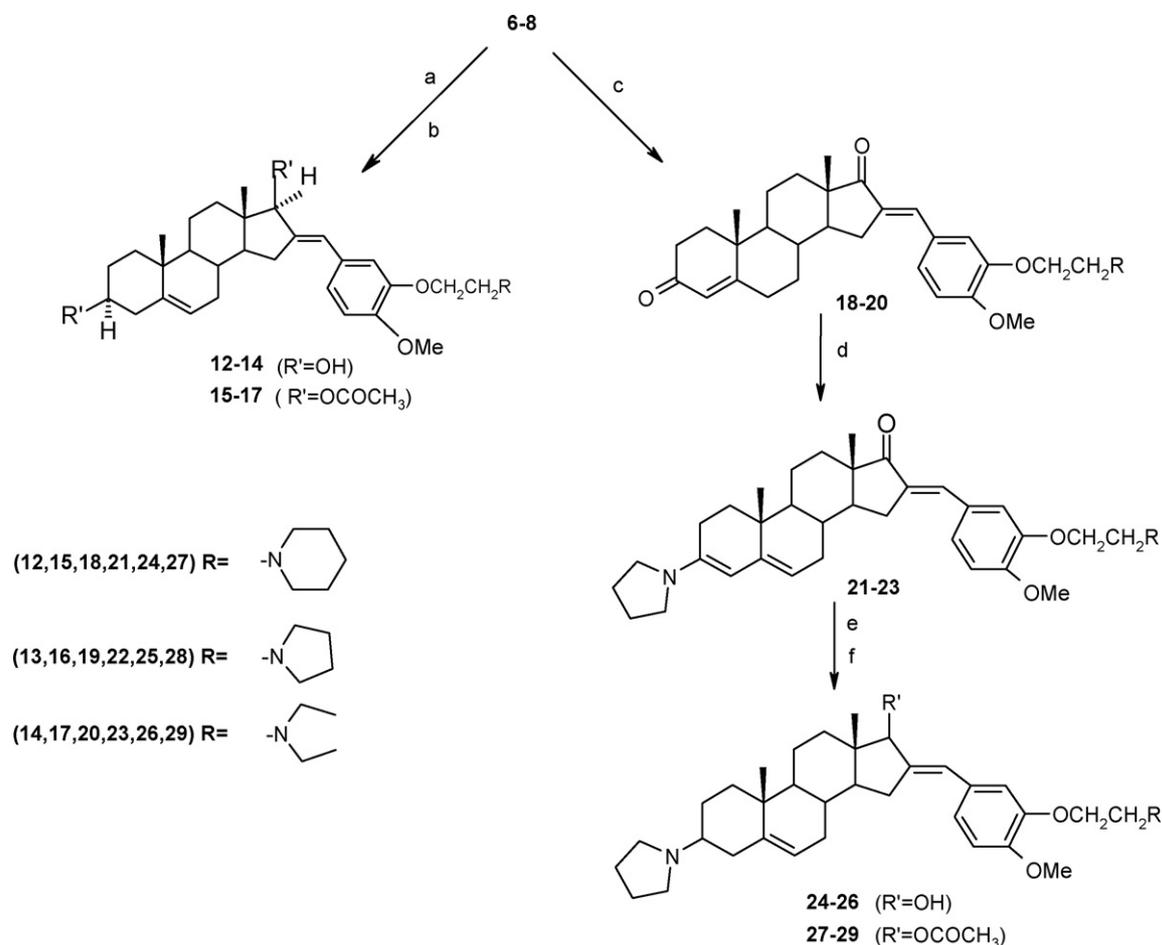
Yield: 70.8%, m.p., 97–98 °C. UV_{max} (MeOH): 265.8 ($\log \epsilon$ 4.28). IR: 1735 (C=O), 1513 (C=C). 1H NMR ($CDCl_3$): 0.76 (s, 3H, H-18); 1.01 (s, 3H, H-19); 2.21 (s, 3H, $-OCOCH_3$); 2.62 (m, 8H, $2 \times -N(CH_2)_2-$); 2.95 (t, 2H, $-CH_2N<$); 3.87 (s, 3H, $-OCH_3$); 4.15 (t, 2H, $-OCH_2-$); 5.36 (m, 2H, H-17 α and H-6); 6.14 (s, 1H, H-vinyl); 6.74–6.80 (m, 3H, H-2', H-5' and H-6'). Anal. calcd for $C_{39}H_{56}N_2O_4$: C, 75.93; H, 9.15; N, 4.54; found: C, 75.81; H, 9.22; N, 4.49.

