Synthesis, Evaluation and QSAR Studies of 16-(4 & 3,4-Substituted) Benzylidene Androstene Derivatives as Anticancer Agents

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Abstract: In a systematic effort aimed at identifying new steroidal cytotoxic agents with potent antipoliferative activity against cancer cells and developing their QSAR models, series of 4-nitro, 4-isopropyl, 4-methoxy and 3,4-dimethoxy substituted benzylidene androst-5-ene derivatives were synthesized. The selected compounds were evaluated for antineoplastic activity against a panel of three human cell lines-breast, CNS and lungs at NCI, Bethesda, USA. The results presented herein reports that compounds **7, 9, 10, 15,16, 18, 20-25, 30, 32-36** and **44** have been found to be active anticancer agents. The QSAR of 20 compounds was performed separately for each cell line and best-fit QSAR models are developed. The QSAR models obtained have shown significant correlations (r^2 range: 0.9163 to 0.8164) and good predictive performance (q^2 range: 0.8499- 0.6320). The validation of models has also been performed using the test set of compounds **5, 15** and **44**.

Key Words: Steroids, androstane, anticancer, *in vitro* activity, QSAR.

INTRODUCTION

Among the devastating and pandemic diseases, cancer is a major disease and according to WHO it is considered as the forth largest killer disease. Treatment of cancer has been one of the primary goals of medicine for last two decades [1]. The steroids represent a class of compounds with numerous and widespread biological effects, anticancer activity being one such important effect. Among themselves steroids can vary markedly in their efficacy, adverse effect, pharmacokinetics and pharmacodynamics. There is a continued interest in the development of potent anticancer steroid.

Steroid hormones (eg. estrogen and progesterone) have been related to the increased risk of some types of cancers. Several *in vitro* and *in vivo* studies indicate a correlation between estrogen and carcinogenesis [2]. In particular, estrogens have been associated with increased cell proliferation [3]. Several successful steroidal molecules have been synthesized and deemed as anticancer drugs eg. formastane (**1**) [4] and exemestane (**2**) [5]. Several groups have explored the interaction of steroids with estrogen, progesterone, corticoid and androgen receptors using QSAR approaches [6,7]. In previous work on androstene derivatives from our lab, certain 16-substituted androstene were found to be active (*in vitro*) antineoplastic agents [8]. The present study is the continuation of the work, in which we have designed and synthesized certain novel 16-[(4-nitro, 4-isopropyl, 4-methoxy and 3,4-dimethoxy)substituted] benzylidene androstenes. The selected synthesized compounds were evaluated for their *in vitro* anticancer activity. To attribute their activity to particular parameter(s) QSAR models have been developed and validated. It attempts to model the activity of a series of compounds using measured or computed properties of the compound.

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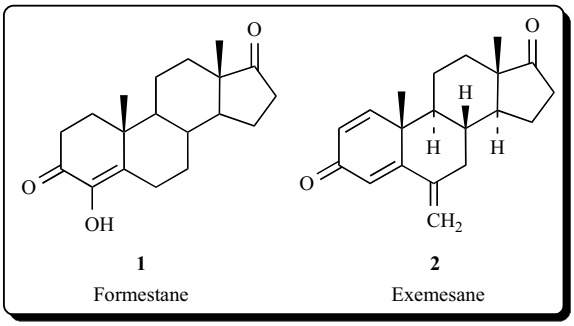

RESULTS

Chemistry

A general outline for the synthesis of these 16-para-substituted benzylidene derivatives is given in scheme **1**. Aldol condensation [9] of 4-methoxybenzaldehyde and 3,4-dimethoxybenzaldehyde was carried out with dehydroepiandrosterone (**3**) to obtain the compounds **4** and **15** respectively. This was followed by oppenauer oxidation [10] of **4** and **15** using cyclohexanone/ toluene system which afforded 3,17-diones **5** and **16.** On treatment with pyrrolidine in methanol, the α,β-unsaturated ketones **5** and **16** gave enamines **6** and **17** respectively. Both the enamines were reduced with sodium borohydride to give **7** and **18**, which were then subjected to esterification to get **8** and **19**.

Reduction of **4** with sodium borohydride yielded the compound **13** and subsequent acetylation with acetic anhydride yielded compound **14**. The direct acetylation of **4** and **15** with acetic anhydride in dry pyridine afforded **11** and **22**.

The detailed methods of synthesis of 4-nitro and 4-isopropyl substituted benzylidene androstene derivatives **24-28, 31, 33-39, 42, 44** and **45** have already been discussed in previous communication [11].

