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Bioorganic & Medicinal Chemistry 14 (2006) 373–384

Bioorganic & Medicinal Chemistry

Synthesis and antiandrogenic activity of some new 3-substituted androstano[17,16-*c*]-5′-aryl-pyrazoline and their derivatives

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Received 7 May 2005; revised 7 August 2005; accepted 9 August 2005
Available online 22 September 2005

Abstract—A series of androstano[17,16-*c*]pyrazolines and their oxidized derivatives (**2**–**14**) were synthesized using 3β-hydroxyandrostan-17-one as a starting material (**1a**, **b**). The synthesized compounds were evaluated for their antiandrogenic activity compared to that of Cyproterone® as positive control. Some of the compounds exhibited better antiandrogenic activity than the reference drug. The detailed synthesis, spectroscopic data, ED_{50}, and toxicity (LD_{50} and LD_{90}) of the synthesized compounds were reported.

1. Introduction

In a previous work, we found that certain substituted steroidal derivatives showed androgenic, anabolic, and anti-inflammatory activities.[1] Some new heterocyclic compounds containing nitrogen atom have been synthesized and used as antiparkinsonian,[2] antitumor,[3–5] antimicrobial,[6–8] and anti-inflammatory[9,10] activities. Pyrazolines present an interesting group of compounds, many of which possess widespread pharmacological properties such as analgesic, antipyretic, and antirheumatic activities.[11,12] These derivatives are also well known for their pronounced anti-inflammatory properties[13,14] and are used as potent antidiabetic agents.[15,16] In addition, the pharmacological and antitumor activities of many compounds containing pyrazoline rings have been reviewed.[17–19] Recently, the heterocyclic nitrogen derivatives exhibited a general ionophoric potency for divalent cations[20] and used a novel thiocyanate-selective membrane sensor.[21] In view of these reports and in continuation of our previous work in heterocyclic chemistry, we have synthesized some new compounds containing pyrazoline ring fused with steroidal structure for the evaluation of antiandrogenic activity compared to Cyproterone® as standard drug.

2. Results and discussion

2.1. Chemistry

The arylmethylenes **1** and **2** are in the *E*-form (thermodynamically favored, less sterically hindered, and with a more favored formation of the intermediate resonance stabilized anion). Based on the assumption of Barton et al.[22–24] the preparation of *E*-isomer results from Aldol condensation of steroidal and terpinoidal ketones with aromatic aldehydes.

Other evidence for the formation of *E*-isomer came from the pmr-trace where the downfield shift of the arylidene proton (7.75–7.81 ppm) indicated an *E*-isomer (7.61 for *Z*-isomer).

Also, the starting materials were the (8β,9α,14α) 3β-hydroxyandrost-5-ene-3-one (i.e., the absolute configuration is 5α,14α). In other words, A/B ring fusion is α and C/D ring fusion is α. This configuration was retained in all reaction process, where the aromatic protons of the steroidal nucleus resonate at their normal values.[25]

In the condensation of hydrazine with the arylidene system after removal of one molecule of water and formation of the 17-hydrazone, the NH_2 attacks the double bond from α-face leading to the formation of 16*R*, and the phenyl ring rotates backward forming 5′-*S* center and all the asymmetric centers of the starting material are retained (no change in chemical shift in the pmr-trace).

Keywords: Pyrazolines; Moffat oxidation, Antiandrogenic activity; Cyperoterone.

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doi:10.1016/j.bmc.2005.08.024

The evidence for the 16*R* center is that when C–C carbon bond between C-16 and C-5′ is β, the C18 carbon atom appears deshielded at more than 0.7 ppm (cf. at 0.65 ppm for 16α-substituent).[26]

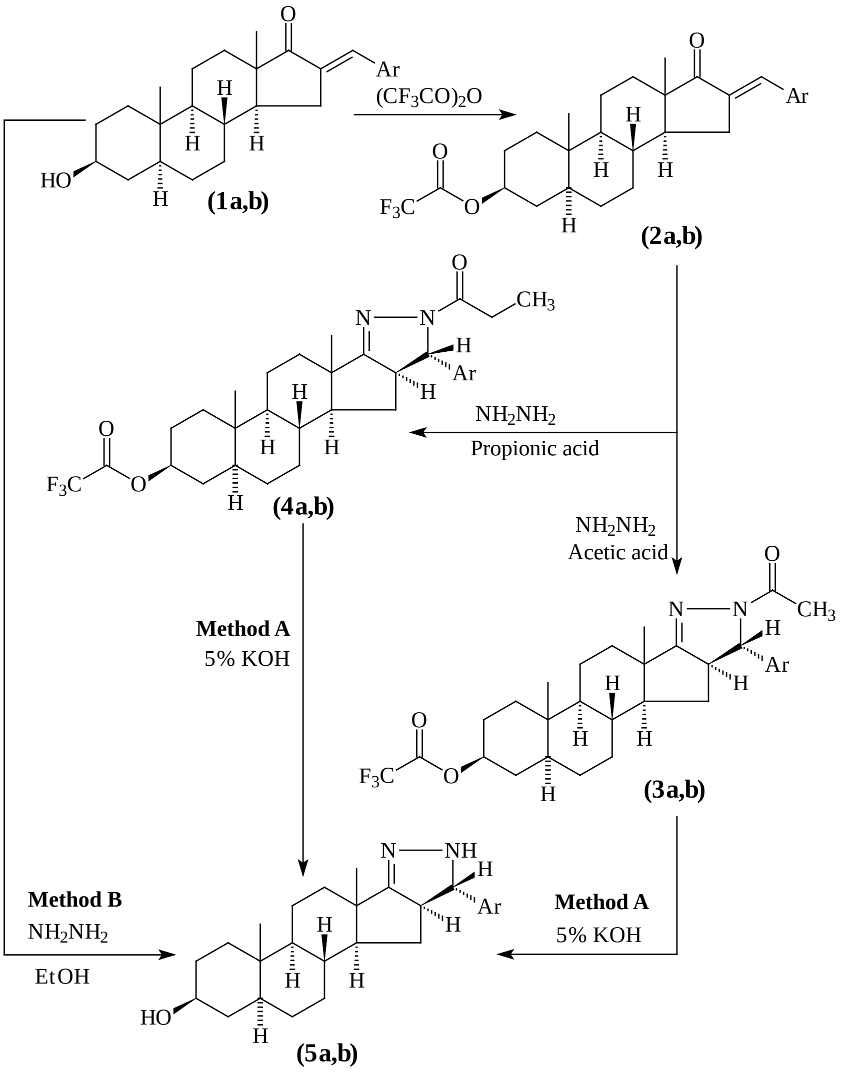
Also, here 16αH, 5′βH (due to backward rotation of the phenyl group to a less sterically hindered cage) is elucidated via determination of J_{vic} for the interaction between C-16 and C-5′ protons. The dihedral angle measurement from the model and the constant of the original Karplus equations (equations 3–5) was 120°. $J_{16\alpha\text{H}-5'\beta\text{H}} \sim 1.2$ CPS (cf. ~1 CPS) if 16βH.[27]

Other evidence for a single isomer came from GC–MS that showed only a single peak compound in the chromatogram and HPLC that showed also high purity for each compound (not less than 98%).

Arylmethylenes of 3β-hydroxyandrostan-17-one derivatives (**1a**,**b**) were synthesized according to a reported procedure.[1] Compounds (**1a**,**b**) were protected by stirring at room temperature with trifluoroacetic anhydride to give the corresponding 3β-trifluoroacetate-16-arylmethylene-androstan-17-one (**2a**,**b**), which was condensed with hydrazine hydrate in refluxing glacial acetic acid or propionic acid to afford the 3β-trifluoroacetate-androstano-*N*-substituted pyrazolines (**3a**,**b**) and (**4a**,**b**), respectively (Scheme 1).

Compounds **3** and **4** were hydrolyzed by refluxing in alcoholic potassium hydroxide 5% to yield 3β-hydroxyandrostanopyrazoline derivatives **5a**,**b**, which can also be obtained directly by condensation of arylmethylene derivatives **1a**,**b** with hydrazine hydrate in refluxing ethanol (Scheme 1).

Oxidation of 3β-hydroxyandrostanopyrazoline derivatives **5a**,**b** with Moffat oxidizing agent in the presence of dicyclohexylcarbodiimide at room temperature gave the corresponding 3-oxo-androstenopyrazoline derivatives **6a**,**b**, which were acetylated with acetylchloride to afford the corresponding 3-oxo-androsteno-*N*-acetylpy-



a, Ar = C_6H_5; **b,** Ar = $C_6H_4OCH_3$(p)

Scheme 1.

razoline derivatives **7a**,**b**. Also, oxidation of compounds **7a**,**b** by dichlorodicyanoquinolinone (DDQ) in the presence of bubbled anhydrous hydrogen chloride gas in dioxane afforded 3-oxo-1,4-diene-androsteno-*N*-acetylpyrazolines (**8a**,**b**), which were hydrolyzed in refluxing 5% alcoholic potassium hydroxide to yield 3-oxo-1,4-diene androstanopyrazoline derivatives **9a**,**b** (Scheme 2).

On the other hand, condensation of compounds (**2a**,**b**) with substituted hydrazines, namely, methylhydrazine or phenylhydrazine, in refluxing glacial acetic acid gave the corresponding 3β-trifluoroacetateandrostano-*N*-substituted pyrazoline derivatives (**10a**–**d**), which were hydrolyzed by 5% alcoholic potassium hydroxide to afford 3β-hydroxyandrostano-*N*-substituted pyrazoline derivatives (**11a**–**d**) (Scheme 3). Oxidation of compounds **11a**–**b** using the Moffat method gave 3-oxo-androstano-*N*-substituted pyrazoline derivatives (**12a**–**d**), which were oxidized with dichlorodicyanoquinolinone (DDQ) to afford the corresponding 3-oxo-1, 4-diene androstano-*N*-substituted pyrazolines **13a**–**d**, while condensation of compounds **12a**–**d** with ethylene triphenylphosphorane by heating at 60 °C in dimethylsulfoxide for 12 h afforded 3-ethylene androstano-*N*-substituted pyrazoline derivatives **14a**–**d** (Scheme 3).