2.9.3. 16-[4-(2-Diethylaminoethoxy)-3-methoxybenzylidene]-3 β -pyrrolidino-5-androsten-17 β -yl acetate (29) (DPJ-RG-1110)

Yield: 72.8%, m.p., 110–111 °C. UV_{max} (MeOH): 266.0 ($\log \epsilon$ 4.38). IR: 1726 (C=O), 1513 (C=C). 1H NMR ($CDCl_3$): 0.80 (s, 3H, H-18); 1.04 (s, 3H, H-19); 1.07 (t, 6H, $-N(CH_2CH_3)_2$); 2.21 (s, 3H, $-OCOCH_3$); 2.64 (m, 8H, $-N(CH_2CH_3)_2$ and $-N(CH_2)_2-3$); 2.92 (t, 2H, $-CH_2N<$); 3.86 (s, 3H, $-OCH_3$); 4.09 (t, 2H, $-OCH_2-$); 5.36 (s, 2H, H-17 α and H-6); 6.14 (d, 1H, H-vinyl); 6.86–6.93 (m, 3H, H-2', H-5' and H-6'). Anal. calcd for $C_{39}H_{58}N_2O_4$: C, 75.68; H, 9.45; N, 4.53; found: C, 75.69; H, 9.54; N, 4.61.



Scheme 1 – Reagents and reaction conditions: (a) MeOH, KOH, RT; (b) $(CH_3CO)O$ /dry pyridine, steam bath, 2H.



Scheme 2 – Reagents and reaction conditions: (a) NaBH₄, MeOH, RT; (b) (CH₃CO)O/dry pyridine, steam bath, 2H; (c) Al(*t*-BuO)₃, cyclohexanone, reflux, 5H; (d) pyrrolidine/MeOH; (e) NaBH₄; (f) (CH₃CO)O/dry pyridine.

3. Results and discussion

3.1. Chemistry

The synthesis of various 16E-arylidenosteroid derivatives have been carried out as depicted in Schemes 1 and 2. Aldol condensation [12] of dehydroepiandrosterone with substituted aldehydes 3–5 at room temperature in alkaline medium afforded 16E-benzylidene steroids 6–8, respectively. The structures of the compounds were established using various spectral, elemental and X-ray crystallographic studies. The methine-bridged proton at C₁₆ appeared at δ 7.38 ppm in the ¹H NMR spectra of all these aldol products. The configuration at C₁₆ with respect to the carbonyl at C₁₇ has been assigned *E* on the basis of earlier reports from our laboratory as well as the X-ray crystallographic studies of some of the synthesized compounds [10,11,13,14]. These aldol products were further subjected to various structural modifications to afford newer derivatives of medicinal interest. Acetylation of 6–8 using acetic anhydride and dry pyridine in a steam bath to afford 3 β -acetoxy derivatives 9–11 and reduction with sodium borohydride in methanol affording 3 β ,17 β -diol products 12–14 were carried out using a simple method [1]. The proton resonance signals for the methine-

bridged proton of the reduced products appeared at an upfield position (δ ~ 6.4 ppm) as compared to their corresponding parent aldol compounds indicating the reduction of 17-keto to 17-hydroxy functionality. Similarly acetylation of 12–14 with acetic anhydride and dry pyridine in a steam bath afforded the corresponding 3 β ,17 β -diacetoxy compounds 15–17, respectively. The –CH– of benzylidene in these diacetoxy compounds was found further little upfield (δ ~ 6.14 ppm) as compared to their 3 β ,17 β -diol counterparts.

In view of the literature reports that 3,17-diketo steroids with higher degree of unsaturation in A-ring exhibit potent aromatase inhibitory activity and significant cytotoxicity, Oppenauer oxidation [15] of 6–8 was carried out to afford α,β -unsaturated-3-keto steroids 18–20, respectively. A singlet for the 4-CH proton at about δ 5.76 ppm was observed in the ¹H NMR spectra of all the Oppenauer products. Further treatment of these 3,17-diketo derivatives with pyrrolidine in refluxing methanol yielded the corresponding unstable dienamines 21–23, which on immediate reduction with sodium borohydride in methanol at room temperature afforded their corresponding 3 β ,16-bis-tertiary amino steroids 24–26. In the process the 17-keto got reduced to 17-hydroxy functionality as indicated by an upfield shift (δ 6.44 ppm) for the vinylic proton of 16-arylidene. The configuration at positions 3 and 17 was assigned β in accordance with earlier reports [16]. Further