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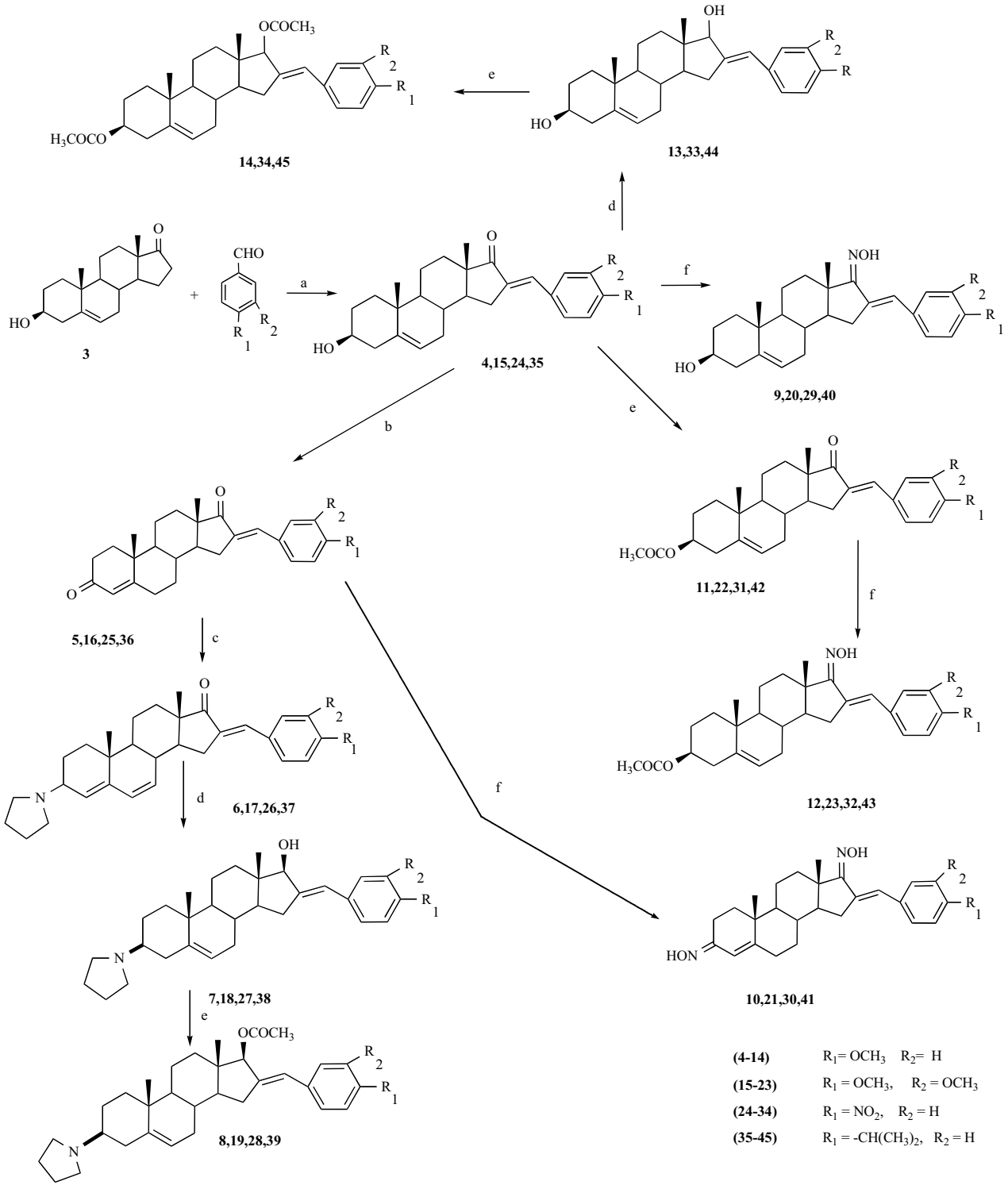

Reagents and Conditions: (a) Sodium hydroxide, shaken at room temperature; (b) Cyclohexanone, room temperaturetrihydrate, reflux in aldehyde

Scheme 1. Outline of synthetic procedure for compounds 4-45.

The detailed account of the synthesis of 17-oximino (**9, 20, 29, 40**), 3-acetoxy-17-oximino (**11, 22, 31, 42**) and 3,17-dioximino derivatives (**10, 21, 30, 41**) has already been reported [12,13].

Anticancer Activity

Cytotoxicity of the 23 synthesized compounds (**5,7-10, 15,16, 18, 20-25, 30-36, 38** and **44)** was checked by *in vitro* Hollow fiber assay against the three human cell lines (breast,

CNS and lungs). The results are given in Table **1**. In the assay nineteen compounds were found to posses anticancer activity.

QSAR Analysis

From the hollow fiber assay of 20 compounds (**7-10, 16, 18, 20-25, 30-36**and **38**), which showed low to moderate to high antineoplastic activity. The wide range of activity of these compounds that is due to the various substituents attached makes this series suitable for a QSAR investigation. The log of biological activity was taken for QSAR analysis. The structures of the compounds were built using ChemDraw software, the energy of the molecules was minimized by MM2 force field. A total of about 1700 different descriptors physicochemical, topological, electro-topological, geometrical, constitutional, 2D and 3D descriptors were calculated by the softwares: Chem3D, Dragon and Codesssa. Stepwise multiple regression analysis method [14] was used to generate QSAR equations using the codessa software. The number of descriptors was reduced to final set of 99, in the final set only those descriptors were chosen which were having $r^2 \geq 0.1$. QSAR models were developed and cross validation was done using Leave-one-out (LOO) method. A set of three compounds (**5, 15** and **44**) was used to check the predictive ability of the developed models.