2.2. Biological assay

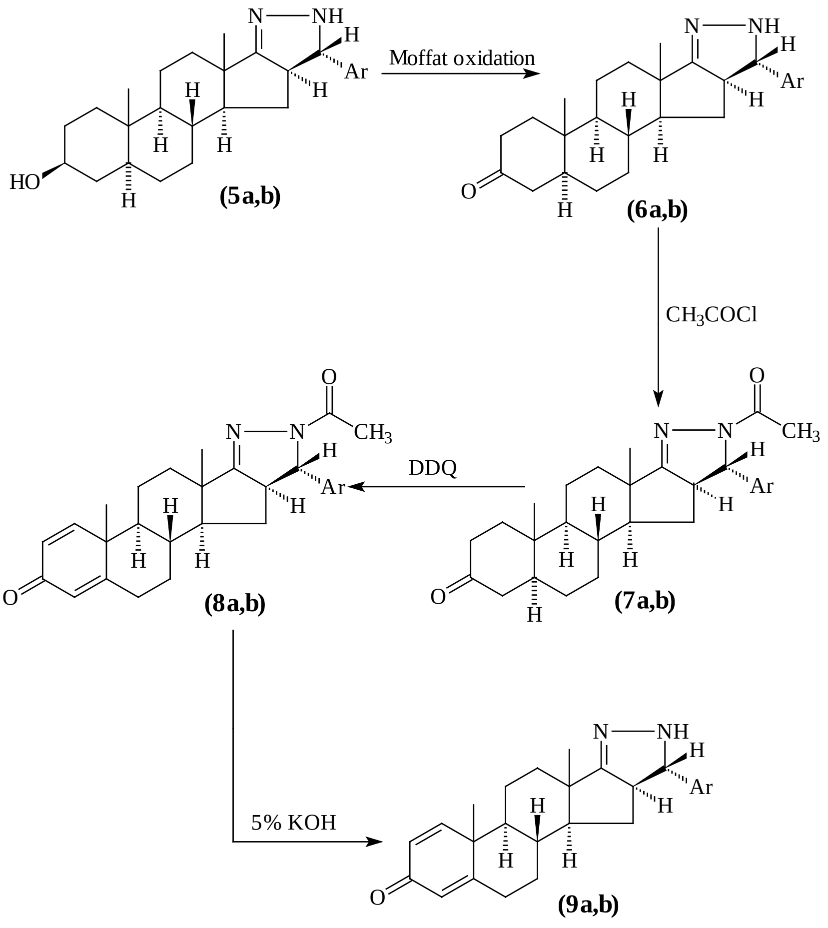
2.2.1. Antiandrogenic activity. All synthesized compounds were tested for their antiandrogenic activities in vivo. Additionally, ED_{50}, LD_{50}, and LD_{90} were determined. All the tested compounds showed antiandrogenic activity. The logarithmic concept was used to calculate the relative potency according to complex concept that seemed to be unreliable for the referee so we recalculate it as he suggests (Table 1). All compounds showed antiandrogenic activities, but less than that of Cyproterone®.

2.2.2. Acute toxicity. The LD_{50} and LD_{90} (rats) were determined by injecting different increasing doses and calculating the dose that kills 50% and 90%, respectively, of the animals.

3. Experimental

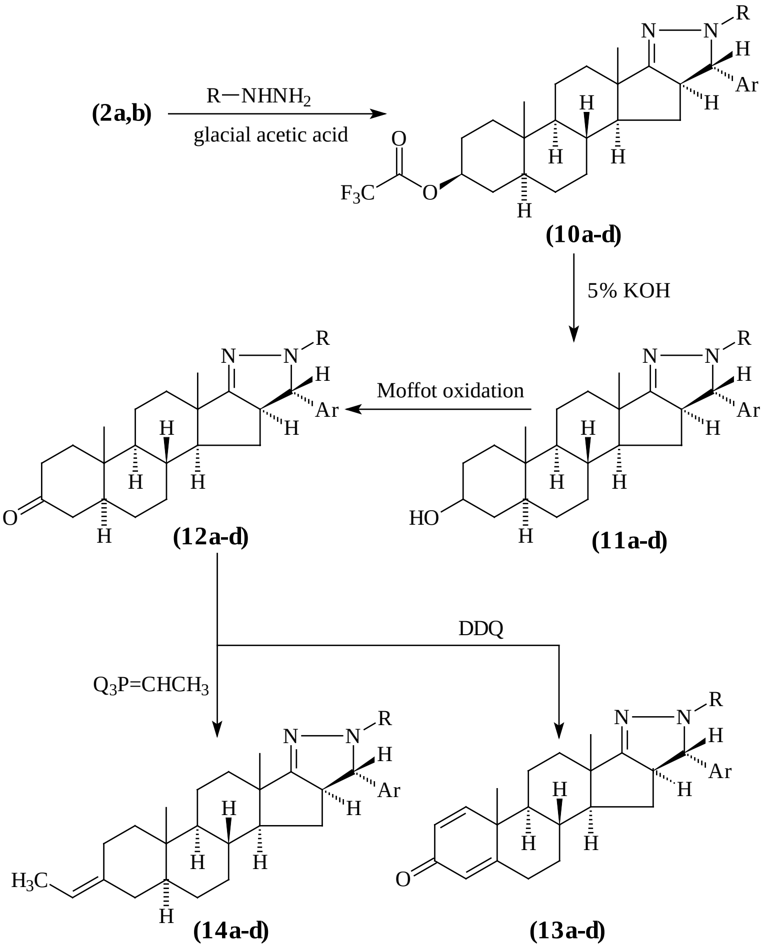
3.1. Synthesis

Melting points were uncorrected and were recorded with an Electrothermal IA 9000 Digital Melting Point Apparatus. Microanalytical data were obtained from the



a, Ar = C_6H_5; **b,** Ar = $C_6H_4OCH_3$(p)

Scheme 2.



	Ar	R
10-14a,	C_6H_5	CH_3
b,	C_6H_5-OCH_3(*p*)	CH_3
c,	C_6H_5	C_6H_5
d,	C_6H_5-OCH_3(*p*)	C_6H_5

Scheme 3.

Microanalytical Unit, Cairo University, Egypt, and their results were found to be in an agreement with proposed structures. The IR spectra (KBr) were recorded on a FT IR-8201 PC Spectrophotometer (Shimadzu). The ^{1}H NMR spectra were measured with Jeol 270 MHz in DMSO-d_6 or $CDCl_3$ and the chemical shifts were recorded in δ ppm relative to TMS. The mass spectra (EI) were run at 70 eV with a Finnigan SSQ GC/MS spectrometer. The reactions were followed by TLC (silica gel, aluminum sheets 60 F_{254}, Merck). HPLC Shimadzu (pump SCL 10 AVP and detector SPD 10 AVP), Japan, column package L3, 4.6 nm × 25 cm, flow rate 1 ml/min, UV detector 254 nm. The starting materials **1a**,**b** were prepared according to the published procedures.[1]

Mobile phase is butyl chloride, water saturated butyl chloride, THF, methanol, and glacial acetic acid [95:95:14:7:6]. Internal standard is a solution of methyl parben in mobile phase having a concentration of 0.04 mg/ml.

For standard preparation transfer, ~12 mg of the tested agents is transferred into a glass-stoppered 50 ml conical flask and 25 ml of internal standard solution is added, sonicated for 5 min, and 1 ml of this solution and 3 ml of mobile phase are combined (see Table 2).

3.1.1. Synthesis of (8β,9α,14α)-3β-trifluoroacet-16(*E*)-aryl-methylene-5α-androstan-17-one (2a,b).

A mixture of the arylmethylene derivatives (**1a**,**b**)[1] (1 mmol) and trifluoroacetic anhydride (5 ml) was kept overnight at room temperature. The reaction mixture was evaporated under reduced pressure up to dryness and the obtained residue was solidified with 1 N sodium bicarbonate (10 ml). The solid formed was collected by filtration, washed with water, dried, and crystallized from methanol to give 3-trifluoroacetate derivatives (**2a**,**b**).

3.1.1.1. (8β,9α,14α)-3β-Trifluoroacet-16(*E*)-phenyl-methylene-5α-androstan-17-one (2a).

