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C_{19}-Steroids as androgen receptor modulators: Design, discovery, and structure-activity relationship of new steroidal androgen receptor antagonists

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Abstract—Dehydroepiandrosterone (DHEA), the most abundant steroid in human circulating blood, is metabolized to sex hormones and other C_{19}-steroids. Our previous collaborative study demonstrated that androst-5-ene-3β,17β-diol (Adiol) and androst-4-ene-3,17-dione (Adione), metabolites of DHEA, can activate androgen receptor (AR) target genes. Adiol is maintained at a high concentration in prostate cancer tissue; even after androgen deprivation therapy and its androgen activity is not inhibited by the antiandrogens currently used to treat prostate cancer patients. We have synthesized possible metabolites of DHEA and several synthetic analogues and evaluated their role in androgen receptor transactivation to identify AR modulators. Steroids with low androgenic potential in PC-3 cell lines were evaluated for anti-dihydrotestosterone (DHT) and anti-Adiol activity. We discovered three potent antiandrogens: 3β-acetoxyandrosta-1,5-diene-17-one 17-ethylene ketal (ADEK), androsta-1,4-diene-3,17-dione 17-ethylene ketal (OAK), and 3β-hydroxyandrosta-5,16-diene (HAD) that antagonized the effects of DHT as well as of Adiol on the growth of LNCaP cells and on the expression of prostate-specific antigen (PSA). In vivo tests of these compounds will reveal their potential as potent antiandrogens for the treatment of prostate cancer.

1. Introduction

Both normal and malignant growth of the prostate gland is completely dependent on available androgens.[1] Therefore, suppression of androgen stimulation of the prostate gland remains a cornerstone of the management of locally advanced or metastatic prostate cancer. Androgen deprivation can be achieved either by suppressing the secretion of testicular androgens by means of surgical or medical castration, or by inhibiting the action of androgens using androgen antagonists such as hydroxyflutamide (HF) or bicalutamide (BC). Androgen antagonists can efficiently block androgen receptor (AR)-mediated gene expression, and therefore offer a potentially useful treatment option for androgen-dependent prostate cancer.[2–4]

The AR has high affinity for its physiological steroidal ligands testosterone (T) and dihydrotestosterone (DHT), the active metabolite of T. HF and BC, potent non-steroidal antiandrogens that inhibit the binding of DHT and T to the AR, are effective temporary treatments for prostate cancer. But following a period of remission, prostate cancer becomes resistant to antiandrogens and growth is termed 'androgen-independent.' This resistance to therapy has been attributed to a modest increase in androgen receptor mRNA expression or to mutations of the androgen receptor gene.[5] It has also been reported that the AR must be capable of binding its ligand to maintain the hormone-refractory stage, and possibly that a modest increase in receptor concentration permits the receptor to function despite the lower levels of androgens in castrated patients.[5] In addition, increased AR levels confer responsiveness to non-canonical ligands such as estrogen, hydrocortisone, or even AR antagonists such as Flutamide, to behave as agonists.[6] Growth of an 'androgen-independent' cell line was suppressed when the AR protein was ablated by

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siRNA.[7] This leads to an important question: are there steroids other than DHT and T, and their metabolites, which could bind to AR and be responsible for the progression of prostate cancer?

In rat whole liver homogenate dehydroepiandrosterone (DHEA) is metabolized to androst-5-ene-3β,17β-diol (Adiol), androst-4-ene-3,17-dione (Adione), and 7-oxygenated DHEAs, diols, triols, and testosterone (T).[8] DHT, estrone, and estradiol are subsequent products in metabolism and their biosynthetic pathway is depicted in Figure 1. Our previous collaborative studies[9,10] have demonstrated that Adiol and Adione could activate AR target genes in the presence of AR, and that AR coactivator (ARA70) could further enhance Adiol's AR-transcriptional activity. Therefore, Adiol is an androgen with intrinsic androgenic activity in human prostate cancer cells.[9,11] The androgenic effects of Adiol were not readily inhibited by HF and BC.[9] Adiol and its sulfate ester are synthesized and secreted from the adrenals. They are also formed in several other organs by reduction of DHEA at position 17. A high concentration of Adiol is maintained in the prostate cancer tissue, even after androgen deprivation therapy,[11] and is thus likely to be the activator of growth in the 'androgen-independent' stage of prostate cancer. The daily production of this androgen in man is estimated at ca. 1.4 mg. It is found in blood plasma at ~1 ng/ml ($\sim 3 \times 10^{-9}$ M), and is a major metabolite of DHEA in human prostate homogenate.[11]

The finding that AR function remains ligand dependent in the normal and upregulated state[5,6] prompted us to synthesize and identify AR modulators. The goal was to find new steroid antagonists with high-binding affinity for the AR, and which will block the activity of 'other antagonists', for example, Adiol on AR transactivation. We focused our attention on synthesizing and evaluating C_{19}-steroidal compounds that are structurally related to DHEA or its metabolites,[8] but in which some metabolically susceptible sites were rendered inactive by appropriate structural modifications. We evaluated their AR ligand specificity with the anticipation that they might yield antiandrogenic response toward the AR, while imparting increased in vivo metabolic stability and pharmacological activity.

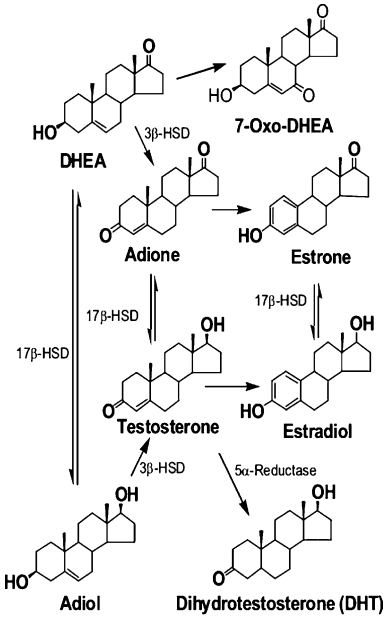
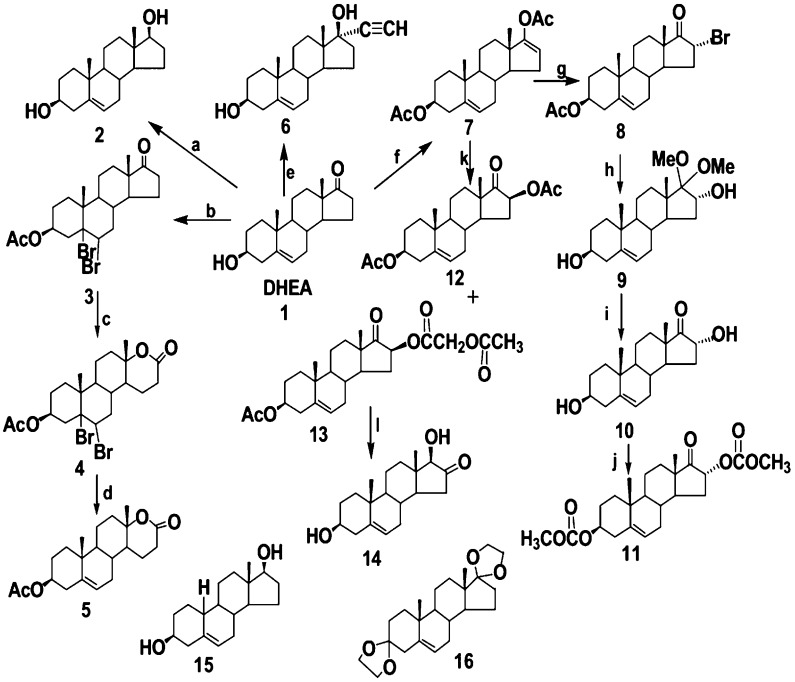


Figure 1. Biosynthetic pathway of C_{19}-steroids and estrogens.

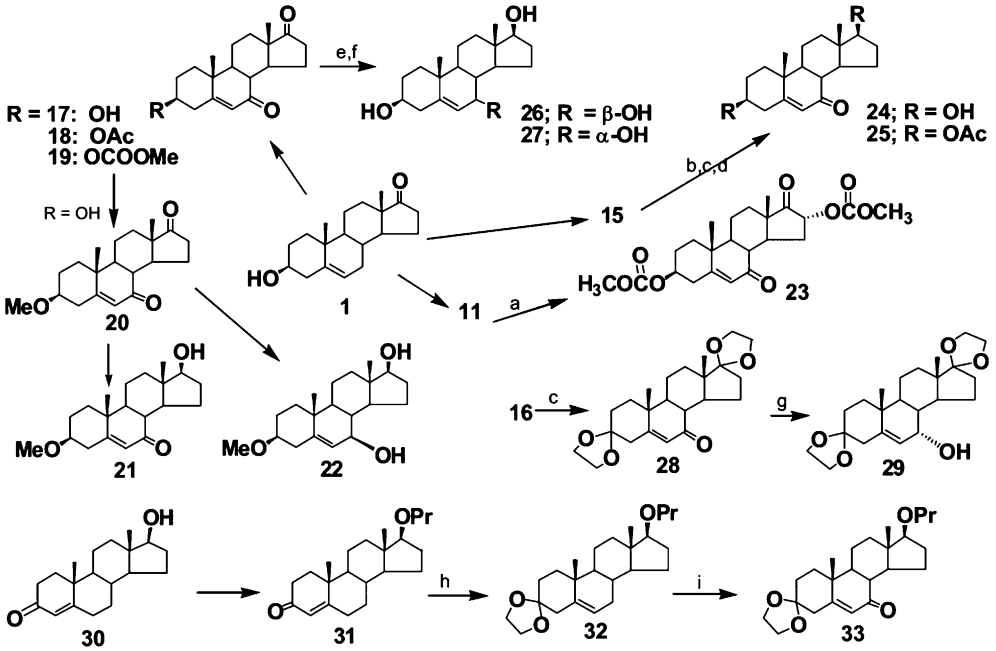
2. Chemistry

DHEA (**1**), the base of a Δ^5 series, was selected for its antiandrogenic potential in prostate cancer cell line studies[9] and because of its beneficial effects in various pathological conditions.[12–16] DHEA metabolites (**2**, **10**, and **14**) as well as some of its synthetic derivatives (**5**, **6**, **12**, and **16**) were prepared from **1**. Their synthesis, as depicted in Scheme 1, is described in Section 6. Adiol (**2**), a major metabolite of **1**, is produced physiologically by the reduction of the metabolically susceptible 17-carbonyl site by 17β-hydroxydehydrogenase (Fig. 1). Adiol, but not DHEA, activates AR target genes in presence of AR.[9] For our biological assays, **2** was synthesized in the laboratory in high purity (100%) and its purity/composition was checked by liquid chromatography-mass spectrometry (LC–MS) in electrospray ionization (ESI) mode. 16-Oxygenated metabolites of DHEA,[8] **10** and **14** (Scheme 1), occur in humans[17] and a function for **10** has been postulated.[18] Metabolite **10** was prepared in four steps from **1**. The other metabolite, **14**, was prepared following the known procedure[19] in three steps from **1** and in 99.9% purity. A new compound **11**, the bis-methyl carbonate of **10**, was synthesized in anticipation of a longer half-life and used for cell line assays. Among synthetic derivatives of **1**, compounds 3β-Acetoxy-17α-oxa-D-homo-androst-5-en-17-one (**5**),[20] 17α-ethynylandrost-5-ene-3β,17β-diol (**6**),[21] 3β,16β-diacetoxyandrost-5-en-17-one (**12**),[19] and androst-5-ene-3,17-dione 3,17-diethylene ketal (**16**)[22] (Scheme 1) were all synthesized from **1** and their structure identification and isomeric purity were established based on NMR, LC–MS data, and reported melting points.[20–22]