The best-fit equation against the **breast** cell line was:

$$\text{Log (BA)} = 257.91+11.608*\text{BELp}^4 - 127.73 *\text{R2u}^+ + 421.87*\text{G1m} + 389.54* \text{FPSA} - 3 \text{ Fractional PPSA (PPSA - 3 / TMSA)(Zefirov's PC)} - 83.626* \text{BEHv1} \quad (1)$$

n= 20, r^2 = 0.9000, q^2 (crossvalidated r^2)= 0.7645 and F-value = 25.20

Table 1. Structure and *In Vitro* Anticancer Activity of the Selected Compounds

S. No.	Structure No.	Activity (in Growth Percentage, Conc. used =0.1 mM)			
		CNS	Lung	Breast	Activity
1	**5**	49	83	62	Inactive
2	**7**	-100	-94	-73	Active
3.	**8**	82	92	90	Inactive
4.	**9**	43	7	17	Active
5.	**10**	-8	-11	5	Active
6.	**15**	31	-2	14	Active
7.	**16**	14	10	5	Active
8.	**18**	-82	- 83	-79	Active
9.	**20**	45	6	19	Active
10.	**21**	8	10	8	Active
11.	**22**	19	11	19	Active
12.	**23**	20	22	18	Active
13.	**24**	-35	35	33	Active
14.	**25**	-37	25	28	Active
15.	**30**	37	9	16	Active
16.	**31**	76	106	90	Inactive
17.	**32**	44	10	22	Active
18.	**33**	-46	-15	-90	Active
19.	**34**	17	65	82	Active
20.	**35**	3	0	0	Active
21.	**36**	91	47	27	Active
22.	**38**	110	108	101	Inactive
23.	**44**	5	5	1	Active

They showed a positive linear dependence on the electrostatic parameters (e.g. PPSA, FPSA and BELp4), Getaway parameter ($R2u^+$) and Whim descriptors (G1m, L1s).

A higher dependency on 3D, WHIM and Electrostatic parameters are observed in models for **CNS** activity. The best fit QSAR model found in this case was:

Log(BA) = - 8.9186 + 1.3766*MOR20u + 36.654*HATS8u – 32.963*H7m + 0.0069251 *DPSA-2 Difference in CPSAs(PPSA2 – PNSA2) Zefirov's PC – 664.97*GATS8m (2)

n= 20, r^2 = 0.8705, q^2 = 0.7592 and F-value = 18.82

Highly significant positive linear dependence on topological parameters (piPC10), WHIM (G1m) and 2D autocorrelations (GATS) has been observed in **lungs** cell line antineoplastic activity. The best equation was:

Log(BA)= -59.317+41.610*Relative number of double bonds + 397.68*G1m + 0.46212*PPSA- 3 atomic charge weighted PPSA(Zefirov's PC) – 0.004627* piPC10 +822.65 *GATS 5m (3)

n= 20, r^2 = 0.9163, q^2 = 0.8499 and F-value = 30.67

The expansion of the descriptors which were found significant as present in eqn. 1, 2 and 3 is given in Table **2**.

A plot of the experimental activity and the predicted activity as calculated by software using the equation 1, 2 and 3 for breast, CNS and lung cell line is given in Figs. (**1**, **2** and **3**) respectively.

To further validate the QSAR models we have predicted the anticancer activities of test set (**5, 15** and **44**) using the equations 1-3. The experimental and predicted results of this are given in Table **3**.

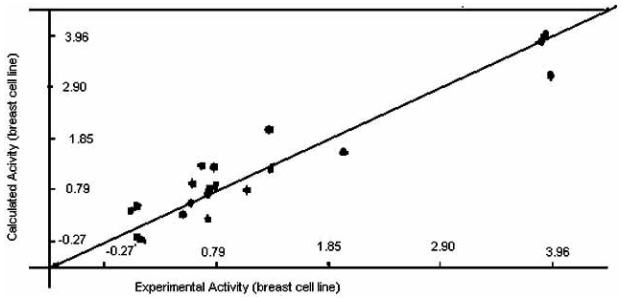

Fig. (1).

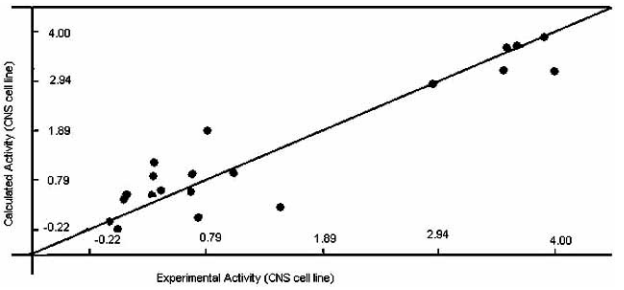

Fig. (2).

DISCUSSION

In our present study, QSAR models have been developed that are based on molecular, sub-molecular, physicochemical, 2D and 3D properties obtained from the various softwares. In contrast to using the entire the matrix of structural parameters as in PLS analysis an automatic forward inclu-

Table 2. The Expanded Version of Descriptors as Stated in eqn. 1,2 and 3

S. No.	Descriptor	Description
1	$BELp^4$	Lowest eigenvalue number 4 of Burden matrix/weighted by atomic polarizabilities (Burden eigenvalues)
2	R2u+	R maximal autocorrelation of lag 2/unweighted (GETAWAY descriptors)
3	G1m	First component symmetry directional WHIM index/weighted by atomic masses (WHIM descriptors)
4	FPSA – PPSA -3 / TMSA)	Charged partial surface area descriptors
5	EHv1	Highest eigenvalue number 1 of Burden matrix/weighted by atomic van der Waals volumes (Burden eigenvalues)
6	MOR20u	3D-MoRSE – signal 20/unweighted (3D- MoRSE descriptors)
7	HATS8u	Leverage – weighted autocorrelation of lag 8/unweighted (GETAWAY descriptors)
8	H7m	H autocorrelation of lag 7/weighted by atomic masses (GETAWAY descriptors)
9	DPSA- CPSAs	Charged partial surface area descriptors
10	GATS8m	Geary autocorrelation - lag 8/weighted by atomic masses (2D autocorrelation)
11	piPC10	Molecular multiple path count of order 10 (Walk and path counts)
12	GATS 5m	Geary autocorrelation - lag 5/weighted by atomic masses (2D autocorrelation)