Yield 78%, mp: 231 °C (MeOH); $[\alpha]_D^{25}$ +91 (*c* 1, MeOH); IR (KBr): 1738, 1660 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.85 (s, 3H, CH_3), 0.95

Table 1. Evaluation of the ED_{50}, LD_{50}, LD_{90}, and antiandrogenic activity of the newly synthesized compounds

Compound No.	ED_{50} mg/kg	LD_{50} mg/kg	LD_{90} mg/kg	Relative antiandrogenic potency to Cyproterone® (100%)
2a	6.55 ± 0.048	630 ± 0.019	1190 ± 0.011	25.95
2b	6.18 ± 0.049	655 ± 0.018	1188 ± 0.011	27.50
3a	6.14 ± 0.047	710 ± 0.018	1214 ± 0.011	27.68
3b	6.32 ± 0.051	753 ± 0.019	1413 ± 0.011	26.89
4a	6.25 ± 0.044	673 ± 0.019	1111 ± 0.011	27.20
4b	6.12 ± 0.048	663 ± 0.017	1088 ± 0.012	27.77
5a	6.18 ± 0.041	708 ± 0.019	1213 ± 0.013	27.50
5b	6.21 ± 0.042	725 ± 0.019	1340 ± 0.013	27.37
6a	5.13 ± 0.039	813 ± 0.019	1460 ± 0.017	33.13
6b	5.40 ± 0.038	800 ± 0.019	1416 ± 0.012	30.14
7a	5.30 ± 0.039	730 ± 0.019	1318 ± 0.012	32.07
7b	5.20 ± 0.037	741 ± 0.020	1322 ± 0.018	32.69
8a	4.18 ± 0.039	980 ± 0.020	1680 ± 0.019	40.66
8b	4.12 ± 0.038	1012 ± 0.031	1730 ± 0.017	41.62
9a	3.91 ± 0.039	1109 ± 0.031	1814 ± 0.016	43.47
9b	4.00 ± 0.038	983 ± 0.033	1704 ± 0.016	42.50
10a	7.15 ± 0.051	688 ± 0.019	1210 ± 0.016	23.77
10b	7.14 ± 0.051	791 ± 0.019	1314 ± 0.016	23.80
10c	6.44 ± 0.044	890 ± 0.018	1560 ± 0.014	26.39
10d	6.13 ± 0.041	830 ± 0.018	1340 ± 0.016	27.73
11a	6.80 ± 0.041	810 ± 0.017	1550 ± 0.017	27.50
11b	5.00 ± 0.041	940 ± 0.019	1630 ± 0.016	34.00
11c	5.10 ± 0.041	813 ± 0.019	1510 ± 0.016	33.33
11d	4.41 ± 0.044	866 ± 0.019	1630 ± 0.016	38.54
12a	4.12 ± 0.041	851 ± 0.017	1740 ± 0.016	41.26
12b	3.91 ± 0.031	861 ± 0.016	1810 ± 0.017	43.47
12c	4.10 ± 0.031	638 ± 0.016	1430 ± 0.015	41.46
12d	3.20 ± 0.029	813 ± 0.017	1510 ± 0.013	53.12
13a	3.10 ± 0.022	647 ± 0.018	1280 ± 0.012	54.83
13b	3.56 ± 0.023	618 ± 0.015	1380 ± 0.013	47.75
13c	3.18 ± 0.031	613 ± 0.016	1310 ± 0.014	53.45
13d	3.14 ± 0.032	823 ± 0.016	1460 ± 0.015	54.14
14a	2.16 ± 0.031	941 ± 0.017	1805 ± 0.016	78.70
14b	2.15 ± 0.030	1012 ± 0.018	1811 ± 0.013	79.06
14c	2.30 ± 0.031	1210 ± 0.018	1930 ± 0.011	73.91
14d	2.10 ± 0.032	1216 ± 0.017	2031 ± 0.014	73.85
Cyproterone®	1.70 ± 0.031	1518 ± 0.016	2200 ± 0.012	80.95

Error limits are SEM'S.

(s, 3H, CH_3), 0.98–1.00 (m, 1H, CH), 1.28–1.30 (m, 4H, $2CH_2$), 1.40–1.60 (m, 6H, $3CH_2$), 1.64–1.90 (m, 4H, $2CH_2$), 2.00 (m, 1H, CH), 2.24–2.40 (m, 2H, CH_2), 2.45 (m, 1H, CH), 2.55 (m, 1H, 3α-CH), 3.20 (m, 1H, 5α-CH), 7.35–7.45 (m, 5H, Ar-H), 7.81 (s, 1H, C=CH). MS (EI): *m*/*z* 474 (15%) [M^+]. Anal. Calcd for $C_{28}H_{33}F_3O_3$: C, 70.86; H, 7.01. Found: C, 70.78; H, 7.00.

3.1.1.2. (8β,9α,14α)-3β-Trifluoroacet-16(*E*)-(*p*-methoxyphenyl)-methylene-5α-androstan-17-one (2b). Yield 62%, mp: 216 °C (MeOH); $[\alpha]_D^{25}$ +96 (*c* 1, MeOH); IR (KBr): 1735, 1650 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.81 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.95–1.05 (m, 1H, CH), 1.25–1.28 (m, 4H, $2CH_2$), 1.36–1.58 (m, 6H, $3CH_2$), 1.62–1.88 (m, 4H, $2CH_2$), 1.95 (m, 1H, CH), 2.20–2.35 (m, 2H, CH_2), 2.48 (m, 1H, CH), 2.54 (m, 1H, 3α-CH), 3.10 (m, 1H, 5α-CH), 3.65 (s, 3H, OCH_3), 7.20–7.40 (m, 4H, Ar-H), 7.75 (s, 1H, C=CH). MS (EI): *m*/*z* 519 (25%) [M^+]. Anal. Calcd for $C_{29}H_{35}F_3O_4$: C, 69.03; H 6.99. Found: C, 68.98; H, 6.94.

3.1.2. Synthesis of (16*R*,5′*S*) (8β,9α,14α)-3β-trifluoroacet-1′*H*,5α-androstano-[17,16-*c*]-5′-aryl-*N*-substituted-pyrazoline (3a,b) and (4a,b). A mixture of the arylmethylene derivatives (**2a**,**b**) (4 mmol) and hydrazine hydrate (0.8 ml, 16 mmol) in glacial acetic acid or propionic acid (15 ml) was refluxed for ~7 h. The reaction mixture was poured onto ice water and neutralized with sodium bicarbonate. The formed precipitate was collected by filtration, washed with water, dried, and crystallized from the proper solvent to give the corresponding *N*-substituted pyrazoline derivatives (**3a**,**b**) and (**4a**,**b**), respectively.

3.1.2.1. (16*R*,5′*S*)(8β,9α,14α)-3β-Trifluoroacetate-5α-androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-acetylpyrazoline (3a). Yield 65%, mp: 167 °C (MeOH); $[\alpha]_D^{25}$ +85 (*c* 1, MeOH); IR (KBr): 1745, 1665, 1600 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.90–0.98 (m, 1H, CH), 1.25–1.30 (m, 4H, $2CH_2$), 1.38–1.56 (m, 6H, $3CH_2$), 1.60–1.92 (m, 4H, $2CH_2$), 2.00 (m, 1H, CH), 2.08 (s, 3H, $COCH_3$), 2.20–2.35 (m, 2H, CH_2), 2.40 (m, 1H, CH), 2.50 (m, 1H, pyrazoline-H), 2.55 (m, 1H, 3α-CH), 3.00 (m, 1H, 5α-CH), 3.35 (d, 1H, pyrazoline-H), 7.30–7.60 (m, 5H, Ar-H). MS (EI): *m*/*z* 530 (10%) [M^+]. Anal. Calcd for $C_{30}H_{37}F_3N_2O_3$: C, 67.90; H, 7.03; N, 5.28. Found: C, 67.86; H, 7.00; N, 5.25.

Table 2. HPLC of the synthesized compounds

Compound No.	Relative retention time	Purity
1a	1.0	99.81
1b	1.1	99.13
2a	1.28	98.16
2b	1.2	98.74
3a	1.24	98.28
3b	1.7	98.74
4a	1.78	98.16
4b	1.18	98.14
5a	1.13	99.88
5b	1.85	99.95
6a	1.40	99.19
6b	1.17	98.16
7a	1.16	99.18
7b	1.51	99.58
8a	1.56	99.71
8b	1.48	99.03
9a	1.62	98.06
9b	1.37	99.14
10a	1.31	99.17
10b	1.22	98.90
10c	1.73	99.88
10d	1.63	99.61
11a	1.30	99.71
11b	1.13	99.63
11c	1.47	98.91
11d	1.08	98.81
12a	1.11	98.56
12b	1.29	98.66
12c	1.66	99.15
12d	1.77	99.07
13a	1.63	99.48
13b	1.46	99.32
13c	1.41	99.38
13d	1.12	99.16
14a	1.15	99.56
14b	1.33	99.65
14c	1.22	99.16
14d	1.66	99.11

3.1.2.2. (16*R*,5′S)(8β,9α,14α)-3β-Trifluoroacetate-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxyphenyl)-*N*-acetyl-pyrazoline (3b). Yield 60%, mp: 268 °C (EtOH/AcOEt); $[\alpha]_D^{25}$ +80 (*c* 1, MeOH); IR (KBr): 1740, 1660, 1600 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.8 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.95–1.10 (m, 1H, CH), 1.21–1.28 (m, 4H, $2CH_2$), 1.41–1.60 (m, 6H, $3CH_2$), 1.65–1.88 (m, 4H, $2CH_2$), 2.05 (m, 1H, CH), 2.10 (s, 3H, $COCH_3$), 2.18–2.30 (m, 2H, CH_2), 2.36 (m, 1H, CH), 2.45 (m, 1H, pyrazoline-H), 2.58 (m, 1H, 3α-CH), 3.05 (m, 1H, 5α-CH), 3.25 (d, 1H, pyrazoline-H), 3.65 (s, 3H, OCH_3), 7.25–7.50 (m, 4H, Ar-H). MS (EI): *m*/*z* 560 (35%) [M$^+$]. Anal. Calcd for $C_{31}H_{39}F_3N_2O_4$: C, 66.41; H, 7.01; N, 5.00. Found: C, 66.35; H, 6.98; N, 4.96.

3.1.2.3. (16*R*,5′*S*)(8β,9α,14α)-3β-Trifluoroacetate-5α-androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-proponylpyrazoline (4a). Yield 45%, mp: 261 °C (AcOEt); $[\alpha]_D^{25}$ +98 (*c* 1, MeOH); IR (KBr): 1730, 1668, 1630 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.85 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 0.95–1.00 (m, 1H, CH), 1.22–1.28 (m, 4H, $2CH_2$), 1.50 (t, 3H, CH_3), 1.55–1.60 (m, 6H, $3CH_2$), 1.65–1.90 (m, 4H, $2CH_2$), 1.98 (m, 1H, CH), 2.10 (q, 2H, CH_2), 2.20–2.36 (m, 2H, CH_2), 2.40 (m, 1H, pyrazoline-H), 2.48 (m, 1H, CH), 2.55 (m, 1H, 3α-CH), 3.10 (m, 1H, 5α-CH), 3.50 (d, 1H, pyrazoline-H), 7.30–7.50 (m, 5H, Ar-H). MS (EI): *m*/*z* 544 (20%) [M$^+$]. Anal. Calcd for $C_{31}H_{39}F_3N_2O_3$, C, 68.36; H, 7.22; N, 5.14. Found: C, 68.32; H, 7.18; N, 5.10.