To synthesize more diverse analogues, the metabolically susceptible 7 position in the DHEA molecule was exploited and various 7-oxygenated metabolites of **1** as well as synthetic 7-oxygenated derivatives were prepared as shown in Scheme 2. 7-Oxo derivatives (**17**–**21**, **23**–**25**, **28**, and **33**) were prepared by our patented procedures involving either the use of common household bleach[23] as an oxidant or by allylic oxidation using chromium(VI) compounds with *N*-hydroxyphthalimide[24,25] in organic solvents at room temperature. Stereoselective reduction of 7-oxo derivatives using sodium borohydride/cerium trichloride heptahydrate provided pure androst-5-ene-3β,7β,17β-triol (**26**) whereas, K-selectride afforded the corresponding 3β,7α,17β-triol (**27**).[26] Compound **28** was synthesized from **16** using our green oxidation procedure.[27] Steroid **28** was reduced at the 7-position by sodium borohydride to afford the mixture of 7α- and 7β-hydroxy diastereomers (β/α = 6:4). The 7α-diastereomer (**29**) was separated from the mixture by column chromatography and was isolated in pure



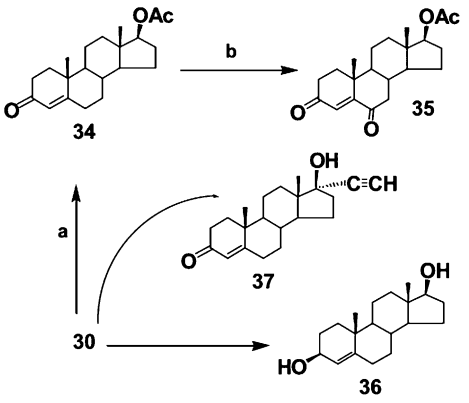
Scheme 1. DHEA and Δ^5-derivatives. Reagents and conditions: (a) $NaBH_4$, MeOH, rt; (b) Br_2, CCl_4, 0–5 °C; (c) MMPP, DCM–MeOH, rt; (d) NaI, THF; (e) sodium acetylide in xylene, DMSO; (f) isopropenyl acetate, *p*-TSA; (g) Br_2, CCl_4, −10 °C; (h) MeOH, NaOMe; (i) AcOH–H_2O, rt; (j) pyridine, methylchloroformate; (k) $Pb(OAc)_4$, AcOH; (l) 2 N NaOH, MeOH.



Scheme 2. 7-Oxygenated C_{19}-steroids. Reagents and conditions: (a) PDC, NOH-phthalimide, Acetone; (b) i—Ac_2O, *p*-TSA, microwave, 0.67 min; (c) clorox bleach, *tert*-butyl hydroperoxide, ethyl acetate; (d) MeOH, NaOH; (e) $NaBH_4$, $CeCl_3$ $7H_2O$, MeOH (f) K-selectride; THF (g) $NaBH_4$, MeOH–DCM; (h) diethylene glycol, toluene, *p*-TSA; (i) N–OH–phthalimide, benzoyl peroxide, air, acetone.

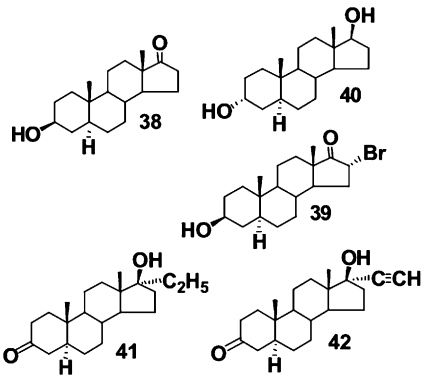
isomeric form, which was further confirmed by LC–MS analysis and spectral data. 7-Oxygenated derivative **33** was prepared from testosterone propionate **31** in two steps. Ketalization of the 3-ketone of **31** afforded Δ^5-ketal **32**, which was oxidized under mild conditions involving *N*-hydroxyphthalimide and air to produce compound **33**.

Compounds selected from the Δ^4 series of C_{19}-steroids are shown in Scheme 3. Steroids **36** and **37** were purchased from Steraloids Inc., to be evaluated in our prostate cancer cell line studies. 17β-Acetoxyandrost-4-ene-3,6-dione (**35**) was synthesized from testosterone-17β-acetate (**34**) using household bleach and *tert*-butyl hydroperoxide.[27]



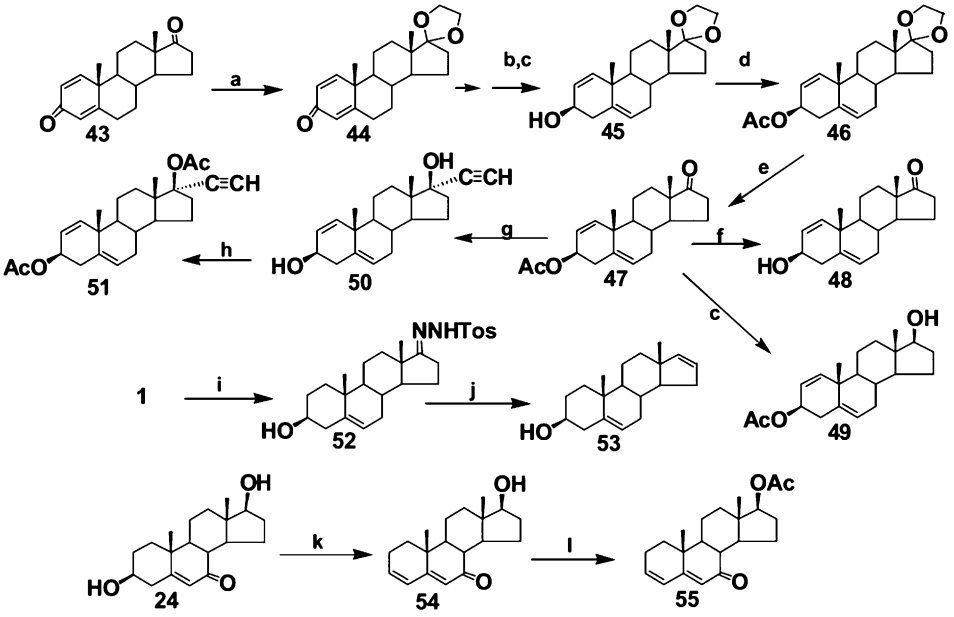
Scheme 3. Δ^4, C_{19}-steroids. Reagents and conditions: (a) Ac_2O, *p*-TSA, microwave, 2 min; (b) clorox bleach, *tert*-butyl hydroperoxide.

Knowing the potent androgenic response of DHT, a 5α-H metabolite of T, prompted us to examine the response of 5α-H metabolites and/or synthetic derivatives of **1** and **30** in prostate cancer cell line assays. A variety of 5α-H steroids (Scheme 4): 3β-hydroxy-5α-androstan-17-one (**38**), 16α-bromo-3β-hydroxy-5α-androstan-17-one (**39**), 5α-androstane-3α,17β-diol (**40**), 17β-hydroxy-17α-ethyl-5α-androstan-3-one (**41**), and 17β-hydroxy17α-ethynyl-5α-androstan-3-one (**42**) were thus selected and purchased from commercial sources for their AR-transcriptional activity evaluation.

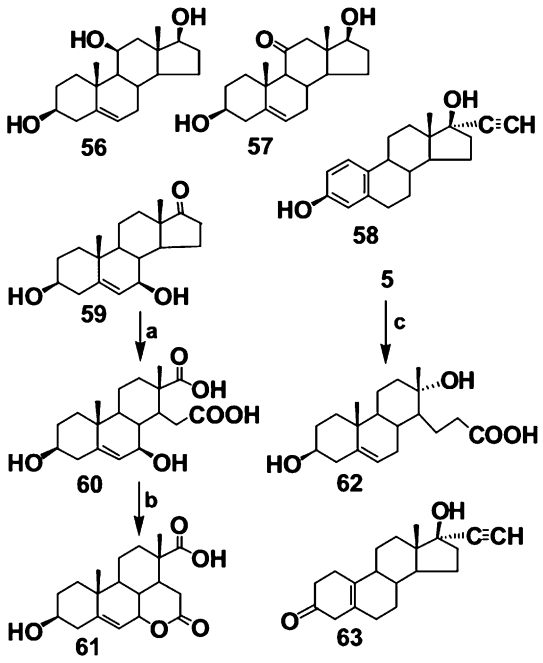


Scheme 4. 5α-H, C_{19}-steroids.

Scheme 5 shows the sequence of steps for the synthesis of conjugated/unconjugated homo- and heteroannular androstadienes. Compound 3β-hydroxyandrosta-1,5-dien-17-one 17-ethylene ketal (**45**) was prepared from androsta-1,4-diene-3,17-dione (**43**) following a reported procedure.[28] Compound **45** on acetylation gave product **46** (ADEK), which on deketalization produced compound **47**. Compound **47** on alkaline hydrolysis yielded compound **48**, but on borohydride reduction afforded steroid **49**. The diene **51**, a 17α-ethynyl derivative of **47**, was synthesized using sodium acetylide in xylene and subsequent acetylation of diene **50** in 1 min using our microwave procedure.[29] The compound androsta-5,16-dien-3β-ol (**53**, HAD) was synthesized from **1** in two simple steps following the procedure of Caglioti.[30] The tosylhydrazone of **1** was prepared by refluxing **1** and tosylhydrazine in alcohol, which on subsequent reduction with lithium aluminum hydride produced $\Delta^{5,16}$ diene **53**[31] in good yield. 7-Oxygenated conjugated diene **54** was prepared by selective dehydration procedure developed for the detection and quantitation of free 7-oxygenated steroids from their bio-conjugates[32] in biological matrices, in nanogram quantities by LC–MS. Selective dehydration of the 3-hydroxyl group of 3β,17β-dihydroxyandrost-5-en-7-one (**24**) using perchloric acid at room temperature afforded diene **54** in excellent yield and purity. Compound **54** on subsequent acetylation in a conventional microwave oven, in 40 s, afforded **55** in excellent yield and purity.



Scheme 5. C_{19}-Androstadienes. Reagents and conditions: (a) diethylene glycol, *p*-TSA, benzene; (b) *t*-BuOK, DMSO, 5–10 °C; (c) $NaBH_4$, MeOH; (d) Ac_2O, pyridine, DMAP, rt, 1 h; (e) *p*-TSA, acetone, H_2O, rt; (f) K_2CO_3, MeOH, H_2O (g) sodium acetylide, DMSO; (h) Ac_2O, *p*-TSA, microwave, 3.0 min; (i) $TsNHNH_2$, MeOH, reflux; (j) $LiAlH_4$, THF; (k) $HClO_4$ (70%), MeOH, rt; (l) Ac_2O, *p*-TSA, microwave, 0.67 min.



Scheme 6. Other C_{19}-steroids. Reagents and condition: (a) Iodine, KOH, MeOH, H_2O; (b) 5 N H_2SO_4; (c) 1 N NaOH, MeOH, reflux.