Table 1 – Growth percentage at 10⁻⁴ molar concentration in the 3-cell line *in vitro* screening

S. no.	Compound	NSC no.	Growth percentage		
			Breast (MCF-7)	Non-small cell lung (NCI-H460)	CNS (SF-268)
1	6 (DPJ-RG-1071)	727078	98	125	112
2	7 (DPJ-RG-1147)	727088	103	102	122
3	8 (DPJ-RG-1097)*	727083	0	0	3
4	9 (DPJ-RG-1111)*	727082	0	0	0
5	10 (DPJ-RG-1175)*	728323	0	0	0
6	11 (DPJ-RG-1146)*	727087	0	0	0
7	12 (DPJ-RG-1214)*	730472	0	0	0
8	13 (DPJ-RG-1217)*	730476	0	0	1
9	14 (DPJ-RG-1216)*	730474	8	1	4
10	15 (DPJ-RG-1215)*	730473	56	6	101
11	16 (DPJ-RG-1218)*	730477	0	0	2
12	17 (DPJ-RG-1226)*	730475	4	1	85
13	18 (DPJ-RG-1072)*	727079	0	0	0
14	19 (DPJ-RG-1148)*	727089	0	0	0
15	20 (DPJ-RG-1098)*	727084	36	5	39
16	24 (DPJ-RG-1074)	727080	97	111	117
17	25 (DPJ-RG-1122)	727090	80	73	103
18	26 (DPJ-RG-1100)*	727085	57	23	74
19	27 (DPJ-RG-1087)*	727081	14	3	24
20	28 (DPJ-RG-1170)*	728322	0	0	0
21	29 (DPJ-RG-1110)*	727086	0	0	0

* Compounds active in 3-cell line assay.

acetylation of 24–26 using acetic anhydride and dry pyridine in a steam bath and careful processing of reaction mixture in ice, afforded 17 β -acetoxy derivatives 27–29, respectively.

3.2. Biological activity

The compounds 6–20 and 24–29, selected by National Cancer Institute, Bethesda, Maryland, USA on the basis of degree of novelty of the structure and computer modeling techniques for evaluation of antineoplastic activity, were first 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 (CNS) [17,18]. The results in the 3-cell line *in vitro* screening are shown in Table 1.

The 3-cell line actives, which reduced the growth of any one of the cell lines to approximately 32% or less, were passed on for evaluation in the full panel of 60-cell lines over a minimum of 5-log dose range at ten fold dilutions, the highest being 10⁻⁴ M. A 48 h continuous drug exposure protocol was used, and a sulforhodamine B (SRB) protein assay was used to estimate the cell viability or growth [17,18]. Mean log dose response parameters such as GI50 (drug concentration resulting in a 50% reduction in the net protein increase), TGI (drug concentration of total growth inhibition) and LC50 (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

Table 2 – Mean log dose response parameters such as GI50, TGI and LC50 of the 60-cell line assay

S. no.	Compound	Mean log ₁₀ GI50 (molar)	Mean log ₁₀ TGI (molar)	Mean log ₁₀ LC50 (molar)
1	8 (DPJ-RG-1097)	-5.59	-4.43	-4.00
2	9 (DPJ-RG-1111)	-5.48	-4.95	-4.48
3	10 (DPJ-RG-1175)	-5.39	-4.91	-4.50
4	11 (DPJ-RG-1146)	-5.30	-4.74	-4.30
5	12 (DPJ-RG-1214)	-6.23	-5.67	-5.09
6	13 (DPJ-RG-1217)	-5.01	-4.59	-4.35
7	14 (DPJ-RG-1216)	-5.18	-4.63	-4.39
8	15 (DPJ-RG-1215)	-4.78	-4.47	-4.35
9	16 (DPJ-RG-1218)	-5.05	-4.64	-4.38
10	17 (DPJ-RG-1226)	-5.05	-4.61	-4.36
11	18 (DPJ-RG-1072)	-5.42	-4.81	-4.34
12	19 (DPJ-RG-1148)	-5.68	-5.17	-4.56
13	20 (DPJ-RG-1098)	-5.55	-4.94	-4.34
14	26 (DPJ-RG-1100)	-5.30	-4.69	-4.18
15	27 (DPJ-RG-1087)	-5.87	-5.36	-4.87
16	28 (DPJ-RG-1170)	-5.55	-5.13	-4.75
17	29 (DPJ-RG-1110)	-5.85	-5.34	-4.81

Table 3 – Data summarizing the preliminary *in vivo* hollow fiber assay reports

S. no.	Compound	NSC no.	IP score	SC score	Total score	Cell kill
1	27 (DPJ-RG-1087)	727081	8	0	8	No
2	29 (DPJ-RG-1110)	727086	12	8	20	No

the cell lines are well documented, were tested against each cell line: NSC 19893 (5-Fluorouracil) and NSC 123127 (Adriamycin).