3.1.2.4. (16*R*,5′*S*)(8β,9α,14α)-3β-Trifluoroacetate-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxyphenyl)-*N*-proponyl-pyrazoline (4b). Yield 55%, mp: 274 °C (MeOH); $[\alpha]_D^{25}$ +151 (*c* 1, MeOH); IR (KBr): 1735, 1670, 1630 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.82 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.90–0.95 (m, 1H, CH), 1.24–1.30 (m, 4H, $2CH_2$), 1.45 (t, 3H, CH_3), 1.50–1.55 (m, 6H, $3CH_2$), 1.60–1.85 (m, 4H, $2CH_2$), 1.95 (m, 1H, CH), 2.05 (q, 2H, CH_2), 2.18–2.35 (m, 2H, CH_2), 2.35 (m, 1H, pyrazoline-H), 2.45 (m, 1H, CH), 2.50 (m, 1H, 3α-CH), 3.05 (m, 1H, 5α-CH), 3.40 (d, 1H, pyrazoline-H), 3.70 (s, 3H, OCH_3), 7.30–7.45 (m, 4H, Ar-H). MS (EI): *m*/*z* 574 (18%) [M$^+$]. Anal. Calcd for $C_{32}H_{41}F_3N_2O_4$: C, 66.88; H, 7.19, N, 4.88. Found: C, 66.82; H, 7.15; N, 4.82.

3.1.3. Synthesis of (16*R*,5′*S*)(8β, 9α,14α)-3β-Hydroxy-5α-androstano[17,16-*c*]-1′*H*-5′-aryl-pyrazoline (5a,b). *Method A*: A solution of the appropriate compounds (**3a**,**b**) or (**4a**,**b**) (4 mmol) in alcoholic potassium hydroxide (5%, 15 ml) was refluxed for 2–4 h. The reaction mixture was acidified with hydrochloric acid, and the obtained solid was filtered off, washed with water, dried, and crystallized from the proper solvent to give compounds (**5a**,**b**).

Method B: A mixture of the arylidenes (**1a**,**b**) (4 mmol) and hydrazine hydrate (16 mmol) in ethanol (25 ml) was refluxed for ~5 h. The solvent was evaporated under reduced pressure to dryness, and the residue was solidified with water, filtered off, washed with water, dried, and crystallized from methanol to give compounds (**5a**,**b**).

3.1.3.1. (16*R*,5′*S*)(8β,9α,14α)-3β-Hydroxy-5α-androstano[17,16-*c*]-1′*H*-5′-phenyl-pyrazoline (5a). Yield 65%, mp: 251 °C (AcOEt); $[\alpha]_D^{25}$ +65 (*c* 1, MeOH); IR (KBr): 3435, 3230, 1610 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.75 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.95–1.00 (m, 1H, CH), 1.24–1.28 (m, 4H, $2CH_2$), 1.40–1.60 (m, 6H, $3CH_2$), 1.65–1.85 (m, 4H, $2CH_2$), 1.98 (m, 1H, CH), 2.25 (m, 1H, pyrazoline-H), 2.28–2.45 (m, 2H, CH_2), 2.50 (m, 1H, CH), 2.55 (m, 1H, 3α-CH), 3.00 (m, 1H, 5α-CH), 3.45 (d, 1H, pyrazoline-H), 7.30–7.55 (m, 5H, Ar-H), 10.10 (s, 1H, NH, exchangeable with D_2O), 10.55 (s, 1H, OH, exchangeable with D_2O). MS (EI): *m*/*z* 392 (20%) [M$^+$]. Anal. Calcd for $C_{26}H_{36}N_2O$, : C, 79.50; H, 9.17; N, 7.14. Found: C, 79.44; H, 9.12; N, 7.10.

3.1.3.2. (16*R*,5′*S*)(8β,9α,14α)-3β-Hydroxy-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)pyrazoline (5b). Yield 70%, mp: 188 °C (MeOH); $[\alpha]_D^{25}$ +95 (*c* 1, MeOH); IR (KBr): 3440, 3240, 1620 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.90–0.95 (m, 1H, CH), 1.20–1.26 (m, 4H, $2CH_2$), 1.42–1.62 (m, 6H, $3CH_2$), 1.60–1.90 (m, 4H, $2CH_2$), 2.05 (m, 1H, CH), 2.30 (m, 1H, pyrazoline-H), 2.30–2.40 (m, 2H, CH_2), 2.48 (m, 1H, CH), 2.52 (m, 1H, 3α-CH), 3.10 (m, 1H, 5α-CH), 3.30 (d, 1H, pyrazoline-H), 3.65 (s, 3H, OCH_3), 7.20–7.40 (m, 4H, Ar-H), 9.85 (s, 1H, NH, exchangeable with D_2O), 10.40 (s, 1H, OH, exchangeable with D_2O). MS (EI): *m/z* 422 (30%) [M^+]. Anal. Calcd for $C_{27}H_{38}N_2O_2$: C, 76.73; H, 9.06; N, 6.63. Found: C, 76.70; H, 8.98; N, 6.58.

3.1.4. Synthesis of (16*R*,5′*S*)(8β,9α,14α)-3-oxo-5α-androstano[17,16-*c*]-1′*H*-5′aryl-pyrazoline (6a,b). *Moffat method*: The appropriate pyrazoline derivatives (**5a**,**b**) (2 mmol) were dissolved in a mixture of [benzene (3 ml), dimethylsulfoxide (3 ml), pyridine (0.16 ml), and TFA (0.01 ml)] and then dicyclohexylcarbodiimide (1.24 g, 6 mmol) was added. The reaction mixture was kept overnight at room temperature, diethyl ether (50 ml) was added and then oxalic acid (0.54 g, 6 mmol) in methanol (5 ml), and after 30 min, water (50 ml) was added. The obtained dicyclohexylurea was removed by filtration. The product was extracted from filtrate with ether and washed with 5% sodium bicarbonate, then with water. The ethereal solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The formed residue was crystallized from ethanol to give the corresponding oxo-compounds (**6a**,**b**) in pure form.

3.1.4.1. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-5α-androstano[17,16-*c*]-1′*H*-5′-phenylpyrazoline (6a). Yield 55%, mp: 305 °C (MeOH); $[\alpha]_D^{25}$ +101 (*c* 1, MeOH); IR (KBr): 3280, 1740, 1620 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.80 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.90–0.95 (m, 1H, CH), 1.24–1.28 (m, 4H, $2CH_2$), 1.40–1.60 (m, 6H, $3CH_2$), 1.64–1.92 (m, 4H, $2CH_2$), 2.05 (m, 1H, CH), 2.22–2.40 (m, 2H, CH_2), 2.50 (m, 1H, pyrazoline-H), 2.55 (m, 1H, CH), 3.05 (m, 1H, 5α-CH), 3.40 (d, 1H, pyrazoline-H), 7.35–7.60 (m, 5H, Ar-H), 10.30 (s, 1H, NH, exchangeable with D_2O). MS (EI): *m/z* 390 (45%) [M^+]. Anal. Calcd for $C_{26}H_{34}N_2O$: C, 79.95; H, 8.77; N, 7.17. Found: C, 79.90; H, 8.74; N, 7.14.

3.1.4.2. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)pyrazoline (6b). Yield 80%, mp: 256 °C (MeOH/AcOEt); $[\alpha]_D^{25}$ +130 (*c* 1, MeOH); IR (KBr): 3245, 1745, 1615 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.75 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.95–1.00 (m, 1H, CH), 1.25–1.30 (m, 4H, $2CH_2$), 1.36–1.58 (m, 6H, $3CH_2$), 1.60–1.88 (m, 4H, $2CH_2$), 2.10 (m, 1H, CH), 2.20–2.42 (m, 2H, CH_2), 2.25 (m, 1H, pyrazoline-H), 2.50 (m, 1H, CH), 3.00 (m, 1H, 5α-CH), 3.35 (d, 1H, pyrazoline-H), 3.70 (s, 3H, OCH_3), 7.25–7.50 (m, 4H, Ar-H), 10.35 (s, 1H, NH, exchangeable with D_2O). MS (EI): *m/z* 420 (25%) [M^+]. Anal. Calcd for $C_{27}H_{36}N_2O_2$: C, 77.10; H, 8.63; N, 6.66. Found: C, 76.95; H, 8.58; N, 6.60.

3.1.5. Synthesis of (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-5α-androstano[17,16-*c*]-1′*H*-5′-aryl-*N*-acetylpyrazoline (7a,b). A mixture of the compounds (**6a**,**b**) (5 mmol) and acetyl chloride (10 ml) was stirred overnight at room temperature. The excess acetyl chloride was evaporated under reduced pressure, the residue was solidified with sodium bicarbonate, and the obtained solid was filtered off, washed with water, dried, and crystallized from the proper solvent to give compounds (**7a**,**b**).