Scheme 6 shows C_{19}-steroid derivatives with more diversity. 11-Oxygenated steroids in a Δ^5-series such as **56** and **57**, 17α-ethynyl 19-nor steroid with an aromatic A-ring **58**, and steroid **63** with a 5–10 double bond were purchased from Steraloids Inc. The 16,17-seco steroids **61** and **62** were synthesized in the laboratory. Lactone **61** is similar to lactone quassolidane, a parent substance of many natural biological active products isolated from the species *Simarubaceae*.[33–35] It was synthesized from 3β,7β-dihydroxyandrost-5-en-17-one (**59**), a reduced product of compound **17** (Scheme 2). The oxidative D-ring opening of the corresponding 7β-hydroxy compound with potassium hypoiodite produced the 16,17 diacid (**60**), which on subsequent acidification with 5N-sulfuric acid underwent lactonization to produce the 7,16-lactone **61**. The 3β,13α-dihydroxy-17,13-seco-androst-5-ene-17-carboxylic acid (**62**) was prepared by D-ring cleavage of D-homo-13,17-lactone (**5**, Scheme 1) in refluxing methanol containing 1 N sodium hydroxide.

3. Biology

C_{19}-Steroids starting from DHEA (**1**, Scheme 1), its possible metabolites **2**, **14** (Scheme 1); **17**, **24**, **26**, **27** (Scheme 2); **36** (Scheme 3); **38** (Scheme 4), and synthetic C-19 steroids **5**, **6**, **11**, **12**, **15**, **16** (Scheme 1); **19**, **21**, **22**, **23**, **25**, **29**, **33** (Scheme 2); **35**, **37** (Scheme 3); **39**–**42** (Scheme 4); **43**, **44**, **46**–**51**, **53**–**55** (Scheme 5); and **56**–**58**, **61**–**63** (Scheme 6) were first investigated for their ability to induce AR-transcriptional activity. Biological activities of some of the DHEA metabolites and synthetic steroidal derivatives have been reported.[36–38] The reporter assays were developed to investigate androgenic activity in PC-3 cells transfected with wild type AR (wtAR) expression plasmid pSG5-AR and androgen response element-reporter plasmid mouse mammalian tumor virus (MMTV). After transfection, cells were cultured for 24 h with 1 nM DHT or steroidal compounds at 1000 nM. Chloramphenicol acetyltransferase (CAT) assays or luciferase (Luc) assays were performed. AR-transcriptional activity (% relative induction) data of these 43 steroids from Schemes 1–6 (1000 nM) have been shown in Table 1, and Figure 2 graphically exhibits the activity profile (% relative CAT or Luc activity) of these steroids, which are placed in Groups I–VI (shown on the X-axis) together with AR agonist DHT (1 nM, Group 0).

Some of the compounds **6**, **14**, **19**, **27**, **44**, **46**, **47**, **48**, **53**, and **58**, from Schemes 1, 2, 5, and 6, that showed low induction in the AR-transcriptional activity in PC-3 cells, were further screened for their ability to modulate DHT- and Adiol-induced AR transactivation in transiently transfected PC-3 cells with wtAR expression plasmid (pSG5-AR) and androgen response element-reporter plasmid (MMTV) in the presence of DHT (1 nM) or Adiol (2.5 or 50 nM). The transcription-antagonistic activity of these steroidal derivatives has been reported.[36–38] Anti-DHT and anti-Adiol activity (% relative induction) of these 10 steroids (1000 nM) has been shown in Table 2, and Figure 3 graphically exhibits anti-androgenic activity profile of these selected steroids at 1000 nM (OAK at 500 nM), and the response of HF, and BC, on the 1 nM DHT- or 50 or 2.5 nM Adiol-induced transcription in PC-3 cells.

Androstadienes (Scheme 5) **44** (ADEK, 1 μM), **46** (OAK, 0.05–1 μM), and **53** (HAD, 1 μM) with double bonds in ring A (**46**), A and B (**44**) or B and D (**53**) exhibited more potent AR-antagonistic activity than compounds obtained by blocking metabolically susceptible sites in DHEA (Figs. 2 and 3, and Tables 1 and 2). Compounds **44**, **46**, and **53** were further investigated and compared with HF and BC for their anti-DHT and anti-Adiol activity in various prostate cancer cell lines and the biological data have been recently communicated.[38] A 3- to 6-fold increase over mock treatment, in AR transcription in PC-3, LNCaP, and CWR22R cell lines by Adiol (2.5 nM) was impressively repressed (~25–50%) by compounds **44**, **46**, and **53**, whereas HF and BC failed to inhibit Adiol-induced effect significantly.[38] These compounds were also tested for DHT-induced PSA expression using Western blot analysis and showed a suppressive effect. The effect of these steroids on the induction/suppression of cell growth was studied in LNCaP cells, with or without DHT,[38] incubated with steroidal compounds, HF and BC. HAD, OAK, and ADEK as well as BC showed no significant growth induction in the absence of DHT, but suppressed the stimulation by DHT of LNCaP cell growth. Ligand-binding assays were performed in LNCaP or COS-1 cells transfected with wtAR in the presence of 1 nM [^{3}H]-R1881 and various concentrations (1–10,000 nM) of DHT, HF, HAD, OAK, and ADEK. These compounds inhibited PSA expression and growth of prostate cancer

Table 1. Effects of Steroids on the induction of AR-transcriptional activity in PC-3 Cells

Compound[a]	Transcriptional activity (% induction)	
	CAT	Luc
5	—	10
6	2	
11	—	3.6
12	—	16
14	—	15
15	115	—
16	—	37
17	8	—
19	3	—
21	5	—
22	10	—
23	—	56
24	70	—
25	8	—
26	—	25
27	8	—
29	—	44
33	—	90
35	—	90
36	115	—
37	50	—
38	—	78
39	—	74
40	150	—
41	75	—
42	35	—
43	—	62
44	—	44
46	—	8
47	—	12
48	—	15
49	—	38
50	—	43
51	—	13
53	—	11
54	20	—
55	18	—
56	10	—
57	18	—
58	2	—
61	—	11
62	—	6
63	90	—

[a] Steroids from Schemes 1–6. PC-3 cells were transfected with wtAR expression plasmid pSG5-AR and MMTV-CAT or MMTV-Luc. Twenty-four hours after transfection, cells were cultured without hormone or with 1 nM DHT or 1000 nM individual steroids.[36,37] CAT or Luc activity is % response relative to DHT (100%). Values are the mean of at least three determinations.

cells, and have sufficient binding affinity to compete with androgens. Active compounds (**44**, **46**, and **53**) were also screened for their affinity for other steroid receptors such as estrogen, progesterone, and glucocorticoid receptors and except for a little estrogenic activity no other receptor-mediated activity was found. Details of these assays, antiandrogenic activity profile, PSA expression, binding affinity, and receptor specificity of the active compounds are described in our recent publication.[38]

4. Results and discussion

A ligand sensitive AR is involved in the regulation of prostate growth, muscle and bone mass, and spermatogenesis in males. Whereas AR agonists are therapeutically useful in the treatment of osteoporosis, cachexia, contraception, and androgen deficiency,[39] the antagonists are required for the treatment of prostate cancer. During our quest for identifying AR modulators from the array of natural and synthetic C_{19}-steroids, it was found[9] that DHEA (**1**) and 7-oxo-DHEA (**17**) did not activate AR target genes in the absence or presence of coactivator (ARA70) in DU145 cells cotransfected with reporter gene and expression plasmid (wtAR, mtAR877, or mtAR708), whereas, Adiol (**2**) and T (**30**) did show intrinsic activity.[9] It was discovered that Adiol was a strong activator of AR, and that HF (1 μM) and BC (1 μM), the non-steroidal antiandrogens presently used in prostate cancer therapy, failed to inhibit this Adiol (2 nM)-induced response.[9] The polar hydroxyl group at position 17 of the DHT has been proposed to form a hydrogen bond to the Thr-877 and Asn-705 residues of the AR ligand-binding domain (LBD).[40,41] It was observed that various steroidal compounds with hydroxyl groups at positions 3 and 17, such as **15**, **36**, and **40** (Table 1 and Fig. 2), induced AR-transcriptional activity (115–150%) more strongly than the known AR-agonist DHT (100%), probably because of the similar bonding affinity of the 17-hydroxyl group to the LBD. Protection of the 17-hydroxyl group in the compounds **33** and **35** brought down the induction to 90%. Whereas, additional oxo-group substitution at position 7 or 16 seemed to lessen AR-transactivation drastically as was observed in the case of compounds **26**, **27** and **11**, **12**, **14** (8–25%, Table 1 and Fig. 2). Disubstitution at position 17 in the compounds **6**, **16**, **29**, **37**, **42**, and **44** also resulted in lower induction (2–50%, Table 1 and Fig. 2) possibly because of steric hindrance. Among these compounds, **40** (Group IV, Scheme 4 and Fig. 2) from the 5α-H series was identified as the most potent agonist (150% induction as compared to DHT 100%). Androsta-1,5-diene compounds (Scheme 5) were designed and most of them (**44**, **46**, **47**, **48**, **49**, and **50**) were synthesized starting from androsta-1,4-diene-3,17-dione (**43**, Scheme 5). Among this group of compounds, those with 17-keto or ketal group (**46**–**48**, Group 5, Fig. 2) have shown less than 20% induction in the AR-transcription activity. Compounds such as **49** and **50** in the same group, with a free hydroxyl group at position 17, exhibited higher induction (~40%, Table 1 and Fig. 2), which was much lower than the effect of androstenediol compounds (**15**, **36**, and **40**, Table 1 and Fig. 2). Also androsta-5,16-diene (**53**, Table 1) showed 11% and androsta-3,5-dienes (**54** and **55**, Table 1) showed ~20% induction in the AR-transcription activity in the PC-3 cell line assays. Compounds selected from Scheme 6 had diverse structures and they also exhibited interesting activity profiles as shown in Table 1 and Figure 2.