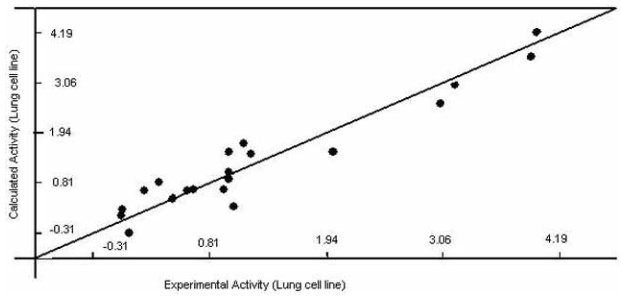

Fig. (3).

sion MLR technique was used to arrive at the primary property determinants with minimal number of independent variables or descriptors.

The calculated values of r^2 are showing high correlations with good predictive ability (q^2 range: 0.8499- 0.6320). It is also worth mentioning that no large numerical changes of the coefficients of these descriptors are observed. Thus, our models have significant predictive ability.

The predictive ability of these models when checked against the test set showed mixed results (Table **2**). These models showed good predictive ability when tested for the steroidal derivatives containing ortho- and para- substitutions in the 16-benzylidene nucleus (3,4-dimethoxy benzylidene derivative (**15**)) or when the substitute is bulkier group at para position (**44**); in comparison to comparatively lesser bulkier substitute (p- OCH_3, **5**).

Thus, it can be concluded that these QSAR models having good r^2 and q^2 values can predict the activity of the steroidal derivatives with multiple and /or bulkier substitution in 16-benzylidene nucleus more satisfactorily, when compared to their predictive performance of lesser bulkier substitution.

EXPERIMENTAL SECTION

General

All the aldehydes and aluminium isopropoxide were procured from Fluka Chemie. Dehydroepiandrosterone acetate was obtained ex gratis from Cipla Ltd., Mumbai (India). Melting points (melting point apparatus MPI, Veego, Mumbai, India) reported are uncorrected. ^{1}H-NMR spectra were recorded on AC-300F, 300 MHz, (Bruker, Fallanden, Switzerland). IR and UV spectra were recorded on Perkin-Elmer 882 (Perkin-Elmer, Ltd., England) and Lamda 15 spectrophotometer (Perkin-Elmer, Germany) models respectively. Ultraviolet spectra were recorded in methanol (λ_{max} in nm). Elemental analyses were carried out on a Perkin-Elmer-2400 (Perkin-Elmer, USA) and the results were within ±0.4% of theoretical values for C, H and N. Mass spectra were recorded on a V6-11-250J70 S and CEC-21-110B Finnigan Mat 1210 (US) or Micro Mass 7070 (U.K.) at 70eV using a direct inlet system.

General Procedure for Synthesis of Compounds 4 and 15

A mixture of dehydroepiandrosterone (**3**) (0.5 g), aldehyde (0.75 g) and sodium hydroxide (0.75 g) in methanol (10 ml) was stirred for 1.5 h at room temperature. The precipitation was observed in the reaction mixture. The reaction mixture was added to ice-cold water. The precipitate was filtered, washed with water and crystallized from methanol.

16-(4-Methoxybenzylidene)-17-oxo-5-androsten-3β-ol (4)

As a starting material p-methoxybenzaldehyde was taken 0.75 g (5.51 mmol) to yield **4** (0.4 g, 61.64 %), m.p. 220ºC.UV_{max} (MeOH): 321.4 nm (log ε 4.40). IR_{vmax} (KBr): 3500, 2935, 1705, 1620, 1595 and 1260 cm^{-1}.^{1}H-NMR ($CDCl_3$): δ 0.97 (s, 3H, 18-C*H*$_3$), 1.08 (s, 3H, 19-C*H*$_3$), 3.52 (m, 1H, 3α-*H*), 3.85 (s, 3H, -OC*H*$_3$), 5.40 (d, 1H, 6-C*H*), 6.94 (d, 2H, J_o=8.7 Hz, 3-C*H* and 5-C*H* aromatic protons), 7.40 [s, 1H, vinyl-*H* of 16-(4-methoxybenzylidene)] and 7.51 (d, 2H, J_o=8.7 Hz, 2-C*H* and 6-C*H* aromatic protons) ppm. MS: m/z (relative intensity): 407 [M^+]. Calcd for $C_{27}H_{34}O_3$: C, 79.76; H, 8.43. Found: C, 78.72; H, 8.31.