3.1.5.1. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-5α-androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-acetyl-pyrazoline (7a). Yield 68%, mp: 230 °C (MeOH); $[\alpha]_D^{25}$ +156 (*c* 1, MeOH); IR (KBr): 1745, 1670, 1640 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.85 (s, 3H, CH_3), 1.0 (s, 3H, CH_3), 1.05–1.15 (m, 1H, CH), 1.22–1.28 (m, 4H, $2CH_2$), 1.40–1.61 (m, 6H, $3CH_2$), 1.64–1.90 (m, 4H, $2CH_2$), 1.98 (m, 1H, CH), 2.20 (s, 3H, $COCH_3$), 2.25–2.38 (m, 2H, CH_2), 2.40 (m, 1H, pyrazoline-H), 2.52 (m, 1H, CH), 3.05 (m, 1H, 5α-CH), 3.45 (d, 1H, pyrazoline-H), 7.20–7.40 (m, 5H, Ar-H). MS (EI): *m/z* 432 (60%) [M^+]. Anal. Calcd for $C_{28}H_{36}N_2O_2$: C, 77.73; H, 8.39; N, 6.48. Found: C, 77.68; H, 8.34; N, 6.40.

3.1.5.2. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)-*N*-acetylpyrazoline (7b). Yield 60%, mp: 130 °C (MeOH); $[\alpha]_D^{25}$ +70 (*c* 1, MeOH); IR (KBr): 1740, 1670, 1630 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.80 (s, 3H, CH_3), 1.0 (s, 3H, CH_3), 1.05–1.10 (m, 1H, CH), 1.24–1.30 (m, 4H, $2CH_2$), 1.42–1.60 (m, 6H, $3CH_2$), 1.62–1.92 (m, 4H, $2CH_2$), 2.10 (m, 1H, CH), 2.20 (s, 3H, $COCH_3$), 2.30 (m, 1H, pyrazoline-H), 2.32–2.40 (m, 2H, CH_2), 2.50 (m, 1H, CH), 3.15 (m, 1H, 5α-CH), 3.35 (d, 1H, pyrazoline-H), 3.65 (s, 3H, OCH_3), 7.20–7.40 (m, 4H, Ar-H). MS (EI): *m/z* 462 (25%) [M^+]. Anal. Calcd for $C_{29}H_{38}N_2O_3$: C, 75.28; H, 8.28; N, 6.06. Found: C, 75.18; H, 8.22; N, 6.00.

3.1.6. Synthesis of (16*R*,5′*S*)(8β,9α,14α)-3-oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-aryl-*N*-acetylpyrazoline (8a,b). Anhydrous hydrogen chloride was bubbled for a few seconds into a mixture of the compounds (**7a**,**b**) (6 mmol) and dichlorodicyanoquinone (DDQ) (1.8 g) in dioxane (40 ml). The mixture was kept for 30 min at room temperature with stirring, the precipitate hydroquinone was filtered off and the filtrate was diluted with water, then extracted with ether, the ethereal solution was washed with 1% sodium hydroxide, water, and dried over anhydrous sodium sulfate. The ether part was evaporated under reduced pressure to dryness and then crystallized from the proper solvent to give the corresponding 3-oxo-diene derivatives (**8a**,**b**).

3.1.6.1. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-phenyl-*N*-acetylpyrazoline (8a). Yield 58%, mp: 238 °C (EtOH/pet.ether 40–60 °C); $[\alpha]_D^{25}$ +71 (*c* 1, MeOH); IR (KBr): 1720, 1665, 1630 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.90 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 1.10–1.15 (m, 1H, CH), 1.25–1.30 (m, 4H, $2CH_2$), 1.40–1.60 (m, 2H, CH_2), 1.65–1.70 (m, 2H, CH_2), 1.75 (m, 1H, CH), 1.85–2.00 (m, 2H, CH_2), 2.10 (s, 3H, $COCH_3$), 2.25 (m, 1H, pyrazoline-H), 2.30–2.45 (m, 1H, CH), 3.30 (d, 1H, pyrazoline-H), 5.70 (s, 1H, H-4), 6.20 (d, 1H, H-2), 7.0 (d, 1H, H-1), 7.35–7.50 (m, 5H, Ar-H). MS (EI): *m/z* 428 (15%) [M^+]. Anal. Calcd for $C_{28}H_{32}N_2O_2$: C,78.47; H, 7.53; N, 6.45. Found: C, 78.43; H, 7.49; N, 6.50.

3.1.6.2. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)-*N*-acetylpyrazoline (8b). Yield 60%, mp: 217 °C (MeOH); $[\alpha]_{\mathrm{D}}^{25}$ +95 (*c* 1, MeOH); IR (KBr): 1730, 1670, 1635 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.95 (s, 3H, CH$_3$), 1.10 (s, 3H, CH$_3$), 1.15–1.20 (m, 1H, CH), 1.25–1.30 (m, 4H, CH$_2$), 1.36–1.58 (m, 2H, CH$_2$), 1.60–1.70 (m, 2H, CH$_2$), 1.76 (m, 1H, CH), 1.90–2.05 (m, 2H, CH$_2$), 2.15 (s, 3H, COCH$_3$), 2.30 (m, 1H, pyrazoline-H), 2.35–2.45 (m, 1H, CH), 3.40 (d, 1H, pyrazoline-H), 3.60 (s, 3H, OCH$_3$), 5.75 (s, 1H, H-4), 6.20 (d, 1H, H-2), 7.10 (d, 1H, H-1), 7.30–7.55 (m, 4H, Ar-H). MS (EI): *m*/*z* 458 (15%) [M$^+$]. Anal. Calcd for $C_{29}H_{34}N_2O_3$: C, 75.95; H, 7.47; N, 6.11. Found: C, 75.90; H, 7.42; N, 6.05.

3.1.7. Synthesis of (16*R*,5′*S*)(8β,9α,14α)-3-oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-aryl-pyrazoline (9a,b). A solution of compounds (**8a**,**b**) (4 mmol) in 5% alcoholic potassium hydroxide (15 ml) was refluxed for 2–3 h. The reaction mixture was neutralized with hydrochloric acid, the obtained solid was filtered off, washed with water, dried, and crystallized from the proper solvent to give compounds (**9a**,**b**).

3.1.7.1. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-phenyl-pyrazoline (9a). Yield 70%, mp: 284 °C (MeOH); $[\alpha]_{\mathrm{D}}^{25}$ +47 (*c* 1, MeOH); IR (KBr): 3415, 1730 cm^{-1}. ^{1}H NMR (DMSO-*d*$_6$): δ 0.70 (s, 3H, CH$_3$), 0.90 (s, 3H, CH$_3$), 0.95–1.05 (m, 1H, CH), 1.24–1.32 (m, 4H, 2CH$_2$), 1.38–160 (m, 2H, CH$_2$), 1.65–1.75 (m, 2H, CH$_2$), 1.78 (m, 1H, CH), 1.85–2.00 (m, 2H, CH$_2$), 2.30 (m, 1H, pyrazoline-H), 2.35–2.45 (m, 1H, CH), 3.45 (d, 1H, pyrazoline-H), 5.65 (s, 1H, H-4), 6.15 (d, 1H, H-2), 7.10 (d, 1H, H-1), 7.30–7.45 (m, 5H, Ar-H), 10.40 (s, 1H, NH, exchangeable with D$_2$O). MS (EI): *m*/*z* 386 (35%) [M$^+$]. Anal. Calcd for $C_{26}H_{30}N_2O$: C, 80.79; H, 7.82; N, 7.25. Found: C, 80.75; H, 7.78; N, 7.18.

3.1.7.2. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)pyrazoline (9b). Yield 65%, mp: 315 °C (MeOH); $[\alpha]_{\mathrm{D}}^{25}$ +135 (*c* 1, MeOH); IR (KBr): 3410, 1725 cm^{-1}. ^{1}H NMR (DMSO-*d*$_6$): δ 0.85 (s, 3H, CH$_3$), 0.90 (s, 3H, CH$_3$), 0.95–1.00 (m, 1H, CH), 1.22–1.31 (m, 4H, 2CH$_2$), 1.44–162 (m, 2H, CH$_2$), 1.66–1.70 (m, 2H, CH$_2$), 1.75 (m, 1H, CH), 1.90–2.10 (m, 2H, CH$_2$), 2.35 (m, 1H, pyrazoline-H), 2.40–2.50 (m, 1H, CH), 3.50 (d, 1H, pyrazoline-H), 3.60 (s, 3H, OCH$_3$), 5.70 (s, 1H, H-4), 6.20 (d, 1H, H-2), 7.05 (d, 1H, H-1), 7.25–7.40 (m, 4H, Ar-H), 10.35 (s, 1H, NH, exchangeable with D$_2$O). MS (EI): *m*/*z* 416 (35%) [M$^+$]. Anal. Calcd for $C_{27}H_{32}N_2O_2$: C, 77.84; H, 7.74; N, 6.73. Found: C, 77.80; H, 7.70; N, 6.68.

3.1.8. Synthesis of (16*R*,5′*S*)(8β,9α,14α)-3β-trifluoroacetate-5α-androstano[17,16-*c*]-1′*H*-5′-aryl-*N*-substituted-pyrazoline (10a–d). A mixture of the arylmethylene derivatives (**2a**,**b**) (4 mmol) and hydrazine derivatives (5 mmol), namely, methyl or phenyl hydrazines in glacial acetic acid (15 ml) was refluxed for 5–7 h. The reaction mixture was poured into ice water, the obtained solid was filtered off, washed with water, dried, and crystallized from the proper solvent to give *N*-substituted pyrazoline derivatives (**10a**–**d**).

3.1.8.1. (16*R*,5′*S*)(8β,9α,14α)-3β-Trifluoroacetate-5α-androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-methylpyrazoline (10a). Yield 55%, mp: 251 °C (EtOH/AcOEt); $[\alpha]_{\mathrm{D}}^{25}$ +19 (*c* 1, MeOH); IR (KBr): 1735, 1645 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.75 (s, 3H, CH$_3$), 0.85 (s, 3H, CH$_3$), 0.92–1.10 (m, 1H, CH), 1.24–1.30 (m, 4H, 2CH$_2$), 1.40–1.58 (m, 6H, 3CH$_2$), 1.60–1.88 (m, 4H, 2CH$_2$), 2.00–2.10 (m, 1H, CH), 2.20 (s, 3H, NCH$_3$), 2.24–2.36 (m, 2H, CH$_2$), 2.40 (m, 1H, pyrazoline-H), 2.50 (m, 1H, CH), 2.55 (m, 1H, 3α-CH), 3.10 (m, 1H, 5α-CH), 3.40 (d, 1H, pyrazoline-H), 7.20–7.45 (m, 5H, Ar-H). MS (EI): *m*/*z* 502 (15%) [M$^+$]. Anal. Calcd for $C_{29}H_{37}F_3N_2O_2$: C, 69.29; H, 7.42; N, 5.57. Found: C, 69.22; H, 7.38; N, 5.54.