From our first PC-3 cell line assays, some of the compounds such as **6**, **14**, **19**, **27**, **46**, **47**, **48**, and **53**, and **58**, that showed only marginal induction (2–15%, at 1000 nM) of AR transcription[36–38] (Table 1 and

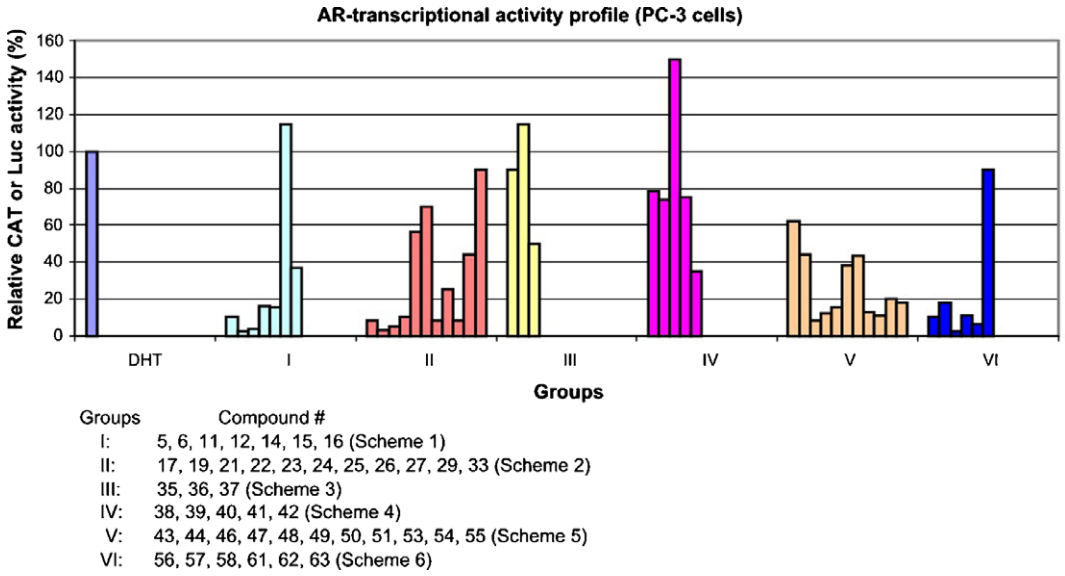


Figure 2. Activity profile of the Steroids on the transcriptional activity of AR. PC-3 cells were transfected with wtAR expression plasmid pSG5-AR and MMTV-CAT (for compounds **6**, **15**, **17**, **19**, **21**, **22**, **24**, **25**, **27**, **36**, **37**, **40**–**42**, **54**–**58**, and **63**) or MMTV-Luc. Twenty-four hours after transfection, cells were cultured without hormone or with 1 nM DHT or with 1000 nM individual steroids. CAT or Luc activity was determined.[36,37] CAT or Luc activity expressed in %. Induction on the Y-axis. X-axis is divided into DHT (Group 0) and Groups I–VI. Each group on the X-axis represents a group of steroids from respective Schemes (i.e., Schemes 1–6). The left-most bar on the X-axis represents DHT as 100%, and every other single bar represents activity of a single steroid in the left to right sequence listed below the chart. Values are means of at least three determinations.

Table 2. Effects of selected steroids on DHT- and Adiol-induced transcriptional activity of AR

Compound[a]	Relative CAT[b] or Luc activity (%)	
	anti-DHT[c]	anti-Adiol
HF	25	90
BC	30	87
6[b]	80	25[d]
14	85	30[d]
19[b]	90	60[d]
27[b]	72	—
44	25	40
46	30	30
47	72	—
48	65	—
53	22	50
58[b]	38	40[d]

[a] Selected steroid's relative antiandrogenic response on the DHT- or Adiol-induced AR-transcriptional activity.

[b] CAT or Luc activity was determined in PC-3 cells transiently cotransfected with pSG5-AR and MMTV-CAT[b] or MMTV-Luc.

[c] For anti-DHT activity, after 24 h cells were cultured with 1000 nM individual steroid along with 1 nM DHT.

[d] For anti-Adiol activity 1000 nM of individual steroid and 50 nM Adiol[36] in PC-3 cells were cultured. HF (1 μM), BC (1 μM) or steroid **44** (0.5 μM), **46** (1 μM), **53** (1 μM), were cultured along with 2.5 nM Adiol.[38] Induction caused by DHT or Adiol set as 100%. The values represent means of at least three determinations.

Fig. 2) were selected for further evaluation of their ability to inhibit DHT- and Adiol-induced[36–38] AR-transcriptional activity in the PC-3 cell line assays and their relative antiandrogenic activity profile (CAT or Luc) is shown in Figure 3. Compound **44** was also selected to evaluate its antiandrogenic potential since it produced the 20–25% induction at varied (50–1000 nM) concentrations[38]. It was observed that compounds **6**, **14**, **19**, and **27**, which showed 2–15% induction in the AR transcription in PC-3 cell line assays (Table 1 and Fig. 2), were not able to inhibit DHT-induced AR transactivation (72–90% induction, Table 2). Although, they did show better response in inhibiting Adiol-induced effect (25–60% induction, Table 2 and Fig. 3). Androstadienes **44**, **46**, and **53** exhibited better anti-DHT (22–30% induction, Table 2) and anti-Adiol effect (30–50% induction, Table 2). It is noteworthy that despite the structural differences in both ring A and ring D among **44**, **46**, and **53**, the bioactivities were very similar for these three compounds. Anti-DHT and anti-Adiol activity of compounds **44** and **46** can probably be partially attributed to the steric hindrance created by the presence of bulky ethylene ketal substituent at position 17. This possible explanation is further supported by the fact that the compound **58** showed consistent suppression (induction ~40%, Table 2) of DHT/Adiol-stimulation (Fig. 3), probably because of the disubstitution at position 17, which creates steric hindrance at that position, and also to the presence of aromatic A-ring. However, further detailed studies are needed to completely understand this phenomenon and to grasp the intricacies and details of the structure-activity relationship.

Blocking of possible metabolically susceptible sites in the base DHEA molecule (positions 6, 7, 16, 17, and 11) or opening the steroidal D-ring in the molecule produced mixed results. But the steroidal compounds designed and synthesized with a little distortion in the A, B, and/or D-ring by introducing double bonds that may or may not be in conjugation seemed more suitable for suppressing DHT- or Adiol-induced AR-transactivation in cell line assays (Table 2 and Fig. 3). It seems that this small amount of rigidity in the molecules fixes

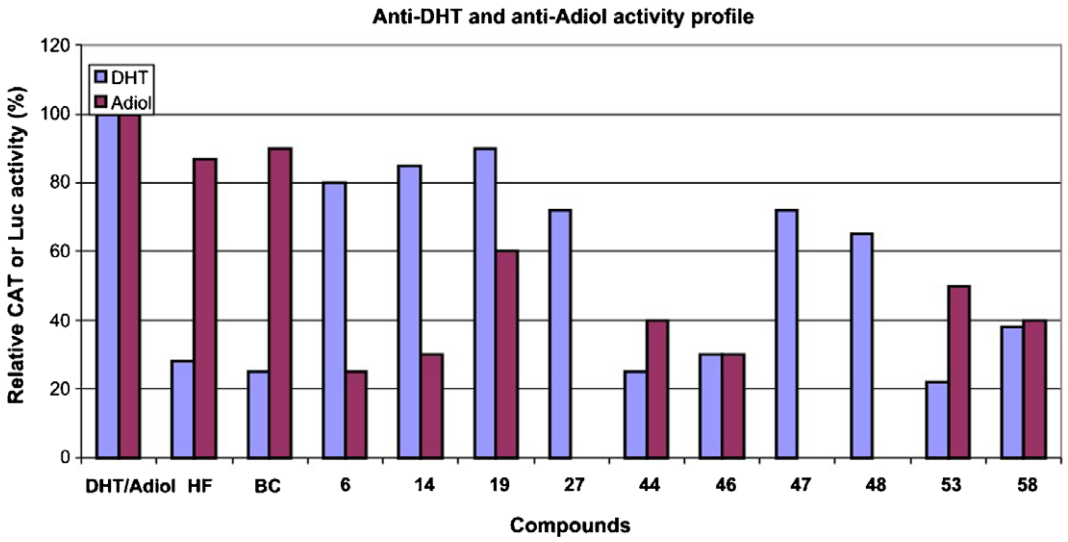


Figure 3. Antiandrogenic activity profile of selected steroids on the DHT- and Adiol-induced AR-transcriptional activity. CAT or Luc activity was determined in PC-3 cells transiently cotransfected with pSG5-AR and MMTV-CAT (compounds **6**, **19**, **27**, and **58**) or MMTV-Luc. For anti-DHT activity, after 24 h cells were cultured with 1000 nM of individual steroid along with 1 nM DHT. For anti-Adiol activity cells were cultured with 1000 nM of steroids **6**, **14**, **19**, **27**, and **58** along with 50 nM Adiol,[36] or compound HF (1 μM), BC (1 μM), **44** (0.5 μM), **46** (1 μM), and **53** (1 μM), along with 2.5 nM Adiol. On the X-axis the left-most bars indicate induction caused by DHT and Adiol set as 100% followed by the effects of HF, BC, and selected steroids on the anti-DHT and anti-Adiol activity. The values represent means of at least three determinations.

them in the appropriate position and location that AR prefers to interact with them and not the co-regulators. We identified three antiandrogens **44**, **46**, and **53** (OAK, ADEK, HAD) from our reporter assays that were able to interrupt androgen binding to the AR, suppress the DHT- and Adiol-induced transactivation of wild type and mutant ARs in prostate cancer cells, and inhibit PSA expression in LNCaP cancer cells better than HF or BC[38]. Their detailed activity on cell lines in comparison with HF and BC, as well as their PSA expression and binding affinity, have been reported in a recent communication.[38]

5. Conclusion

Since the discovery of androgenic activity of naturally occurring steroids, Adiol and Adione, in human prostate cancer cells[9] our interest was stimulated toward identifying steroidal AR modulators. DHT, T, and Adione, all belong to the family of C_{19}-steroids and all are produced metabolically from DHEA. The inability of standard antiandrogens to prevent the activation of AR by Adiol indicates some differences between the combinations of AR-Adiol and AR-DHT. This was born out by finding differences in the respective proteolysis products of these two combinations.[9] We therefore screened DHEA metabolites and synthetic derivatives in the group of C_{19}-steroids, to obtain information concerning structural requirements for binding to the AR. The results also indicated that a little distortion in the steroid skeleton could produce improved antagonist activity that can thwart the effect of DHT as well as that of Adiol on AR. The present study led to the discovery of three potent antiandrogens, **44**, **46**, and **53**, which showed better antiandrogenic potential than HF and BC, and could block DHT- as well as Adiol-induced AR transactivation on both wt and mutant ARs with or without co-activators. They did show some estrogen activity but no progesterone or glucocorticoid receptor activity, which gives us further impetus to design and synthesize better antiandrogens with no estrogenic activity.

6. Experimental

Reaction chemicals and organic solvents were purchased from Aldrich Chemical Company. Deionized water (18.2 MΩ-cm) was used for the reactions and for LC–MS detection and analysis. Steroids were purchased either from Steraloids Inc., New Port, USA, or were synthesized in this laboratory. Melting points (°C) were determined in open capillary tubes in an electrically heated and stirred Thiele-type bath and are uncorrected. Chemical structure of each individual compound, previously known or unknown, was established based on NMR spectral studies and the literature references, and confirmed by molecular mass measurements and their fragmentation patterns in the positive and/or negative ion mode using an online LC–MS instrument. Reaction mixtures and final products were analyzed for identification, selectivity, % conversion, and % purity by LC resolution, followed by mass measurement. NMR spectra were recorded at 200 and 300 MHz on a Bruker spectrometer and 400 MHz on a Varian FT-Unity-Innova (ui400) instrument. Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane (TMS = 0) as internal standard. Coupling constants are given in Hertz (Hz). The following abbreviations are used: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), and m (multiplet). Flash column chromatography was performed on silica gel (70–230 mesh).