16-(3,4-Dimethoxybenzylidene)-17-oxo-5-androsten-3β-ol (15)

3,4-Dimethoxybenzaldehyde was taken (0.75 g, 4.49 mmol) to obtain 15 (0.75 g, 55%), m.p. 270-272ºC.UV_{max} (MeOH): 333.4 nm (log ε 4.4) and 244.0 nm (log ε 4.04). IR_{vmax} (KBr): 3510, 2900, 1720, 1610, 1510, 1240 and 1110 cm^{-1}.^{1}H-NMR ($CDCl_3$): δ 0.98 (s, 3H, 18-C*H*$_3$), 1.07 (s, 3H, 19-C*H*$_3$), 3.52 (m, 1H, 3α-*H*), 3.91 (s, 3H, -OC*H*$_3$), 3.92 (s, 3H, -OC*H*$_3$) 5.40 (d, 1H, 6-C*H*), 6.92 (d, 1H, J_o=8.5 Hz, 5-C*H* aromatic proton), 7.06 (d, 1H, J_m=1.6 Hz, 2-C*H* aromatic proton), 7.17 (dd, H, J_m=1.6 Hz, J_o=6.6 Hz, J_m=1.8 Hz, 6-C*H* aromatic proton) and 7.39 [s, 1H, vinyl-*H* of 16-(3,4-dimethoxybenzylidene)] ppm. MS: m/z (relative intensity): 437 [M^+]. Calcd for $C_{33}H_{45}NO_5$: C, 77.03; H, 8.31. Found: C, 76.93; H, 8.31.

General Procedure for Synthesis of Compounds 5 and 16

The aldol product was dissolved in a mixture of cyclohexanone (10 ml) and dry toluene (150 ml). Traces of mois-

Table 3. The Predicted and Experimental Activity Values of the Test Compounds

S. No.	Compound No.	Predicted Values of Anticancer Activity (log (% Inhibition))			Experimental Values of Anticancer Activity (log (% Inhibition))		
		CNS	Lungs	Breast	CNS	Lungs	Breast
1.	5	2.7975	2.5844	0.8372	1.7076	1.2304	1.5798
2.	15	1.4714	2.3541	1.2719	1.8389	2.0086	1.9345
3.	44	2.0775	2.3549	2.8112	1.9777	1.9777	1.9956

ture were removed by azeotropic distillation. The distillation was continued at a slow rate while adding a solution of aluminium isopropoxide (1.0 g) in dry toluene drop wise. The reaction mixture was refluxed for 4h and allowed to stand at room temperature for 12 h. The slurry was filtered and the residue was washed thoroughly with dry toluene. The combined filtrate and the washings were steam distilled until the complete removal of organic solvents was affected. The residue was allowed to stand overnight and then it was filtered, washed with the water, dried and crystallized from methanol.

16-(4-Methoxybenzylidene)-4-androstene-3,17-dione (5)

16-(4-Methoxybenzylidene)-17-oxo-5-androsten-3β-ol (**4**, 1.0 g) was used to obtain **5** 0.8 g, 80.39 %), m.p. 237-240ºC. UV_{max} (MeOH): 322.0 nm (log ε 4.44) and 237.6 nm (log ε 4.36).$IR_{\nu max}$ (KBr): 2930, 1705, 1595 and 1250 cm^{-1}.^{1}H-NMR ($CDCl_3$): δ 1.0 (s, 3H, 18-C*H*$_3$), 1.25 (s, 3H, 19-C*H*$_3$), 3.85 (s, 3H, -OC*H*$_3$), 5.76 (s, 1H, 4-C*H*), 6.93 (d, 2H, J_o=8.8 Hz, 3-C*H* and 5-C*H* aromatic protons), 7.42 [s, vinyl-*H* at 16-(4-methoxybenzylidene)] and 7.50 (d, 2H, J_m=8.8 Hz, 2-C*H* and 6-C*H* aromatic protons) ppm. MS: m/z (relative intensity): 405 [M^+]. Calcd for $C_{27}H_{32}O_3$: C, 80.16; H, 7.97. Found: C, 80.00; H, 7.71.

16-(3,4-Dimethoxybenzylidene)-4-androstene-3,17-dione (16)

16-(3,4-Dimethoxybenzylidene)-17-oxo-5-androsten-3β-ol (**15**, 1.0 g) was oxidised to get **16** (1.0 g, 100 %), m.p. 118-120ºC. UV_{max}(MeOH): 334.2 nm (log ε 4.33) and 241.2 nm (log ε 4.44). $IR_{\nu max}$ (KBr): 2935, 1710, 1660, 1610, 1590, 1550 and 1240 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.01 (s, 3H, 18-C*H*$_3$), 1.25 (s, 3H, 19-C*H*$_3$), 3.92 (s, 3H, -OC*H*$_3$), 3.93 (s, 3H, -OC*H*$_3$), 5.76 (s, 1H, 4-C*H*), 6.92 (d, 1H, J_o=8.6 Hz, 5-C*H* aromatic proton), 7.06 (d, 1H, J_m=1.5 Hz, 2-C*H* aromatic proton), 7.17 (dd, 1H, J_m=1.3 Hz, J_o=6.7 Hz, 6-C*H* aromatic proton) and 7.40 [s, 1H, vinyl-*H* of 16-(3,4-dimethoxybenzylidene)] ppm. MS: m/z (relative intensity): 83 (base peak). Calcd for $C_{28}H_{34}O_4$: C, 77.03; H, 7.89. Found: C, 76.72; H, 7.79.

General Procedure for Synthesis of Compounds 6 and 17

Freshly distilled pyrrolidine (1.0 ml) was added to a refluxing solution of dione (**5** and **16**, 0.5 g) in methanol (100 ml). Refluxing was continued for 15 min. On cooling in ice the crystalline material obtained was filtered, washed with methanol and dried to afford **6** and **17**.