3.1.8.2. (16*R*,5′*S*)(8β,9α,14α)-3β-Trifluoroacetate-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxyphenyl)-*N*-methylpyrazoline (10b). Yield 70%, mp: 315 °C (AcOEt); $[\alpha]_{\mathrm{D}}^{25}$ +113 (*c* 1, MeOH); IR (KBr): 1745, 1640 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.70 (s, 3H, CH$_3$), 0.80 (s, 3H, CH$_3$), 0.90–1.00 (m, 1H, CH), 1.22–1.28 (m, 4H, 2CH$_2$), 1.38–1.61 (m, 6H, 3CH$_2$), 1.64–1.90 (m, 4H, 2CH$_2$), 1.98–2.05 (m, 1H, CH), 2.30 (s, 3H, NCH$_3$), 2.25–2.35 (m, 2H, CH$_2$), 2.40 (m, 1H, pyrazoline-H), 2.45 (m, 1H, CH), 2.54 (m, 1H, 3α-CH), 3.05 (m, 1H, 5α-CH), 3.35 (d, 1H, pyrazoline-H), 3.60 (s, 3H, OCH$_3$), 7.30–7.35 (m, 4H, Ar-H). MS (EI): *m*/*z* 532 (15%) [M$^+$]. Anal. Calcd for $C_{30}H_{39}F_3N_2O_3$: C, 67.64; H, 7.38; N, 5.26. Found: C, 67.60; H, 7.34; N, 5.22.

3.1.8.3. (16*R*,5′*S*)(8β, 9α,14α)-3β-Trifluoroacetate-5α-androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-phenylpyrazoline (10c). Yield 60, mp: 138 °C (EtOH); $[\alpha]_{\mathrm{D}}^{25}$ +86 (*c* 1, MeOH); IR (KBr): 1730, 1640 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.70 (s, 3H, CH$_3$), 0.85 (s, 3H, CH$_3$), 0.92–0.98 (m, 1H, CH), 1.24–1.28 (m, 4H, 2CH$_2$), 1.41–1.60 (m, 6H, 3CH$_2$), 1.62–1.88 (m, 4H, 2CH$_2$), 2.05–2.10 (m, 1H, CH), 2.24–2.30 (m, 2H, CH$_2$), 2.35 (m, 1H, pyrazoline-H), 2.40 (m, 1H, CH), 2.55 (m, 1H, 3α-CH), 3.00 (m, 1H, 5α-CH), 3.50 (d, 1H, pyrazoline-H), 7.25–7.65 (m, 10H, Ar-H). MS (EI): *m*/*z* 564 (35%) [M$^+$]. Anal. Calcd for $C_{34}H_{39}F_3N_2O_2$: C, 72.31; H, 6.96; N, 4.96. Found: C, 72.26; H, 6.92; H, 4.90.

3.1.8.4. (16*R*,5′*S*)(8β,9α,14α)-3β-Trifluoroacetate-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxyphenyl)-*N*-phenylpyrazoline (10d). Yield 55%, mp: 158 °C (EtOH); $[\alpha]_{\mathrm{D}}^{25}$ +127 (*c* 1, MeOH); IR (KBr): 1740, 1635 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.75 (s, 3H, CH$_3$), 0.90 (s, 3H, CH$_3$), 0.95–1.00 (m, 1H, CH), 1.22–1.30 (m, 4H, 2CH$_2$), 1.40–1.58 (m, 6H, 3CH$_2$), 1.60–1.90 (m, 4H, 2CH$_2$), 2.00–2.10 (m, 1H, CH), 2.25 (m, 1H, pyrazoline-H), 2.28–2.36 (m, 2H, CH$_2$), 2.42 (m, 1H, CH), 2.50 (m, 1H, 3α-CH), 3.05 (m, 1H, 5α-CH), 3.40 (d, 1H, pyrazoline-H), 3.65 (s, 3H, OCH$_3$), 7.35–7.60 (m, 9H, Ar-H). MS (EI): *m*/*z* 594 (45%) [M$^+$]. Anal. Calcd for $C_{35}H_{41}F_3N_2O_3$: C, 70.68; H, 6.95; N, 4.71. Found: C, 70.62; H, 6.90; N, 4.67.

3.1.9. Synthesis of (16*R*,5′*S*)(8β,9α,14α)-3β-hydroxyl-5α-androstano[17,16-*c*]-1′*H*-5′-aryl-*N*-substituted-pyrazoline (11a–d). A solution of the fluoroacetate pyrazoline derivatives (**10a**–**d**) (1 mmol) in 5% alcoholic KOH (10 ml) was refluxed for ~2 h, and then neutralized with hydrochloric acid. The precipitated solid was filtered off, washed with water, dried, and crystallized from the proper solvent to give compounds (**11a**–**d**).

3.1.9.1. (16*R*,5′*S*)(8β,9α,14α)-3β-Hydroxyl-5α-androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-methylpyrazoline (11a). Yield 65%, mp: 154 °C (MeOH); $[\alpha]_D^{25}$ +96 (*c* 1, MeOH); IR (KBr): 3515, 1650 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.80 (s, 3H, CH$_3$), 0.90 (s, 3H, CH$_3$), 0.95–1.10 (m, 1H, CH), 1.25–1.30 (m, 4H, 2CH$_2$), 1.40–1.60 (m, 6H, 3CH$_2$), 1.65–1.90 (m, 4H, 2CH$_2$), 1.95–2.00 (m, 1H, CH), 2.15 (s, 3H, NCH$_3$), 2.30 (m, 1H, pyrazoline-H), 2.35–2.42 (m, 2H, CH$_2$), 2.48 (m, 1H, CH), 2.54 (m, 1H, 3α-CH), 3.05 (m, 1H, 5α-CH), 3.45 (d, 1H, pyrazoline-H), 7.25–7.45 (m, 5H, Ar-H), 10.55 (s, 1H, OH, exchangeable with D$_2$O). MS (EI): *m*/*z* 406 (18%) [M$^+$]. Anal. Calcd for $C_{27}H_{38}N_2O$: C, 79.75; H, 9.42; N, 6.89. Found: C, 79.70; H, 9.38; N, 6.84.

3.1.9.2. (16*R*,5′*S*)(8β,9α,14α)-3β-Hydroxyl-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)-*N*-methylpyrazoline (11b). Yield 55%, mp: 257 °C (AcOEt); $[\alpha]_D^{25}$ +43 (*c* 1, MeOH); IR (KBr): 3510, 1645 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.80 (s, 3H, CH$_3$), 0.95 (s, 3H, CH$_3$), 1.00–1.15 (m, 1H, CH), 1.24–1.32 (m, 4H, 2CH$_2$), 1.38–1.62 (m, 6H, 3CH$_2$), 1.65–1.88 (m, 4H, 2CH$_2$), 1.95–2.00 (m, 1H, CH), 2.25 (s, 3H, NCH$_3$), 2.28–2.38 (m, 2H, CH$_2$), 2.40 (m, 1H, pyrazoline-H), 2.46 (m, 1H, CH), 2.55 (m, 1H, 3α-CH), 3.10 (m, 1H, 5α-CH), 3.55 (d, 1H, pyrazoline-H), 3.75 (s, 3H, OCH$_3$), 7.25–7.40 (m, 4H, Ar-H), 10.50 (br s, 1H, OH, exchangeable with D$_2$O). MS (EI): *m*/*z* 436 (10%) [M$^+$]. Anal. Calcd for $C_{28}H_{40}N_2O_2$: C, 77.02; H, 9.23; N, 6.42. Found: C, 76.96; H, 9.18; N, 6.37.

3.1.9.3. (16*R*,5′*S*)(8β,9α,14α)-3β-Hydroxyl-5α-androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-phenylpyrazoline (11c). Yield 72%, mp 181 °C (MeOH); $[\alpha]_D^{25}$ +131 (*c* 1, MeOH); IR (KBr): 3490, 1635 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.75 (s, 3H, CH$_3$), 0.85 (s, 3H, CH$_3$), 0.90–1.00 (m, 1H, CH), 1.22–1.32 (m, 4H, 2CH$_2$), 1.42–1.60 (m, 6H, 3CH$_2$), 1.65–1.90 (m, 4H, 2CH$_2$), 1.98–2.05 (m, 1H, CH), 2.25 (m, 1H, pyrazoline-H), 2.28–2.40 (m, 2H, CH$_2$), 2.48 (m, 1H, CH), 2.50 (m, 1H, 3α-CH), 3.05 (m, 1H, 5α-CH), 3.35 (d, 1H, pyrazoline-H), 7.15–7.45 (m, 10H, Ar-H), 10.60 (br s, 1H, OH, exchangeable with D$_2$O). MS (EI): *m*/*z* 468 (26%) [M$^+$]. Anal. Calcd for $C_{32}H_{40}N_2O$: C, 82.00; H, 8.60; N, 5.98. Found: C, 81.95; H, 8.20; N, 5.93.