6.1. LC–MS–ESI method for analyzing steroidal substrates

Chromatography of all the reaction mixtures and the isolated products was carried out on an Agilent 1100 LC–MS system comprised of a capillary pump (G1376A) operated in normal mode, a quaternary pump (G1311A), column oven (G1313A), autosampler (G1315A), diode array detector (G1315A), and a single quadrupole mass detector (G1946A). Data were acquired and processed using LC/MSD Chemstation version A.09.03 software. LC was performed on a Zorbax-XDB C_8 analytical column (2.1 × 100 mm, 3.5 μm), protected with a Zorbax XDB-C_8 guard column, and maintained at 40 °C. The column flow rate was set at 0.3 ml/min and the eluent was monitored at 205, 240, and 280 nm with a reference wavelength of 360 nm. An acetonitrile–water (ACN/W) linear gradient (%) ACN/W: 30:70 at $t = 0$, 90:10 at $t = 15$ min, and 30:70 at $t = 16$ min followed by a 10 min post-run time was used for the analyses. Formic acid (0.1%) at 0.04 ml/min was added post-column. Compounds were analyzed for their molecular mass and fragmentation using electro-spray ionization (ESI) in positive or negative mode. Operating conditions were: drying gas (N_2) 10 L/min; drying gas temperature 350 °C; nebulizer pressure 30 psi; capillary voltage −4500 V (for positive mode) and +3000 V (for negative mode); gain 2, and fragmentor 80 V. The samples were run in scan mode.

6.2. Androst-5-en-3β,17β-diol (Adiol, 2)

The compound was synthesized in the laboratory by sodium borohydride (1.5 mol. equiv) reduction of DHEA in methanol (MeOH) at 20 °C. Mp 181–82 °C (lit.[42] 180–81 °C).

6.3. 3β-Acetoxy-17α-oxa-D-homo-androst-5-en-17-one (5)

Levy and Jacobson[20] described its synthesis in 1947 utilizing peracetic acid as the oxidizing agent. Our synthetic process involved inexpensive and safe water-soluble monoperoxyphthalic acid magnesium salt hexahydrate (MMPP), which provides relatively faster reaction, easy work-up, and pure product in excellent yield.

DHEA 3-acetate (5.0 g, 0.015 mol) was employed as the starting material, which was first cooled to 0–5 °C in carbon tetrachloride (CCl_4) and a solution of bromine (0.9 ml, 0.0175 mol) in 10 ml CCl_4 was added slowly to the reaction mixture. It was stirred for 30 min at ambient temperature and then concentrated to 10 ml volume. The 5α,6β-dibromo-3β-acetoxyandrostan-17-one (**3**) was crystallized out by diluting the content in the flask with cold petroleum ether (bp 35–60 °C). Yield 97% (7.15 g), mp 161–62 °C (decomp.) (lit.[20] 162.9–163.2°). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.5 (m, 1H, 3α-H), 4.85 (dd, 1H, $J = 4.64$, 1.95 Hz, 6α-H), 2.06 (s, 3H, OAc), 1.49 (s, 3H, 19-CH_3), 0.91 (s, 3H, 18-CH_3).

To a stirred solution of compound **3** (2.0 g, 4.0 mmol) in dichloromethane (DCM)–MeOH (1:4, 100 ml,) at room temperature, water (10 ml) and MMPP (8.0 g) were added. After 24 h and 36 h, additional quantities of MMPP (4.0 g and 2.0 g) were added. After stirring for 48 h, solvent was evaporated and the mixture was extracted with DCM. The organic phase was washed with water and brine, dried over anhydrous magnesium sulfate, and solvent was removed in vacuo. The residue was crystallized from acetone–diethyl ether to afford product **4** (1.87 g, 91%) as a white solid, mp 167–70 °C (decomp.) lit.[20] 170.5–70.9° (decomp.). ^{1}H NMR and LC–MS showed two isomeric peaks (α- and β-) in the ratio of 1:2. LC (λ_{205}): 6α-Br and 6β-Br. MSD (both isomers fragmented similarly) (ESI, +ve): *m/z* 527, 529, 531 (t, $[M+Na]^+$), 447, 449 (d, $[M+Na-HBr]^+$), 367 $[M+Na-2\times HBr]^+$, 345 $[M+H-2\times HBr]^+$, 307 $[M+Na-2\times HBr-AcOH]^+$, 285 $[M+H-2\times HBr-AcOH]^+$, 267 $[M+H-2\times HBr-AcOH-H_2O]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): Major isomer (6β-Br) δ 5.5 (m, 1H, 3α-H), 4.84 (dd, $J = 4.4$, 2.2 Hz, 1H, 6α-H), 2.06 (s, 3H, OAc), 1.43 (s, 3H, 19-CH_3), 1.36 (s, 3H, 18-CH_3), Minor isomer (6α-Br) δ 5.2 (m, 1H, 3α-H), 4.82 (overlapped 6β-H), 2.08 (s, 3H, OAc), 1.30 (s, 3H, 19-CH_3), 1.22 (s, 3H, 18-CH_3).

Compound **4** was debrominated with sodium iodide in tetrahydrofuran, and product 3β-acetoxy-17α-oxa-D-homo-androst-5-en-17-one (**5**) was purified by column chromatography on silica gel (eluent-15% acetone in petroleum ether). Yield 85%, mp 184–85 °C (lit.[20] 183–85 °C), LC (λ_{205}) purity 99%. MSD (ESI, +ve): *m/z* 369 $[M+Na]^+$, 287 $[M+H-AcOH]^+$, 269 $[M+H-AcOH-H_2O]^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38 (t, $J = 2.4$ Hz, 1H, 6-H), 4.6 (m, 1H, 3α-H), 2.04 (s, 3H, OAc), 1.33 (s, 3H, 19-CH_3), 1.00 (s, 3H, 18-CH_3); ^{13}C NMR δ 171.6 (CO-17), 170.7 (CO-Ac), 139.8 (C-5), 121.7 (C-6), 83.3 (C-13), 73.7 (C-3), 49.08, 46.83, 34.58 (CH's), 39.05, 38.0, 36.9, 31.2, 29.0, 27.8, 22.08, 20.3 (CH_2's), 21.6, 20.1, 19.4 (CH_3's).

6.4. 17α-Ethynylandrost-5-ene-3β,17β-diol (6)

To a stirred solution of DHEA (**1**, 0.15 g, 0.5 mmol) in dimethylsulfoxide (DMSO, 4.0 ml), a solution of sodium acetylide (18 w% slurry in xylene/mineral oil, 2 ml) was added slowly under nitrogen atmosphere. After being stirred for 2 h at room temperature, the mixture was cooled and quenched with a cold saturated solution of ammonium chloride and extracted with ethyl acetate containing 10% petroleum ether. The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated in vacuum. The residue on trituration with cold petroleum ether formed an off-white solid, which was purified by crystallization from acetone–petroleum ether. Yield 80% (0.13 g), mp 239–41 °C (lit.[21] 240–42 °C).

6.5. 3β,16α-Dicarbomethoxyandrost-5-en-17-one (11)

Synthesis of this new compound was accomplished in five steps from DHEA.

DHEA (22.0 g) was dissolved in a mixture of isopropenyl acetate (300 ml) and toluene-p-sulfonic acid (*p*-TSA, 1.6 g), and the solution was gently refluxed with continuous but slow distillation. A steady stream of vapors

was collected by upward distillation using a pressure-equalizing funnel. Volume in the flask was maintained at 200 ml by the addition of fresh isopropenyl acetate for a period of 16 h. Solvent was evaporated and the residue was dissolved in a mixture of ethyl acetate and toluene (4:1), washed thoroughly with cold saturated sodium bicarbonate solution, and with water and brine. The dried solution was concentrated to 10 ml and diluted with ether. Compound was crystallized from methanol into a white shiny solid. Mother liquor on further concentration and crystallization afforded additional quantities of the desired 3β,17-diacetoxyandrosta-5,16-diene (**7**). Conversion 90%, yield 79% (20.3 g), mp 146–48 °C (lit.[43] 146–47 °C). ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.48 (dd, $J = 2.1$, 1.7 Hz, 1H, 6-H), 5.4 (d, $J = 5$ Hz, 1H, 16-H), 4.62 (m, 1H, 3α-H), 2.2 (s, 3H, 17-OAc), 2.04 (s, 3H, 3-OAc), 1.05 (s, 3H, 19-CH_3), 0.92 (s, 3H, 18-CH_3).

Compound **7** (2.0 g) was dissolved in dry carbon tetrachloride (60 ml) and the solution was cooled to −10 °C. To this vigorously stirred solution, a solution of bromine (0.25 ml) in carbon tetrachloride (10 ml) was added in 2 min. The solution was stirred for a further 15 min, and then an aqueous solution of sodium hydrogen sulfite was added. The bromo compound was extracted with methylene chloride, which was then washed with sodium bicarbonate solution and brine. The solution was dried over anhydrous magnesium sulfate and concentrated in vacuo to 10 ml. 3β-Acetoxy-16α-bromoandrost-5-en-17-one (**8**) was precipitated as a white solid by the addition of ether. Yield 87% (1.9 g), mp 172–73 °C (decomp.) (lit.[44] 173–75 °C). LC (λ_{205}): purity 96%. MSD (ESI, +ve): *m/z* 431, 433 $[M+Na]^+$, 349, 351 $[M+H-AcOH]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.4 (d, $J = 5$ Hz, 1H, 6-H), 5.4 (dd, $J = 5.4$, 2.9 Hz, 1H, 16β-H), 4.62 (m, 1H, 3α-H), 2.04 (s, 3H, 3-OAc), 1.05 (s, 3H, 19-CH_3), 0.93 (s, 3H, 18-CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 213.3 (CO-17), 170.5 (CO-Ac), 140.0 (C-5), 121.6 (C-6), 73.6 (C-3), 49.9, 48.2, 46.2, 30.7 (CH's), 38.0, 36.8, 34.2, 32.2, 30.6, 27.7, 20.2 (CH_2's), 21.4, 19.3, 13.9 (CH_3's).

Compound **8** (1.0 g, 2.4 mmol) was dissolved in hot methanol (20 ml) and the solution was added to a hot solution of sodium methoxide (methanol 20 ml and sodium 1.0 g). The solution was stirred at reflux temperature for 15 min, concentrated to half in vacuo, and poured into cold water (300 ml). The product was extracted with ethyl acetate–petroleum ether mixture (4:1) and the organic solution was washed successively with dilute hydrochloric acid, sodium bicarbonate, and water. The dried solution was concentrated and cooled to give 3β,16α-dihydroxy-17,17-dimethoxyandrost-5-ene (**9**) as a white solid. LC (λ_{205}), purity 98%. MSD (ESI, +ve): *m/z* 373.3 $[M+Na]^+$, 351.3 $[M+H]^+$, 333.3 $[M+H-H_2O]^+$, 319.2 $[M+H-MeOH]^+$, 301.2 $[M+H-H_2O-MeOH]^+$, 287.3 $[M+H-2\times MeOH]^+$, 269.2 $[M+H-2\times MeOH-H_2O]^+$, 251.1 $[M+H-2\times MeOH-2\times H_2O]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.3 (d, $J = 5$ Hz, 1H, 6-H), 4.3 (t, $J = 8.9$, 1H, 16β-H), 3.5 (m, 1H, 3α-H), 3.46, (s, 3H, 17-OCH_3), 3.37 (s, 3H, 17-OCH_3), 0.99 (s, 3H, 19-CH_3), 0.79 (s, 3H, 18-CH_3).