16-(4-Methoxybenzylidene)-3-pyrrolidino-3,5-androstadien-17-one (6)

The compound **6** (mmol) yielded **7** (0.4 g, 70.72 %), m.p. 212-216ºC.UV_{max} (MeOH): 315.2 nm. $IR_{\nu max}$ (KBr): 2950, 1710, 1620 and 1250 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.0 (s, 3H, 18-C*H*$_3$), 1.05 (s, 3H, 19-C*H*$_3$), 3.70 (s, 3H, -OC*H*$_3$), 4.20 (s, 1H, 4-C*H*), 5.70 (br, 1H, 6-C*H*), 6.80-7.60 [m, 5H,one vinyl-*H* of 16-(4-methoxybenzylidene), 2-C*H*, 3-C*H*, 5-C*H* and 6-C*H* aromatic protons)] ppm.

16-(3,4-Dimethoxybenzylidene)-3-pyrrolidino-3,5-androstadien-17-one (17)

Starting from **16** (mmol), **17** was obtained (0.5 g, 89.11%), m.p. 185ºC. UV_{max} (MeOH): 290.6 nm. $IR_{\nu max}$ (KBr): 2950, 1710, 1620, 1520 and 1250 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.98 (s, 6H, 18-C*H*$_3$ and 19-C*H*$_3$), 4.12 (s, 3H, -OC*H*$_3$), 4.17 (s, 3H, -OC*H*$_3$), 4.79 (s, 1H, 4-C*H*), 5.08 (t, 1H, 6-C*H*) and 7.28 [m, 4H,one vinyl-*H* of 16-(3,4-dimethoxybenzylidene), 2-C*H*, 5-C*H* and 6-C*H* aromatic protons] ppm.

General Procedure for Synthesis of Compounds 7 and 18

Sodium borohydride (1.0 g) was added in small quantities to a stirred suspension of enamines (**6** and **17**) in methanol (50 ml) at room temperature. Stirring was continued for further 4h. Excess of methanol was removed by distillation under reduced pressure until a small volume of reaction mixture was obtained, which was poured into 50 ml of ice-cold water. The precipitated product was filtered, washed with water, dried and crystallized from methanol.

16-(4-Methoxybenzylidene)-3β-pyrrolidino-5-androsten-17β-ol (7)

Reduction of **6** (0.4g, mmol) yielded **7** (0.25 g, 61.95 %) m.p. 198-204ºC. UV_{max} (MeOH): 264.0 nm (log ε 4.49). $IR_{\nu max}$ (KBr): 3310, 2940, 1660, 1590 and 1240 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.71 (s, 3H, 18-C*H*$_3$), 1.03 (s, 3H, 19-C*H*$_3$), 2.62 (m, 4H, *N*-methylenes of pyrrolidine function), 3.81 (s, 3H, -OC*H*$_3$), 4.04 (d, 1H, 17α-H), 5.36 (d, 1H, 6-C*H*), 6.45 (s) and 6.46 (s) [1:0.9 area ratio, integrating for 1H, vinyl-*H* of 16-(4-methoxybenzylidene)], 6.89 (t, 2H, J_o=8.7 Hz, 3-C*H* and 5-C*H* aromatic protons) and 7.32 (d, 2H, J_o=8.7 Hz, 2-C*H* and 6-C*H* aromatic protons) ppm. MS: m/z (relative intensity): 462 [M^+]. Calcd for $C_{31}H_{43}NO_2$: C, 80.65; H, 9.39; N, 3.03. Found: C, 79.65; H, 8.93; N, 2.64.

16-(3,4-Dimethoxybenzylidene)-3β-pyrrolidino-5-androsten-17β-ol (18)

Sodium borohydride reduction of 16-(3,4-dimethoxybenzylidene)-3-pyrrolidino-3,5-androstadien-17-one (**17**, 0.5 g, mmol) gave **18** (0.41 g, 82.82 %), m.p. 235-240ºC.UV_{max} (MeOH): 265.6 nm (log ε 4.45). $IR_{\nu max}$ (KBr): 3190, 2950, 1660, 1510, 1260 and 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.72 (s, 3H, 18-C*H*$_3$), 1.04 (s, 3H, 19-C*H*$_3$), 2.65 (br, 4H, *N*-methylenes of pyrrolidine function), 3.89 [s, 6H, 2x(OC*H*$_3$)], 4.05 (s, 1H, 17α-*H*), 5.37 (s, 1H, 6-C*H*), 6.45 (s) and 6.46 (s) [1:1 area ratio, integrating for 1H, vinyl-*H* of 16-(3,4-dimethoxybenzylidene)], 6.85 (dd, 1H, J_o=8.3 Hz, J_m=1.70 Hz, 6-C*H* aromatic proton), 6.92 (d, 1H, J_0=8.3 Hz, 5-C*H* aromatic proton) and 6.98 (d, 1H, J_m=1.7 Hz, 2-C*H* aromatic proton) ppm. MS: m/z (relative intensity): 491[M^+]. Calcd. for $C_{32}H_{45}O_3N$: C, 78.16; H, 9.23; N, 2.85. Found: C, 78.01; H, 9.65; N, 3.18.