Out of all the compounds screened for 60-cell line assay, 27 and 29 qualified for preliminary *in vivo* testing using hollow fiber assay [19] and have shown interesting IP and SC scores. 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. These derivatives lie outside the category of adequately studied class of antitumor agents and are among the small percentage, which have been selected for testing in the *in vivo* hollow fiber assays. Data summarizing the *in vivo* hollow fiber assay performed by the Developmental Therapeutic Program on the compounds is given in Table 3. A standard panel of 12 tumor cell lines was used for the preliminary *in vivo* hollow fiber screening of the *in vitro* actives. These include NCI-H23, NCI-H522, MDA-MB-231, MDA-MB-435, SW-620, COLO 205, LOX, UACC-62, OVCAR-3, OVCAR-5, U251 and SF-295. A total of three different tumor lines are prepared for each experiment so that each mouse receives three intraperitoneal (IP) implants (one for each tumor line) and three subcutaneous (SC) implants (one of each tumor line). Each compound is assessed in a total of four experiments (3-cell lines/experiment \times 4 experiments = 12 cell lines). The test compound was administered into athymic nude mice implanted with twelve selected human tumor cell lines encased in hollow fibers. After 6–8 days, the fibers were collected, cells were removed and growth inhibition was measured using MTT assay. The percent net growth for each cell line in each treatment group was calculated and compared to the percent net growth in the vehicle treated controls. Taxol was the positive control used in the hollow fiber assay. A 50% or greater reduction in percent net growth in the treated samples compared to the vehicle control samples is considered a positive result. Each positive result is given a score of 2 and all of the scores are totaled for a given compound. The maximum possible score for an agent is 96 (12 cell lines \times 2 sites \times 2 dose levels \times 2 (score)).

Compounds giving promising results in these assays are referred to Biological Evaluation Committee for cancer drugs to select for further *in vivo* evaluation using xenograft models. A compound is referred for xenograft testing if it has a combined IP + SC score of 20 or greater, a SC score of 8 or greater, or produces cell kill of any cell line at either dose level evaluated. The total pattern of activity of the compounds is also taken into consideration for further *in vivo* evaluation. Biological Evaluation Committee for Cancer Drugs selected compound 29 for further detailed *in vivo* xenograft testing.

It is apparent from the results depicted in Tables 1–3 that the synthesized 16E-arylidenerosteroids 6–20 and 24–29 exhibit

significant cytotoxicity. As expected and in accordance with our previous reports, introduction of hydroxyl group in steroid skeleton leads to reduction in cytotoxicity as observed for compounds 6 and 7 (–OH is present at 17 position) and 24 and 25 (–OH at 3 position). These compounds were not able to reduce the growth of any one of the cell lines to the required percentage in 3-cell line assay and did not qualify for evaluation in full panel of 60-cell line. In contrast, monoacetoxy steroids 9–11 (acetoxy at 3-position), 27–29 (acetoxy at 17-position) and 15–17 (acetoxy at 3- and 17-position) exhibit potent cytotoxicity. Similarly diketone compounds 18–20 with higher degree of oxidation in ring A exhibited more activity. Of all the three series of steroidal derivatives, the ones substituted with diethylamino moiety like 8, 11, 14, 17, 20, 23, 26 and 29 appear to be the most promising one. This observation is further supported by the fact that out of hydroxy substituted compounds 6–8 and 24–26, only 8 and 26 showed good cytotoxicity, which may be attributed to presence of diethylamino functionality in side chain. It can be said that open ring analogues are better cytotoxic agents than their cyclic counterparts.

Out of all 60-cell line actives, only bis-tertiary amino steroids 27 and 29 qualified for *in vivo* hollow fiber assay. Two well-placed tertiary amino groups further enhance the cytotoxic potential of the compounds, which is also the aim of the current study. The most promising compound 29 possessing diethylamino group with a total IP and SC score of 20 in *in vivo* hollow fiber assay was selected for further detailed *in vivo* xenograft testing. The test agent suppressed tumor growth but unfortunately failed to meet the established antitumor activity criteria.

In conclusion, 16-arylidenerosteroids represents a novel class of cytotoxic agents. Further research efforts should be directed towards rational structural modifications of such compounds to produce better antineoplastics.

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