Compound **9** was used as such in the next step, it was taken up in a mixture of glacial acetic acid (9.0 ml) and water (1.0 ml), and the reaction mixture was stirred at room temperature for 3 h. Acetic acid was removed completely under vacuum and the residue was taken up in cold water and the precipitated white solid was filtered, washed with sodium bicarbonate and water. The product 3β,16α-dihydroxy-androst-5-en-17-one (**10**) was obtained in 89% yield (0.67 g) and was crystallized from methanol, mp 180–84 °C (lit.[45] 182–86 °C). LC (λ_{205}): purity 98%. MSD (ESI, +ve): *m/z* 327.2 $[M+Na]^+$, 305.2 $[M+H]^+$, 287.3 $[M+H-H_2O]^+$, 269.2 $[M+H-2\times H_2O]^+$, 251.1 $[M+H-3\times H_2O]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.4 (d, $J = 5.12$ Hz, 1H, 6-H), 4.4 (dd, $J = 7.6$, 2.2 Hz, 1H, 16β-H), 3.52 (m, 1H, 3α-H), 1.04 (s, 3H, 19-CH_3), 0.99 (s, 3H, 18-CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 220.0 (CO-17), 141.0 (C-5), 121.2 (C-6), 71.28, 71.19 (CH's-3, 16), 50.1, 48.6 (CH's), 42.1, 37.1, 31.4, 31.2, 30.9, 30.4, 20.0 (CH_2's), 19.4, 13.9 (CH_3's).

To a cooled (0 °C) and stirred solution of 16α-hydroxy-DHEA (**10**) (0.4 g, 1.3 mmol) in dry pyridine (5.0 ml), a solution of methyl chloroformate (1.0 ml) in dichloromethane (5.0 ml) was added slowly in 1 h. The reaction mixture was stirred for 4 h, diluted with some more dichloromethane and then quenched with ice-cold water. Organic layer was separated, washed in sequence with dilute hydrochloric acid, saturated sodium bicarbonate, and water, and dried over anhydrous magnesium sulfate. Solvent was removed completely under vacuum and the residue was stirred with cold diethyl ether to yield 3β,16α-dicarbomethoxyandrost-5-en-17-one (**11**) as a white solid in 71% yield (0.39 g). Crystallized from methanol, mp 195–97 °C, LC (λ_{205}): purity 99.5%. MSD (ESI, +ve): *m/z* 443.2 $[M+Na]^+$, 367.3 $[M+Na-MeOH-CO_2]^+$, 269.2 $[M+H-2\times MeOH-2\times CO_2]^+$, 251.3 $[M+H-2\times MeOH-2\times CO_2-H_2O]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.4 (d, $J = 4.88$ Hz, 1H, 6-H), 5.3 (dd, $J = 8.8$, 1.8 Hz, 1H, 16β-H), 4.5 (m, 1H, 3α-H), 3.82, (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 1.04 (s, 3H, 19-CH_3), 0.99 (s, 3H, 18-CH_3).

6.6. 3β,16β-Diacetoxyandrost-5-en-17-one (12)

The compound was prepared as described in the lit.[19] It was obtained mixed with 3β-acetoxy,16β-(acetoxy)acetoxyandrost-5-en-17-one (**13**), and was separated by column chromatography on silica gel using acetone–petroleum ether (1:9) and characterized using LC–MS for purity and mass measurement and NMR for structure assignments. Compound **12**, mp 167–69 °C (lit.[19] 167–70 °C), LC (λ_{205}): purity 99.2%. MSD (ESI, +ve): *m/z* 411.2 $[M+Na]^+$, 389.2 $[M+H]^+$, 329.2 $[M+H-AcOH]^+$, 311.2 $[M+H-AcOH-H_2O]^+$, 269.1 $[M+H-2\times AcOH]^+$, 251.1 $[M+H-2\times AcOH-H_2O]^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.41 (d, $J = 5.6$ Hz, 1 H, 6-H), 4.99 (t, $J = 8.8$ Hz, 1H, 16β-H), 4.6 (m, 1H, 3α-H), 2.12 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.06 (s, 3H, 19-CH_3), 0.98 (s, 3H, 18-CH_3); ^{13}C NMR (400 MHz, $CDCl_3$) δ 214.7 (CO-17), 170.7, 170.5 (CO-Ac), 140.2 (C-5), 121.8 (C-6), 74.8, 73.8 (CH's-3, 16), 50.4, 46.3, 30.9 (CH's), 38.2, 37.1, 31.8, 31.0, 29.6, 27.9, 20.3

(CH_2's), 21.6, 21.0, 19.5, 14.4 (CH_3's). Minor product **13**, mp 194–96 °C (lit.[19] 195–97 °C). LC (λ_{205}): purity 99.0%. MSD (ESI, +ve): *m/z* 469.2 $[M+Na]^+$, 447.2 $[M+H]^+$, 387.2 $[M+H-AcOH]^+$, 369.2 $[M+H-AcOH-H_2O]^+$, 269.1 $[M+H-AcOH-HOCOCH_2OCOCH_3]^+$, 251.1 $[M+H-AcOH-H_2O-HOCOCH_2OCOCH_3-H_2O]^+$.

6.7. 3β,17β-Dihydroxyandrost-5-en-16-one (14)

The compound was prepared by saponification of compound **12** (4.0 g) in methanol (60 ml) with 2 N sodium hydroxide (10 ml) at room temperature in 2 h. Methanol was partially evaporated and the contents were poured into ice water and neutralized with acetic acid. The white solid was filtered and washed with cold water, sucked dry, and then crystallized from methanol to give compound **14** (2.3 g, 72%). Mp 203–5 °C (lit.[19] 201–4 °C). LC (λ_{205}): purity 99.9%. MSD (ESI, +ve): *m/z* 327.2 $[M+Na]^+$, 305.2 $[M+H]^+$, 287.3 $[M+H-H_2O]^+$, 269.1 $[M+H-2\times H_2O]^+$, 251.1 $[M+H-3\times H_2O]^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.4 (d, J = 5.2 Hz, 1H, 6-H), 3.8 (s, 1H, 17α-H), 3.5 (m, 1H, 3α-H), 1.05 (s, 3H, 19-CH_3), 0.76 (s, 3H, 18-CH_3); ^{13}C NMR (400 MHz, $CDCl_3$) δ 218.0 (CO-17), 141.4 (C-5), 121.0 (C-6), 86.4, 71.5 (CH's-3, 17), 50.3, 45.4, 31.1 (CH's), 42.1, 37.2, 36.5, 35.9, 31.9, 31.4, 20.5 (CH_2's), 19.6, 11.5 (CH_3's).

6.8. Androst-5-ene-3,17-dione 3,17-diethylene diketal (16)

The compound was prepared by the ketalization of androst-5-ene-3,17-dione (2.0 g, 6.9 mmol) in refluxing toluene (100 ml) using ethylene glycol (14 ml) and toluene-p-sulfonic acid (0.1 g). The reaction mixture was worked-up after 5 h and the product was crystallized from methanol, yield 2.4 g (92%), mp 172–74 °C (lit.[22] 171–72.5 °C). LC (λ_{205}): purity 99.2%. MSD (ESI, +ve): *m/z* 397.2 $[M+Na]^+$, 375.2 $[M+H]^+$, 313.2 $[M+H-HOCH_2CH_2OH]^+$, 251.1 $[M+H-2\times HOCH_2CH_2OH]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.4 (t, J = 2.5 Hz, 1H, 6-H), 3.7 (m, OCH_2–CH_2O), 4.0 (m, OCH_2–CH_2O), 1.06 (s, 3H, 19-CH_3), 0.86 (s, 3H, 18-CH_3).

6.9. Synthesis of compounds 17–22 and 26 is reported elsewhere.[23,27,46]

6.10. 3β,16α-Dicarbomethoxyandrost-5-ene-7,17-dione (23)

A mixture of 3β,16α-dicarbomethoxyandrost-5-en-17-one (**11**, Scheme 2, 0.58 g) and *N*-hydroxyphthalimide (0.5 g) in acetone was treated with pyridinium dichromate (0.75 g). The oxidant was added in two portions at 8 h interval and the reaction mixture was stirred at room temperature for 24 h. Solvent was removed completely in vacuo and the solid residue was stirred with ethyl acetate containing 10% hexane for 15 min, filtered and washed twice with the same solvent. The combined organic layer was washed in sequence with water, saturated sodium bicarbonate solution, and brine, and dried. Compound **23** was crystallized from acetone–diethyl ether, yield 68%, mp 132–34 °C. LC (λ_{236}): purity 98.2%. MSD (ESI, +ve): *m/z* 359.2 $[M+H-CH_3OCOOH]^+$, 283.3 $[M+H-2\times CH_3OCOOH]^+$, 265.1 $(M+H-2\times CH_3OCOOH-H_2O)^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.76 (d, J = 1.7 Hz, 1H, 6-H), 5.3, 5.26 (dd, J = 9.2, 1.5 Hz, 1H, 16α-H), 4.6 (m, 1H, 3α-H), 3.82 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 1.24 (s, 3H, 19-CH_3), 1.0 (s, 3H, 18-CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 212.5 (CO-17), 199.8 (CO-7), 164.6 (C-5), 155.4, 154.8 (2× CO), 126.5 (C-6), 75.6, 75.3 (CH's-3, 16), 55.2, 54.8 (2× OCH_3), 49.7, 44.0, 43.2 (CH's), 42.1, 37.7, 35.6, 31.8, 30.327.1, 20.1 (CH_2's), 17.4, 14.1 (CH_3's).

6.11. 3β,17β-Dihydroxyandrost-5-en-7-one (24)

Compound **2** was acetylated[29] and subsequently oxidized at position 7, following the sodium hypochlorite procedure,[27] to obtain 3β,17β-diacetoxyandrost-5-en-7-one (**25**). Saponification of **25** yielded compound **24**. Crystallized from acetone–petroleum ether, mp 202–4 °C (lit.[47] 201–4 °C), yield 62%. LC (λ_{242}): purity 99.6%. MSD (ESI, +ve): *m/z* 327.2 $[M+Na]^+$, 305.2 $[M+H]^+$, 287.1 $[M+H-H_2O]^+$, 269.1 $[M+H-2\times H_2O]^+$, 251.1 $[M+H-3\times H_2O]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.7 (d, J = 1.7 Hz, 1H, 6-H), 3.6 (t, J = 5.0 Hz, 1H, 17α-H), 3.6 (m, 1H, 3α-H), 1.22 (s, 3H, 19-CH_3), 0.77 (s, 3H, 18-CH_3).

6.12. Androst-5-ene-3β,7β,17β-triol (26)

The compound was prepared by reduction of 3β-hydroxyandrost-5-ene-7,17-dione (**17**, 0.4 g, 1.32 mmol) in methanol (15 ml) and DCM (5 ml) using sodium borohydride (0.2 g, 5.3 mmol) and cerium trichloride heptahydrate (0.48 g, 1.32 mmol) at 0–5 °C in 30 min. Yield 88%, mp 235–37 °C (lit.[26] 247–48 °C). LC (λ_{205}): purity 99.2%. MSD (ESI, +ve): *m/z* 329.2 $[M+Na]^+$, 289.2 $[M+H-H_2O]^+$, 271.2 $[M+H-2\times H_2O]^+$, 253.2 $[M+H-3\times H_2O]^+$; ^{1}H NMR (200 MHz, DMSO + D_2O): δ 5.18 (s, 1H, 6-H), 3.60 (d, J = 7.08 Hz, 7α-H), 3.5 (t, J = 7.81 Hz, 1H, 17α-H), 3.35 (m, 1H, 3α-H), 1.02 (s, 3H, 19-CH_3), 0.68 (s, 3H, 18-CH_3).