General Procedure for Synthesis of Compounds 8 and 19

A mixture of 17- hydroxyl-3β-pyrrolidino derivative (**7** and **18**) was taken in acetic anhydride (2.0 ml) and dry pyridine (0.5 ml) was heated on a steam bath for 2h. The reaction mixture was cooled and poured onto crushed ice containing chloroform. It was immediately basified with aqueous potassium hydroxide. The chloroform layer was separated and aqueous layer was extracted with chloroform (3 x 50 ml). The chloroform extracts were combined and dried and chloroform was recovered by distillation under reduced pressure to get solid compound, which was crystallized in chloroform-methanol.

16-(4-Methoxybenzylidene)-3β-pyrrolidino-5-androsten-17β-yl Acetate (DPJ-867) (8)

The compound 16-(4-methoxybenzylidene)-3β-pyrrolidino-5-androsten-17β-ol (**7**, 0.7 g) was taken to afford 8 (0.5 g, 63.45%), m.p. 258-262ºC. UV_{max} (MeOH): 264.0 nm (log ε 4.44). IR_{vmax} (KBr): 2940, 1730, 1600 and 1240 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.79 (s, 3H, 19-C*H*$_3$), 1.02 (s, 3H, 18-C*H*$_3$), 2.22 (s, 3H, 17-OCOC*H*$_3$), 2.63 (m, 4H, -*N*-methylenes of pyrrolidine function), 3.81 (s, 3H, -OC*H*$_3$) 5.36 (d, 1H, 6-C*H*), 6.15 (s) and 6.16 (s) [1:1 area ratio, integrating for 1H, vinyl-*H* of 16-(4-methoxybenzylidene)], 6.87 (d, 2H, J_o=8.7 Hz, 3-C*H* and 5-C*H* aromatic protons) and 7.29 (d, 2H, J_o=8.7 Hz, 2-C*H* and 6-C*H* aromatic protons) ppm. Calcd. for $C_{33}H_{45}NO_3$; C, 78.68; H, 9.01; N, 2.78. Found: C, 78.06; H, 9.04; N, 2.76.

16-(3,4-Dimethoxybenzylidene)-3β-pyrrolidino-5-androsten-17β-yl Acetate (19)

The compound 16-(3,4-dimethoxybenzylidene)-3β-pyrrolidino-5-androsten-17β-ol (**18**, 0.5 g) was taken to afford **19** (0.57 g, 100 %), m.p. 142-144ºC, UV_{max} (MeOH) : 333.6 nm (log ε 4.37) and 244.0 nm (log ε 4.02). IR_{vmax} (KBr) : 2920, 1720, 1700, 1610, 1510, 1250 and 1120 cm^{-1}. ^{1}H-NMR ($CDCl_3$) : δ 0.98 (s, 3H, 18-C*H*$_3$), 1.09 (s, 3H, 19-C*H*$_3$), 2.04 (s, 3H, 3-OCOC*H*$_3$), 3.91 (s, 3H, -OC*H*$_3$), 3.92 (s, 3H, -OC*H*$_3$), 4.61 (m, 1H, 3α-*H*), 5.43 (d, 1H, 6-C*H*), 6.92 (d, 1H, J_o=8.3 Hz, 5-C*H* aromatic proton), 7.06 (d, 1H, J_m=1.5 Hz, 2-C*H* aromatic proton), 7.17 (dd, 1H, J_m=1.6 Hz, J_o=7.1 Hz, J_m=1.8 Hz, 6-C*H* aromatic proton) and 7.39 [s, 1H, vinyl-*H* of 16-(3, 4-dimethoxybenzylidene)] ppm. MS: m/z (relative intensity): 534[M^+]. Calcd for $C_{34}H_{47}NO_4$: C, 76.51; H, 8.88; N, 2.62. Found: C, 75.72; H, 8.71; N, 2.04.

General Procedure for Synthesis of Compounds 11 and 22

To the aldol product, dry pyridine (1.5 ml) and acetic anhydride (1.0 ml) were added and the reaction mixture was heated on a steam bath for 1h. The contents were poured onto crushed ice and allowed to stand. The precipitate obtained was filtered, washed with water, dried and collected.

16-(4-Methoxybenzylidene)-17-oxo-5-androsten-3β-yl Acetate (11)

The compound **4** (0.5 g) was acetylated to yield **11** (0.5 g, 90.63 %), m.p. 224-228ºC.UV_{max} (MeOH): 321.6 nm (log ε 4.48). IR_{vmax} (KBr): 2950, 1730, 1710, 1600 and 1250 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.97 (s, 3H, 18-C*H*$_3$), 1.11 (s, 3H, 19-C*H*$_3$), 2.04 (s, 3H, -OCOC*H*$_3$), 3.85 (s, 3H, -OC*H*$_3$), 4.61 (m, 1H, 3α-*H*), 5.43 (d, 1H, 6-C*H*), 6.94 (d, 2H, J_o=8.8 Hz, 3-C*H* and 5-C*H* aromatic proton), 7.40 [s, 1H, vinyl-*H* at 16-(4-methoxybenzylidene)] and 7.50 (d, 2H, J_o=8.8 Hz, 2-C*H* and 6-C*H* aromatic protons) ppm. MS: m/z (relative intensity): 449 [M^+]. Calcd for $C_{29}H_{36}O_4$: C, 77.64; H, 8.09. Found: C, 77.00; H, 7.88.