3.1.9.4. (16*R*,5′*S*)(8β,9α,14α)-3β-Hydroxyl-5α-andro stano[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)-*N*-phenylpyra zoline (11d). Yield 75%, mp: 275 °C (MeOH); $[\alpha]_D^{25}$ +120 (*c* 1, MeOH); IR (KBr): 3520, 1640 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.80 (s, 3H, CH$_3$), 0.85 (s, 3H, CH$_3$), 0.90–0.98 (m, 1H, CH), 1.24–1.28 (m, 4H, 2CH$_2$), 1.40–1.60 (m, 6H, 3CH$_2$), 1.65–1.7.85 (m, 4H, 2CH$_2$), 2.00–2.10 (m, 1H, CH), 2.20–2.32 (m, 2H, CH$_2$), 2.35 (m, 1H, pyrazoline-H), 2.46 (m, 1H, CH), 2.50 (m, 1H, 3α-CH), 3.05 (m, 1H, 5α-CH), 3.40 (d, 1H, pyrazoline-H), 3.60 (s, 3H, OCH$_3$), 7.30–7.80 (m, 9H, Ar-H), 10.20 (br s, 1H, OH, exchangeable with D$_2$O). MS (EI, 70 eV): *m*/*z* 498 (5%) [M$^+$]. Anal. Calcd for $C_{33}H_{42}N_2O$: C, 79.47; H, 8.49; N, 5.62. Found: C, 79.41; H, 8.43; N, 5.57.

3.1.10. Synthesis of (16*R*,5′*S*)(8β,9α,14α)-3-oxo-5α-androstano[17,16-*c*]-1′*H*-5′-aryl-*N*-substituted-pyrazoline (12a–d). Using the same procedure for the synthesis of derivative (**6**) using compounds (**11a**–**d**) as starting materials.

3.1.10.1. (16*R*,5′*S*)(8β, 9α,14α)-3-Oxo-5α-androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-methyl-pyrazoline (12a). Yield 60%, mp: 312 °C (EtOH); $[\alpha]_D^{25}$ +90 (*c* 1, MeOH); IR (KBr): 1745, 1645 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.80 (s, 3H, CH$_3$), 0.92 (s, 3H, CH$_3$), 0.95–1.00 (m, 1H, CH), 1.24–1.30 (m, 4H, 2CH$_2$), 1.42–1.60 (m, 6H, 3CH$_2$), 1.65–1.92 (m, 4H, 2CH$_2$), 2.05 (m, 1H, CH), 2.10 (s, 3H, NCH$_3$), 2.30 (m, 1H, pyrazoline-H), 2.32–2.42 (m, 2H, CH$_2$), 2.48 (m, 1H, CH), 3.05 (m, 1H, 5α-CH), 3.35 (d, 1H, pyrazoline-H), 7.30–7.50 (m, 5H, Ar-H). MS (EI): *m*/*z* 404 (28%) [M$^+$]. Anal. Calcd for $C_{27}H_{36}N_2O$: C, 80.15; H, 8.97; N, 6.93. Found: C, 79.98; H, 8.94; N, 6.90.

3.1.10.2. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)-*N*-methylpyrazoline (12b). Yield 70%, mp: 287 °C (AcOEt); $[\alpha]_D^{25}$ +73 (*c* 1, MeOH); IR (KBr): 1740, 1640 cm^{-1}. ^{1}H NMR (DMSO-*d*$_6$): δ 0.75 (s, 3H, CH$_3$), 0.85 (s, 3H, CH$_3$), 0.90–1.05 (m, 1H, CH), 1.22–1.28 (m, 4H, 2CH$_2$), 1.40–1.58 (m, 6H, 3CH$_2$), 1.62–1.90 (m, 4H, 2CH$_2$), 2.00 (m, 1H, CH), 2.10 (s, 3H, NCH$_3$), 2.22–2.32 (m, 2H, CH$_2$), 2.35 (m, 1H, pyrazoline-H), 2.46 (m, 1H, CH), 3.00 (m, 1H, 5α-CH), 3.40 (d, 1H, pyrazoline-H), 3.65 (s, 3H, OCH$_3$), 7.25–7.60 (m, 4H, Ar-H). MS (EI): *m*/*z* 434 (18%) [M$^+$]. Anal. Calcd for $C_{28}H_{38}N_2O_2$: C, 77.37; H, 8.81; N, 6.45. Found: C, 77.30; H, 8.76; N, 6.40.

3.1.10.3. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-5α-androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-phenyl-pyrazoline (12c). Yield 75%, mp: 220 °C (AcOEt); $[\alpha]_D^{25}$ +58 (*c* 1, MeOH); IR (KBr): 1735, 1635 cm^{-1}. ^{1}H NMR (DMSO-*d*$_6$): δ 0.80 (s, 3H, CH$_3$), 0.85 (s, 3H, CH$_3$), 0.90–1.00 (m, 1H, CH), 1.24–1.30 (m, 4H, 2CH$_2$), 1.42–1.60 (m, 6H, 3CH$_2$), 1.65–1.92 (m, 4H, 2CH$_2$), 2.10 (m, 1H, CH), 2.24–2.40 (m, 2H, CH$_2$), 2.45 (m, 1H, pyrazoline-H), 2.50 (m, 1H, CH), 3.00 (m, 1H, 5α-CH), 3.50 (d, 1H, pyrazoline-H), 7.30–7.65 (m, 10H, Ar-H). MS (EI): *m*/*z* 466 (35%) [M$^+$]. Anal. Calcd for $C_{32}H_{38}N_2O$: C, 82.36; H, 8.21; N, 6.00. Found: C, 82.26; H, 8.15; N, 5.95.

3.1.10.4. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-5α-androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)-*N*-phenylpyrazoline (12d). Yield 65%, mp: 266 °C (AcOEt); $[\alpha]_D^{25}$ +88 (*c* 1, MeOH); IR (KBr): 1745, 1640 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.75 (s, 3H, CH$_3$), 0.85 (s, 3H, CH$_3$), 0.95–1.05 (m, 1H, CH), 1.24–1.32 (m, 4H, 2CH$_2$), 1.40–1.60 (m, 6H, 3CH$_2$), 1.62–1.90 (m, 4H, 2CH$_2$), 1.98 (m, 1H, CH), 2.30 (m, 1H, pyrazoline-H), 2.32–2.40 (m, 2H, CH_2), 2.48 (m, 1H, CH), 3.10 (m, 1H, 5α-CH), 3.40 (d, 1H, pyrazoline-H), 3.70 (s, 3H, OCH_3), 7.25–7.65 (m, 9H, Ar-H). MS (EI): *m/z* 496 (5%) [M^+]. Anal. Calcd for $C_{33}H_{40}N_2O_2$: C, 79.79; H, 8.12; N, 5.64. Found: C, 79.72; H, 8.00; N, 5.60.

3.1.11. Synthesis of (16*R*,5′*S*)(8β,9α,14α)-3-oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-aryl-*N*-substituted-pyrazoline (13a–d). Using the same procedure for the synthesis of compounds (**8**) using compounds (**12a–d**) as starting materials.

3.1.11.1. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-phenyl-*N*-methylpyrazoline (13a). Yield 62%, mp: 271 °C (EtOH); $[\alpha]_D^{25}$ +35 (*c* 1, MeOH); IR (KBr): 1747, 1642 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.75 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.95–1.00 (m, 1H, CH), 1.25–1.30 (m, 4H, $2CH_2$), 1.40–1.60 (m, 2H, CH_2), 1.65–1.78 (m, 2H, CH_2), 1.38–1.92 (m, 1H, CH), 1.95–2.15 (m, 2H, CH_2), 2.20 (s, 3H, NCH_3), 2.30 (m, 1H, pyrazoline-H), 2.35–2.42 (m, 1H, CH), 3.35 (d, 1H, pyrazoline-H), 5.85 (s, 1H, H-4), 6.35 (d, 1H, H-2), 7.10 (d, 1H, H-1), 7.35–7.65 (m, 5H, Ar-H). MS (EI): *m/z* 400 (15%) [M^+]. Anal. Calcd for $C_{27}H_{32}N_2O$: C, 80.95; H, 8.05; N, 7.00. Found: C, 80.88; H, 7.98; N, 6.94.

3.1.11.2. (16*R*,5′*S*)(8β,9α, 14α)-3-Oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)-*N*-methylpyrazoline (13b). Yield 74%, mp: 206 °C (EtOH); $[\alpha]_D^{25}$ +170 (*c* 1, MeOH); IR (KBr): 1745, 1635 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.70 (s, 3H, CH_3), 0.80 (s, 3H, CH_3), 0.90–0.98 (m, 1H, CH), 1.24–1.29 (m, 4H, $2CH_2$), 1.42–1.60 (m, 2H, CH_2), 1.62–1.76 (m, 2H, CH_2), 1.36–1.92 (m, 1H, CH), 1.94–2.14 (m, 2H, CH_2), 2.25 (s, 3H, NCH_3), 2.28–2.38 (m, 1H, CH), 2.40 (m, 1H, pyrazoline-H), 3.40 (d, 1H, pyrazoline-H), 3.60 (s, 3H, OCH_3), 5.80 (s, 1H, H-4), 6.25 (d, 1H, H-2), 6.95 (d, 1H, H-1), 7.40–7.70 (m, 4H, Ar-H). MS (EI): *m/z* 430 (48%) [M^+]. Anal. Calcd for $C_{28}H_{34}N_2O_2$: C, 78.10; H, 7.96; N, 6.51. Found: C, 77.96; H, 7.91; N, 6.45.