6.13. Androst-5-ene-3β,7α,17β-triol (27)

The compound was prepared by reduction of 3β,17β-dihydroxyandrost-5-en-7-one (**24**, 0.5 g) in anhydrous tetrahydrofuran (THF, 20 ml) using a 1.0 M solution of potassium-tri-*sec*-butylborohydride (K-selectride) in THF (10 ml) at −76 °C for 2 h. Yield 58%, mp 266–67 °C (lit.[26] 271–73 °C). LC (λ_{205}): purity 98.3%. MSD (ESI, +ve): *m/z* 329.2 $[M+Na]^+$, 289.2 $[M+H-H_2O]^+$, 271.2 $[M+H-2\times H_2O]^+$, 253.2 $[M+H-3\times H_2O]^+$; ^{1}H NMR (200 MHz, DMSO): δ 5.46 (d, J = 5.0 Hz, 1H, 6-H), 3.65 (bs, 7β-H), 3.4 (m, 2H, 3α-H, 17α-H), 0.96 (s, 3H, 19-CH_3), 0.68 (s, 3H, 18-CH_3).

6.14. 7α-Hydroxyandrost-5-ene-3,17-dione 3,17-diethylene diketal (29)

Sodium borohydride (0.4 g, 10.5 mmol) was added to the mixture of androst-5-ene-3,7,17-trione 3,17-diethylene diketal[27] (2.0 g, 5.1 mmol) in a mixture of methanol and DCM (2:3, 50 ml). The mixture was stirred at room temperature for 30 min, poured into ice water and the product was extracted with DCM. LC–MS analysis indicated the presence of 7β- and 7α-hydroxy com-

pounds in 60:40 ratio. The 7α-isomer **29** was separated by column chromatography on silica gel using 15% acetone in petroleum ether as eluent, and was crystallized from methanol. LC (λ_{205}): purity 95.4%. MSD (ESI, +ve): *m/z* 413.2 $[M+Na]^+$, 373.1 $[M+H-H_2O]^+$, 311.1 $[M+H-H_2O-HOCH_2CH_2OH]^+$, 249.1 $[M+H-H_2O-2\times HOCH_2CH_2OH]^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 5.57 (dd, $J = 5.13$, 2.0 Hz, 1H, 6-H), 3.95 (m, 4H, OCH_2–CH_2O), 3.9 (m, 5H, OCH_2–CH_2O + 7β-H), 1.0 (s, 3H, 19-CH_3), 0.87 (s, 3H, 18-CH_3).

6.15. 17β-Propionyloxyandrost-5-ene-3,7-dione 3-ethylene ketal (33)

The compound was prepared from testosterone 17β-propionate (**31**), which was ketalized at position 3 using ethylene glycol, toluene, and toluene-p-sulfonic acid to give 17β-propionyloxyandrost-5-en-3-one 3-ethylene ketal (**32**). ^1H NMR (200 MHz, $CDCl_3$): δ 5.34 (t, $J = 2.0$ Hz, 6-H), 4.6 (t, $J = 7.8$ Hz, 17α-H), 3.9 (m, 4H, OCH_2–CH_2O), 2.32 (q, $J = 7.2$ Hz, 2H, CH_2), 1.12 (t, $J = 7.2$ Hz, 3H, CH_3), 1.02 (s, 3H, 19-CH_3), 0.80 (s, 3H, 18-CH_3).

A stirred mixture of **32** (2.0 g, 5.2 mmol), *N*-hydroxyphthalimide (0.84 g, 5.2 mmol), and benzoyl peroxide (0.02 g) in acetone (50 ml) was refluxed. Air was bubbled into the refluxing solution for 8 h. Acetone was removed and residue was taken up in toluene. After cooling at 10 °C for 20 min, phthalimide was filtered out and the organic solution was washed twice with saturated sodium bicarbonate and subsequently with water and brine. Compound **33** was crystallized from ethyl acetate-hexane, yield 58%, mp 258–9 °C. LC (λ_{240}): purity 99.8%. MSD (ESI, +ve): *m/z* 425.2 $[M+Na]^+$, 403.3 $[M+H]^+$, 329.2 $[M+H-C_2H_5COOH]^+$, 267.0 $[M+H-C_2H_5COOH-HOCH_2CH_2OH]^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 5.67 (d, $J = 1.7$ Hz, 1H, 6-H), 4.6 (dd, $J = 7.1$, 1.7 Hz, 1H, 17α-H), 3.96 (m, 4H, OCH_2–CH_2O), 2.32 (q, $J = 7.5$ Hz, 2H, CH_2), 1.14 (t, $J = 7.5$ Hz, 3H, CH_3), 1.21 (s, 3H, 19-CH_3), 1.1 (s, 3H, 18-CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 201.1 (CO-17), 175.0 (CO-Propionyl), 165.0 (C-5), 126.4 (C-6), 108.8 (C-3), 81.7 (C-17), 64.6, 64.5 (CH_2-ketal), 49.6, 44.9, 44.87 (CH's), 41.8, 35.9, 35.6, 31.0, 27.8, 27.6, 25.9, 20.7 (CH_2's), 17.0, 12.1, 9.3 (CH_3's).

6.16. 17β-Acetoxyandrost-4-ene-3,6-dione (35)

The compound was synthesized by the oxidation of testosterone acetate with *tert*-butyl hydroperoxide and household bleach solution as reported[27] earlier.

6.17. Steroidal compounds 36–42 were purchased from Steraloids Inc.

6.18. 3β-Acetoxyandrosta-1,5-dien-17-one 17-ethylene ketal (46, ADEK)

3β-Hydroxyandrosta-1,5-dien-17-one 17-ethylene ketal (**45**) was synthesized as described.[28] To a cooled mixture of crude **45** (4.5 g, 13.6 mmol) in pyridine (10 ml) and acetic anhydride (4.0 ml), dimethylaminopyridine (0.010 g) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into ice water and the compound was extracted with ether. The organic layer was washed with water several times followed by brine and dried over anhydrous magnesium sulfate. Evaporation of the solvent under vacuum below 35 °C gave the crude solid. Crystallization from methanol gave 4.25 g (85%) ADEK (**46**), mp 105–6 °C. LC (λ_{205}): purity 99.7%. MSD (ESI, +ve): *m/z* 395.2 $[M+Na]^+$, 373.2 $[M+H]^+$, 313.2 $[M+H-AcOH]^+$, 311.2 $(M+H-HOCH_2CH_2OH)^+$, 251.2 $(M+H-AcOH-HOCH_2CH_2OH)^+$; ^1H NMR (400 MHz, $CDCl_3$): δ 5.86 (dd, $J = 10.4$, 2.0 Hz, 1-H, 1-H), 5.5 (m, 2H, 2-H+6-H), 5.25 (m, 1H, 3α-H), 3.90 (m, 4H, OCH_2–CH_2O), 2.06 (s, 3H, OAc), 1.11 (s, 3H, 19-CH_3), 0.88 (s, 3H, 18-CH_3); ^{13}C NMR (400 MHz, $CDCl_3$) δ 170.9 (CO-Ac), 137.99 (C-5), 138.0, 125.5, 122.9 (CH's-olefinic), 72.2 (C-3), 119.5 (C-17), 65.4, 64.8 (CH_2-ketal), 50.8, 46.7,32.2 (CH's), 36.1, 34.4, 30.9, 30.7, 22.9, 20.7 (CH_2's), 21.9, 21.6, 14.5 (CH_3's).

6.19. 3β-Acetoxyandrosta-1,5-dien-17-one (47)

To a solution of **46** (0.44 g) in acetone–water (40 ml, 9:1) *p*-TSA (0.12 g) was added and the mixture was stirred at room temperature for 16 h. Acetone was removed and the residue was diluted with some ice water and neutralized to pH 6 using 5% aqueous sodium bicarbonate solution. On further cooling, the separated white solid was collected, washed with water, and dried (0.38 g, 98%). Product **47** crystallized from methanol, mp 185–87 °C. LC (λ_{205}): purity 99.8%. MSD (ESI, +ve): *m/z* 351.2 $[M+Na]^+$, 329.2 $[M+H]^+$, 269.2 $[M+H-AcOH]^+$, 251.1 $(M+H-AcOH-H_2O)^+$; ^1H NMR (400 MHz, $CDCl_3$): δ 5.86 (dd, $J = 10.0$, 2.0 Hz, 1H, 1-H), 5.5 (m, $J = 10.0$, 1.6 Hz, 2H, 2-H+6-H), 5.25 (m, 1H, 3α-H), 2.07 (s, 3H, OAc), 1.13 (s, 3H, 19-CH_3), 0.91 (s, 3H, 18-CH_3); ^{13}C NMR (400 MHz, $CDCl_3$) δ 137.5, 125.9, 122.4 (CH's-olefinic), 72.1 (C-3), 52.0, 47.0, 31.5 (CH's), 36.1, 36.0, 31.6, 30.4, 22.0, 20.6 (CH_2's), 21.9, 21.6, 13.9 (CH_3's).

6.20. 3β-Hydroxyandrosta-1,5-dien-17-one (48)

Potassium carbonate (0.3 g) was added to a solution of **47** (0.25 g) in methanol–water (15 ml, 9:1) and the mixture was stirred at room temperature for 16 h. Methanol was removed and the residue was diluted with ice water and neutralized to pH 6 using 1 N HCl solution. The solution on further cooling gave a white solid, which was filtered, washed with water, dried (0.18 g, 83%), and the product was crystallized from aqueous methanol. Mp 138–40 °C. LC (λ_{205}): purity 99.8%. MSD (ESI, +ve): *m/z* 309.2 $[M+Na]^+$, 287.2 $[M+H]^+$, 269.1 $[M+H-H_2O]^+$, 251.2 $[M+H-2\times H_2O]^+$; ^1H NMR (400 MHz, $CDCl_3$): δ 5.78 (dd, $J = 10.0$, 2.0 Hz, 1H, 1-H), 5.57 (dt, $J = 10.0$ Hz, 1H, 2-H), 5.45 (t, $J = 2.0$ Hz, 1H, 6-H), 4.2 (bt, 1 H, 3α-H), 1.12 (s, 3H, 19-CH_3), 0.91 (s, 3H, 18-CH_3); ^{13}C NMR (400 MHz, $CDCl_3$) δ 221.0 (C-17), 139.4 (C-5), 136.0, 130.0, 121.3 (CH's-olefinic), 69.9 (C-3), 52.1, 47.2, 31.6 (CH's), 40.6, 36.0, 31.6, 30.4, 22.0, 20.6 (CH_2's), 22.1, 13.9 (CH_3's).

6.21. 3β-Acetoxyandrosta-1,5-dien-17β-ol (49)

To a solution of **47** (0.1 g) in methanol (20 ml) sodium borohydride (0.05 g) was added and the mixture was stirred at room temperature for 0.5 h. The solution was diluted with ice water and neutralized to pH 7 with dilute acetic acid. Methanol was evaporated and the compound was extracted with ethyl acetate–petroleum ether mixture (8:2), washed with water, and dried over magnesium sulfate. Solvent was removed completely and the residue was crystallized from methanol to obtained pure product **49** (0.08 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.87 (dd, J = 10.0, 2.0 Hz, 1H, 1-H), 5.5 (m, 2H, 2-H+6-H), 5.25 (m, 1H, 3α-H), 3.65 (t, J = 8.4 Hz, 1H, 17α-H), 2.07 (s, 3H, 3-OAc), 1.12 (s, 3H, 19-CH_3), 0.78 (s, 3H, 18-CH_3); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.0 (CO-Ac), 138.1 (C-5), 138.0, 125.6, 122.9 (CH's-olefinic), 82.0 (C-17), 72.2 (C-3), 51.6, 47.0, 32.0 (CH's), 36.7, 36.1, 31.1, 30.7, 23.6, 20.9 (CH_2's), 21.9, 21.6, 11.3 (CH_3's).