16-(3,4-Dimethoxybenzylidene)-17-oxo-5-androsten-3β-yl Acetate (22)

Compound **15** was acetylated and crystallized from ether to give **22** (0.57 g, 100 %), m.p. 142-144ºC.UV_{max} (MeOH): 333.6 nm (log ε 4.37) and 244.0 nm (log ε 4.02).IR_{vmax} (KBr): 2920, 1720, 1700, 1610, 1510, 1250 and 1120 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.98 (s, 3H, 18-C*H*$_3$), 1.09 (s, 3H, 19-C*H*$_3$), 2.04 (s, 3H, 3-OCOC*H*$_3$), 3.91 (s, 3H, -OC*H*$_3$), 3.92 (s, 3H, -OC*H*$_3$), 4.61 (m, 1H, 3α-*H*), 5.43 (d, 1H, 6-C*H*), 6.92 (d, 1H, J_o=8.3 Hz, 5-C*H* aromatic proton), 7.06 (d, 1H, J_m=1.5 Hz, 2-C*H* aromatic proton), 7.17 (dd, 1H, J_m=1.6 Hz, J_o=7.1 Hz, J_m=1.8 Hz, 6-C*H* aromatic proton) and 7.39 [s, 1H, vinyl-*H* of 16-(3, 4-dimethoxybenzylidene)] ppm. MS: m/z (relative intensity): 479 [M^+]. Calcd for $C_{30}H_{38}O_5$: C, 75.28; H, 8.00. Found: C, 74.48; H, 7.79.

16-(4-Methoxybenzylidene)-5-androstene-3β,17β-diol (13)

To a stirred solution of 16-(4-methoxybenzylidene)-17-oxo-5-androsten-3β-ol (**4**, 0.5 g) in methanol (50 ml) at room temperature, sodium borohydride (1.0 g) was added in small amounts. Stirring was continued for 4 h and excess of solvent was removed by distillation under reduced pressure. The residue was added to ice-cold water. The white precipitate obtained was filtered, washed with water, dried and crystallized in chloroform-methanol to afford **13** (0.40 g, 79.60 %), m.p. 196-200ºC. UV_{max} (MeOH): 264.0 nm (log ε 4.42). IR_{vmax} (KBr): 3350, 2960, 1605, 1250 and 900 cm^{-1}.^{1}H-NMR ($CDCl_3$): δ 0.71 (s, 3H, 18-C*H*$_3$), 1.04 (s, 3H, 19-C*H*$_3$), 3.54 (m, 1H, 3α-*H*), 3.81 (s, 3H, -OC*H*$_3$), 4.05 (t, 1H, 17α-*H*), 5.38 (d, 1H, 6-C*H*), 6.45 (s) and 6.46 (s) [1:0.95 area ratio, integrating for 1H, vinyl-H of 16-(4-methoxybenzylidene)], 6.87 (d, 2H, J_o=8.7 Hz, 3-C*H* and 5-C*H* aromatic protons) and 7.31 (d, 1H, J_o=8.7 Hz, 2-C*H* and 6-C*H* aromatic protons) ppm. MS: m/z (relative intensity): 409[M^+]. Calcd for $C_{27}H_{36}O_3$: C, 79.37; H, 8.88. Found: C, 77.90; H, 8.01.

16-(4-Methoxybenzylidene)-5-androstene-3β,17β-diol Diacetate (14)

Dry pyridine (2.0 ml) and acetic anhydride (2.0 ml) were added to 16-(4-methoxybenzylidene)-5-androstene-3β,17β-diol (**13**, 0.5 g) and the reaction mixture was heated on steam bath for 2h. The contents were poured onto crushed ice and allowed to stand. The precipitate obtained was filtered, washed with water, dried and crystallized in ether to yield **14** (0.40 g, 69.75 %) m.p. 105ºC.UV_{max} (MeOH): 264.0 nm (log ε 4.46). IR_{vmax} (KBr): 2920, 1730, 1240 and 900 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 0.71 (s, 3H, 18-C*H*$_3$), 1.05 (s, 3H, 19-C*H*$_3$), 2.04 (s, 3H, 3-OCOC*H*$_3$), 2.20 (s, 3H, 17-OCOC*H*$_3$), 3.81 (s, 3H-OC*H*$_3$), 4.61 (m, 1H, 3α-*H*), 5.36 (s, 1H, 17α-*H*), 5.40 (d, 1H, 6-C*H*), 6.15 (s) and 6.16 (s) [1:1 area ratio, integrating for 1H, vinyl-*H* of16-(4-methoxybenzylidene)], 6.86 (d, 2H, J_o=8.7 Hz, 3-C*H* and 5-C*H* aromatic protons) and 7.29 (d, 1H, J_o=8.8 Hz, 2-C*H* and 6-C*H* aromatic proton) ppm. MS: m/z (relative intensity): 439 [M^+]. Calcd for $C_{28}H_{38}O_4$: C, 76.68; H, 8.73. Found: C, 74.92; H, 8.41.

Pharmacological Activity

The synthesized compounds were sent to National Cancer Institute, Bethesda, USA, where they were evaluated *in vitro* for anticancer activity against 3-cell lines panel consisting of MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS), using concentration of 0.1 mM and then incubated for 48 h. End-point determinations were made with sulforhodamine B, a protein binding dye. Results of each test agent were reported as the percent of growth of treated cells when compared to the untreated control cells. Compounds that reduced

the growth of any one of the cell lines to 32 % or less (negative numbers indicate cell kill) were termed as active.

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