3.1.11.3. (16*R*,5′*S*)(8β,9α, 14α)-3-Oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-phenyl-*N*-phenylpyrazoline (13c). Yield 65%, mp: 155 °C (EtOH); $[\alpha]_D^{25}$ +25 (*c* 1, MeOH); IR (KBr): 1740, 1640 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.75 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.95–1.05 (m, 1H, CH), 1.25–1.31 (m, 4H, $2CH_2$), 1.44–1.62 (m, 2H, CH_2), 1.62–1.78 (m, 2H, CH_2), 1.38–1.92 (m, 1H, CH), 1.95–2.15 (m, 2H, CH_2), 2.18–2.32 (m, 1H, CH), 2.35 (m, 1H, pyrazoline-H), 3.30 (d, 1H, pyrazoline-H), 5.70 (s, 1H, H-4), 6.20 (d, 1H, H-2), 7.05 (d, 1H, H-1), 7.35–7.75 (m, 10H, Ar-H). MS (EI): *m/z* 462 (25%) [M^+]. Anal. Calcd for $C_{32}H_{34}N_2O$: C, 83.07; H, 7.41; N, 6.06. Found: C, 82.92; H, 7.36; N, 5.98.

3.1.11.4. (16*R*,5′*S*)(8β,9α,14α)-3-Oxo-androst-1,4-diene[17,16-*c*]-1′*H*-5′-(*p*-methoxy-phenyl)-*N*-phenylpyrazoline (13d). Yield 74%, mp: 174 °C (MeOH); $[\alpha]_D^{25}$ +41 (*c* 1, MeOH); IR (KBr): 1740, 1635 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.80 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.95–1.10 (m, 1H, CH), 1.22–1.28 (m, 4H, $2CH_2$), 1.42–1.60 (m, 2H, CH_2), 1.60–1.78 (m, 2H, CH_2), 1.38–1.92 (m, 1H, CH), 1.95–2.10 (m, 2H, CH_2), 2.25 (m, 1H, pyrazoline-H), 2.28–2.35 (m, 1H, CH), 3.45 (d, 1H, pyrazoline-H), 3.70 (s, 3H, OCH_3), 5.75 (s, 1H, H-4), 6.30 (d, 1H, H-2), 7.0 (d, 1H, H-1), 7.35–7.70 (m, 9H, Ar-H). MS (EI): *m/z* 492 (5%) [M^+]. Anal. Calcd for $C_{32}H_{36}N_2O_2$: C, 80.45; H, 7.37; N, 5.69. Found: C, 80.40; H, 7.32; N, 5.62.

3.1.12. Synthesis of 3(*Z*)-ethylidene androstano[17,16-*c*]-1′*H*-5′-aryl-*N*-substituted-pyrazoline (14a–d). To a stirred solution of ethylidene triphenylphosphorane (1.2 mmol) in dimethylsulfoxide (100 ml), cycloketone derivatives (**12a–d**) (1 mmol) in dry benzene (60 ml) were added dropwise, and then heated at 60 °C for 10–12 h. The reaction mixture was cooled, poured into ice water, the formed product was extracted with ethyl acetate, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The obtained residue was crystallized from the proper solvent to give compounds (**14a-d**).

3.1.12.1. 3(*Z*)-Ethylidene androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-methylpyrazoline (14a). Yield 68%, mp: 237 °C (MeOH); $[\alpha]_D^{25}$ +73 (*c* 1, MeOH); IR (KBr): 1642 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.80 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.98–1.05 (m, 1H, CH), 1.24–1.29 (m, 4H, $2CH_2$), 1.40–1.60 (m, 6H, $3CH_2$), 1.70 (d, 3H, CH_3), 1.74–1.90 (m, 4H, $2CH_2$), 2.10 (m, 1H, CH), 2.15 (s, 3H, NCH_3), 2.20–2.32 (m, 2H, CH_2), 2.35 (m, 1H, pyrazoline-H), 2.48 (m, 1H, CH), 3.10 (m, 1H, 5α-CH), 3.40 (d, 1H, pyrazoline-H), 5.45 (q, 1H, CH=C), 7.25–7.55 (m, 5H, Ar-H). MS (EI): *m/z* 416 (5%) [M^+]. Anal. Calcd for $C_{29}H_{40}N_2$: C, 83.59; H, 9.68; N, 6.73. Found: C, 83.52; H, 9.64; N, 6.68.

3.1.12.2. 3(*Z*)-Ethylidene androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxyphenyl)-*N*-methyl-pyrazoline (14b). Yield 56%, mp: 289 °C (EtOH); $[\alpha]_D^{25}$ +117 (*c* 1, MeOH); IR (KBr): 1645 m^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.75 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.95–1.00 (m, 1H, CH), 1.22–1.35 (m, 4H, $2CH_2$), 1.42–1.58 (m, 6H, $3CH_2$), 1.60–1.72 (m, 4H, $2CH_2$), 1.75 (d, 3H, CH_3), 3.05 (m, 1H, CH), 2.25 (s, 3H, NCH_3), 2.30 (m, 1H, pyrazoline-H), 2.32–2.40 (m, 2H, CH_2), 2.50 (m, 1H, CH), 3.10 (m, 1H, 5α-CH), 3.50 (d, 1H, pyrazoline-H), 3.65 (s, 3H, OCH_3), 5.40 (q, 1H, CH=C), 7.30–7.70 (m, 4H, Ar-H). MS (EI): *m/z* 446 (35%) [M^+]. Anal. Calcd for $C_{30}H_{42}N_2O$: C, 80.66; H, 9.48; N, 6.27. Found: C, 80.60; H, 9.42; N, 6.23.

3.1.12.3. 3(*Z*)-Ethylidene androstano[17,16-*c*]-1′*H*-5′-phenyl-*N*-phenylpyrazoline (14c). Yield 66%, mp: 293 °C (MeOH); $[\alpha]_D^{25}$ +46 (*c* 1, MeOH); IR (KBr): 1640, 1600 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.75 (s, 3H, CH_3), 0.85 (s, 3H, CH_3), 0.90–1.00 (m, 1H, CH), 1.24–1.32 (m, 4H, $2CH_2$), 1.42–1.60 (m, 6H, $3CH_2$), 1.68 (d, 3H, CH_3), 1.70–1.95 (m, 4H, $2CH_2$), 2.10 (m, 1H, CH), 2.30 (m, 1H, pyrazoline-H), 2.35–2.45 (m, 2H, CH_2), 2.50 (m, 1H, CH), 3.10 (m, 1H, 5α-CH), 3.50 (d, 1H, pyrazoline-H), 5.50 (q, 1H, CH=C), 7.30–7.65 (m, 10H, Ar-H). MS (EI): *m/z* 478 (5%) [M^+]. Anal. Calcd for $C_{34}H_{42}N_2$: C, 85.30; H, 8.84; N, 5.85. Found: C, 85.00; H, 8.79; N, 5.81.

3.1.12.4. 3(*Z*)-Ethylidene androstano[17,16-*c*]-1′*H*-5′-(*p*-methoxyphenyl)-*N*-phenyl-pyrazoline (14d). Yield 62%, mp: 263 °C (MeOH); $[\alpha]_D^{25}$ +69 (*c* 1, MeOH); IR (KBr): 1645, 1610 m^{-1}. ^1H NMR (DMSO-d_6): δ 0.70 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.95–1.05 (m, 1H, CH), 1.24–1.33 (m, 4H, $2CH_2$), 1.42–1.60 (m, 6H, $3CH_2$), 1.70 (d, 3H, CH_3), 1.74–1.95 (m, 4H, $2CH_2$), 2.00 (m, 1H, CH), 2.30 (m, 1H, pyrazoline-H), 2.34–2.40 (m, 2H, CH_2), 2.50 (m, 1H, CH), 3.10 (m, 1H, 5α-CH), 3.35 (d, 1H, pyrazoline-H), 3.60 (s, 3H, OCH_3), 5.45 (q, 1H, CH=C), 7.30–7.80 (m, 9H, Ar-H). MS (EI): *m*/*z* 508 (40%) [M^+]. Anal. Calcd for $C_{35}H_{44}N_2O$: C, 82.63; H, 8.72: N, 5.51. Found: C, 82.58; H, 8.68; N, 5.45.

3.2. Antiandrogenic activity in female rats

3.2.1. Aim and rational. Neuman and Elger described[28] the method for testing the antiandrogenic activity in ovariectomized female rats. The protection of the anti-androgen Cyperoterone® against the trophic effect of testosterone on uterine and prenuptial growth was equally studied in intact as well as castrated female rats.

3.2.2. Procedure. Female Sprague–Dawley rats weighting 40–45 g were overotomized as described above. After one week, animals were divided into 38 groups (12 animals) of which 36 received new compounds, a group received control, and a group received Cyperoterone®. The treatment was continued over a period of 12 days with daily subcutaneous injection of 0.3 mg testosterone propionate and the same dose of each individual new tested compound. The control received only testosterone propionate. On the 13th day, the animals were sacrificed, and the uteri and perpetual glands were weighed. The increase in female accessory sexual organs due to testosterone treatment was dose-despondently reduced by antiandrogen.[29,30]

Dose–response curves were established for various doses of the antiandrogen at a given dose of testosterone propionate or for various doses of testosterone propionate at a given dose of the antiandrogen. From the dose–response curve, the relative antiandrogenic potency of the newly synthesized compounds was calculated and compared to that of Cyperoterone® as a reference drug.

3.2.3. Determination of acute to xicity (LD_{50} and LD_{90}). Female Sprague–Dawley albino rats were used to determine intraperitoneal LD_{50} and LD_{90} of the tested compounds. Prior to the determination of LD_{50} and LD_{90} values, a range finding screen was conducted using 20 rats treated with each tested compound at dose ranging from 3 to 2000 mg/kg/dose level. Based on the mortality observed within 14 days, the doses used for the LD_{50} and LD_{90} determination were 3, 10, 30, 100, 300, 1000, and 2000 mg/kg of compound administered by intraperitoneal injection as a 10% solution in dimethyl sulfoxide (DMSO). Control animals received intraperitoneal injections of DMSO. For each concentration and control, 10 female rats were injected with the tested compounds for viability twice daily for two weeks. From the mortality data of all tested animals, the intraperitoneal LD_{50} and LD_{90} values for each agent were determined according to Austen and Brocklehurst.[31]

References and notes

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