6.22. 3β,17β-Dihydroxy-17α-ethynylandrosta-1,5-diene (50)

A mixture of 3β-acetoxyandrosta-1,5-dien-17-one (**47**, 0.95 g, 2.9 mmol) and DMSO (20 ml) was stirred at room temperature under nitrogen and treated with an 18% suspension of sodium acetylide in xylene (12 ml). The mixture was stirred for 2 h then poured into cold brine and extracted with ether (3 × 30 ml). The combined extracts were washed with water, dried and concentrated to 5 ml. The crude product **50** was obtained by diluting with petroleum ether. It was redissolved in DCM, filtered, and concentrated to give pure product **50** (0.86 g, 96%), mp 215–17 °C. LC (λ_{205}): purity 99.4%. MSD (ESI, +ve): m/z 335.2 $[M+Na]^+$, 313.2 $[M+H]^+$, 295.2 $[M+H-H_2O]^+$, 277.2 $[M+H-2\times H_2O]^+$; ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO): δ 5.56 (d, J = 10.0 Hz, 1H, 1-H), 5.37 (dd, J = 10.0 Hz, 1H, 2-H), 5.19 (d, J = 2.8 Hz, 1H, 6-H), 3.95 (bt, 1H, 3α-H), 2.36 (d, J = 1.6 Hz, 1H, acetylenic-H), 0.91 (s, 3H, 19-CH_3), 0.67 (s, 3H, 18-CH_3); ^{13}C NMR (400 MHz, $CDCl_3$ + DMSO) δ 139.5 (C-5), 135.5, 130.4, 121.1 (CH's-olefinic), 88.5 (C≡), 79.3 (≡CH), 73,4 (C-17), 69.3 (C-3), 50.9, 46.6, 32.5 (CH's), 40.4, 39.1, 32.6, 30.9, 23.2, 20.8 (CH_2's), 22.1, 12.9 (CH_3's).

6.23. 3β,17β-Diacetoxy-17α-ethynylandrosta-1,5-diene (51)

A mixture of compound **50** (0.12 g, 0.38 mmol) and acetic anhydride (0.8 ml) containing 1.0 mol% toluene-p-sulfonic acid was heated in the conventional microwave oven (925 W) for 3 min at high power setting.[29] A usual work-up yielded the product **51** in quantitative yield (0.15 g), mp 155–57 °C. LC (λ_{205}): purity 95.4%. MSD (ESI, +ve): m/z 419.2 $[M+Na]^+$, 359.2 $[M+Na-AcOH]^+$, 337.2 $[M+H-AcOH]^+$, 277.2 $[M+H-2\times AcOH]^+$; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.88 (dd, J = 10.0, 2.0 Hz, 1H, 1-H), 5.48 (m, 2H, 2-H+6-H), 5.24 (m, 1H, 3α-H), 2.59 (s, 1H, acetylenic-H), 2.07 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.12 (s, 3H, 19-CH_3), 0.9 (s, 3H, 18-CH_3); ^{13}C NMR (400 MHz, $CDCl_3$) δ 170.9, 169.8 (CO-Ac), 138.0 (C-5), 137.9, 125.7, 122.8 (CH's-olefinic), 84.7 (C≡), 83.5 (C-17), 75.1 (≡CH), 72.2 (C-3), 49.4, 46.4, 32.3 (CH's), 37.6, 36.1, 33.1, 31.0, 23.8, 20.9 (CH_2's), 21.88, 21.69, 21.59, 13.7 (CH_3's).

6.24. Androsta-5,16-dien-3β-ol (HAD, 53)

A mixture of DHEA (7.0 g, 24 mmol) and tosylhydrazine (0.3 g, 50 mmol) in methanol (300 ml) was refluxed for 24 h, concentrated to half, cooled, and the product **52** crystallized out. The white crystalline material was filtered, washed with cold methanol, and dried to obtain pure product in 73% yield (8.5 g). Additional quantities were obtained from the mother liquor by crystallization from aqueous methanol. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.83 (d, J = 8.4 Hz, 2H, Ar-H), 7.28 (d, J = 8.5 Hz, 2H, Ar-H), 5.33 (d, J = 4.5 Hz, 1H, 6-H), 3.51 (m, 1H, 3α-H), 2.43 (s, 3H, CH_3), 1.01 (s, 3H, 19-CH_3), 0.8 (s, 3H, 18-CH_3).

To a solution of **52** (1.0 g, 2.2 mmol) in tetrahydrofuran (30 ml), lithium aluminum hydride (1.5 g) was added and the mixture was refluxed for 20 h. The mixture was cooled and the excess of hydride was decomposed with moist ether and water. The organic solution was washed with water, brine and dried over $MgSO_4$. Crystallization from methanol afforded **53** in 80% yield (0.48 g), mp 135–37 °C (lit. 137 °C[31]). LC (λ_{205}): purity 99.8%. MSD (ESI, +ve): m/z 273.3 $[M+H]^+$, 255.2 $[M+H-H_2O]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.86 (m, 1H, 17-H), 5.72 (m, 1H, 16-H), 5.38 (d, J = 5.1 Hz, 1H, 6-H), 3.5 (m, 1H, 3α-H), 1.05 (s, 3H, 19-CH_3), 0.80 (s, 3H, 18-CH_3).

6.25. 17β-Hydroxyandrosta-3,5-dien-7-one (54)

A solution of 3β,17β-dihydroxyandrost-5-en-7-one (**24**, 0.25 g, 0.82 mmol) in methanol (10 ml) was stirred with perchloric acid (70% solution, 0.5 ml) at room temperature for 10 h. The mixture was diluted with cold water and neutralized with sodium bicarbonate. The precipitated solid was extracted with ethyl acetate–petroleum ether (9:1), washed with water, and dried. Solvent was evaporated to dryness and the residue was stirred with cold ether. The precipitated solid was filtered, washed with water, and dried to give 0.19 g (83%) of **54**, mp 170–72 °C (lit.[48] 170–72 °C). LC (λ_{285}): purity 97.3%. MSD (ESI, +ve): m/z 309.1 $[M+Na]^+$, 287.2 $[M+H]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.26 (m, 2H, 3-H+4-H), 5.61 (s, 1H, 6-H), 3.66 (t, J = 8.2 Hz, 1H, 17α-H), 1.14 (s, 3H, 19-CH_3), 0.80 (s, 3H, 18-CH_3).

6.26. 17β-Acetoxyandrosta-3,5-dien-7-one (55)

Acetylation of compound **54** was performed in a conventional microwave oven (925 W) with acetic anhydride (3 mol equiv) containing 0.5 mol% p-TSA, in 40 s at high power setting. Yield 90%, mp 217–19 °C. LC (λ_{280}): purity 99.6%. MSD (ESI, +ve): m/z 351.2 $[M+Na]^+$, 329.2 $[M+H]^+$, 269.2 $[M+H-AcOH]^+$, 251.1 $[M+H-AcOH-H_2O]^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.15 (m, 2H, 3-H+4-H), 5.61 (s, 1H, 6-H), 4.62 (dd, J = 9.0 Hz, 1H, 17α-H), 2.05 (s, 3H, OAc), 1.13 (s, 3H, 19-CH_3), 0.85 (s, 3H, 18-CH_3).

6.27. Steroidal compounds 56–58 and 63 were purchased from Steraloids Inc.

6.28. 3β,7β-Dihydroxy-16,17-seco-androst-5-ene-16,17-dioic acid (16 → 7) lactone (61)

A solution of iodine (3.5 g) in methanol (80 ml) and a solution of potassium hydroxide (2.54 g) in 50% aqueous methanol (80 ml) were added to a solution of 3β,7β-dihydroxyandrost-5-en-17-one[49] (**59**, 0.85 g, 2.8 mmol) in methanol (80 ml) under vigorous stirring. Both solutions were added from separate funnels over a period of 5 h in such a manner that a slight excess of iodine was maintained. The mixture was stirred at room temperature for another 16 h. Methanol was removed and the residue was diluted with ice water and extracted twice with ether. The cool aqueous layer was acidified with 5 N sulfuric acid and once again extracted with ether. The title compound (**61**) was isolated as the major compound (0.42 g, 45%) from the aqueous layer on cooling, mp 235–45 °C (decomp.). The ether extracts (containing free acid and mono-methyl ester) were combined and stirred with potassium hydroxide in methanol. The residue on similar work-up and acidification with 5 N sulfuric acid provided additional quantities of the lactone **61**. LC (λ_{205}): purity 99.0%. MSD (ESI, −ve): *m/z* 333.1 $[M\text{-}H]^-$, 287.1 $[M\text{-}H-HCOOH]^-$; MSD (ESI, +ve): *m/z* 357.2 $[M+Na]^+$, 335.2 $[M+H]^+$, 317.3 $[M+H-H_2O]^+$, 289.2 $[M+H-HCOOH]^+$, 271.3 $[M+H-H_2O-HCOOH]^+$; ^1H NMR (200 MHz, CD_3OD): δ 5.4 (s, 1H, 6-H), 4.6 (d, J = 9.1 Hz, 1H, 7α-H), 3.46 (m, 1H, 3α-H), 1.14 (s, 3H, 19-CH_3), 1.1 (s, 3H, 18-CH_3).

6.29. 3β,13α-Dihydroxy-17,13-seco-androst-5-ene-17-carboxylic acid (62)

3β-acetoxy-17α-oxa-D-homo-androst-5-en-17-one (**5**, 0.2 g, 0.58 mmol) in methanol (20 ml) was refluxed with 1 N sodium hydroxide solution (1 ml) for 1 h. Methanol was evaporated under vacuum and the residue was diluted with brine. The solution was adjusted to pH 5 with acetic acid and the solid was extracted with dichloromethane–methanol (9:1), washed with brine, and dried. The organic solvent was evaporated and the residue taken up in ether and the white solid was collected on a filter. Mp 245–47 °C, LC (λ_{205}): purity 90.4%. MSD (ESI, +ve): *m/z* 345.2 $[M+Na]^+$, 327.2 $[M+Na-H_2O]^+$, 305.2 $[M+H-H_2O]^+$, 287.3 $[M+H-2\times H_2O]^+$, 269.1 $[M+H-3\times H_2O]^+$, 251.2 $[M+H-CH_2{=}CH COOH]^+$; ^1H NMR (400 MHz, $CDCl_3$): δ 5.3 (s, 1H, 6-H), 3.45 (m, 1H, 3α-H), 1.09 (s, 3H, 19-CH_3), 0.95 (s, 3H, 18-CH_3); ^{13}C NMR (400 MHz, $CDCl_3$ + DMSO, 3:1) δ 181.6 (CO), 145.7 (C-5), 125.6 (C-6), 75.8 (C-3), 77.5 (C-13), 57.8, 54.1, 41.8 (CH's), 46.9, 46.8, 42.0, 39.7, 37.4, 36.3, 28.7, 27.6 (CH_2's), 25.9, 24.2 (CH_3's).

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