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Synthesis, characterization and biological evaluation of some 16E-arylidene androstane derivatives as potential anticancer agents

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ABSTRACT

A series of new 16E-arylidene androstane derivatives were synthesized and characterized. The new compounds were screened for their anticancer activities against the human cancer cell lines SW480, A549, HepG2 and HeLa in vitro using the MTT assay. The results of the in vitro study showed that a number of compounds have shown IC_{50} values lower than 20 μM against the four cancer cell lines.

1. Introduction

Cancer is presently the second most important disease leading to death in both developing and developed countries according to the WHO. Among common currently available drugs for the treatment of cancer are bioalkylating agents (chlormethine) [1,2], antimetabolic agents (fluorouracil) [3,4], anticancer antibiotics (doxorubicin) [5], and compounds from plants and their derivatives (paclitaxel) [6]; however, a dose of anticancer drug sufficient to kill tumor cells is often toxic to normal tissue, thus leading to numerous side effects which, in turn, limit treatment efficacy. Long term effectiveness is often limited by dose-related cumulative cardiotoxicity and development of acquired drug resistance [7–9]. Therefore, the new therapeutic agents for the treatment of cancer thus needed should be more active and produce less side effects.

Steroids elicit diverse biological action via various functional groups located around the periphery of their rigid tetracyclic core. They have the ability to regulate a variety of biological processes and thus are potential drug candidates for the treatment of a large number of diseases including breast cancer [10], prostate cancer [11], leukaemia [12,13], autoimmune diseases [14], osteoporosis [15], etc. Most steroid based pharmaceuticals are semi-synthetic compounds, or analogues, developed from the lead compound and prepared by connecting special functional groups to the core structure of the steroid.

It has been reported that some tertiary amino steroids, 2β,16β-dipiperidino-5α-androstane-3α,17β-diol dipivalate (DAP) (Fig. 1) [16] exhibit significant antitumor activity against transplantable tumors in three animal species including mouse, hamster, and rat. The acute toxicity of DAP was similar to that of cyclophosphamide and considerably lower than vinblastine. In an effort to find the new analogues of this type of novel steroid molecule that exhibit higher activity along with simpler chemical reactions and moreover are easily prepared, some 16E-arylidene androstene derivatives have emerged as potent anticancer agents, such as compound DPJ-RG-1110 [17,18], compound I [19] and compound II [20] (Fig. 1). These previous studies indicate that incorporation of a heterocyclic ring in the steroid backbone demonstrated an important impact on the activity. In addition, a substitution of the phenyl ring at the C-16 position also effects the activity. This type of 16E-arylidene androstene-aminosteroid derivative represents a novel class of potential anticancer agents.

In order to elucidate the structure–activity relationship for this type of compound we studied the synthesis and cytotoxic activity of 16E-arylidene androstane-3-aminosteroid derivatives with different substituents and we obtained additional information on the potential pharmacophoric core.

2. Experimental

2.1. General

All the chemicals and solvent were purchased from commercial sources. Solvents and reagents were dried and purified according

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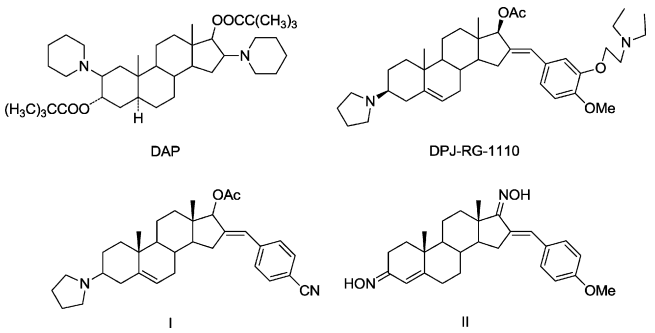


Fig. 1. Structures of DAP and DPJ-RG-1110.

to the literature methods. Melting points were determined on a XT-4 apparatus (uncorrected). ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian Mercury VX400 apparatus in $CDCl_3$ with TMS as internal standard. Mass spectra were recorded on a Shimadzu LCMS-2020 spectrometer. X-ray diffraction data were collected on Bruker SMART APEX CCD diffractometer. Elemental analysis was carried out on a VarioEL III (German) instrument. Silica gel was used for analytical and flash chromatography.

2.2. General procedure for the synthesis of compounds 2a–h (Scheme 1)

To a stirred solution of epiandrosterone (1.45 g, 5.00 mmol) and sodium hydroxide (1.00 g, 25.00 mmol. 5.0 equiv) in methanol was added the corresponding aldehyde (7.50 mmol, 1.5 equiv). The resulting solution was stirred for 12 h at 40 °C until the reaction was completed (monitored by TLC). Cold water was added to the reaction mixture and the precipitate obtained was filtered, washed with water, dried and crystallized from methanol to yield 2a–h.

2.2.1. 16-(4-methyl-benzylidene)-5α-androstan-3β-ol-17-one (2a)

White solid. yield 91%. m.p. 165–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 3.59–3.65 (m, 1H, C_3-OH), 7.42 (s, 1H, =CH), 7.22 (d, 2H, J = 7.92 Hz), 7.44 (d, 2H, J = 8.04 Hz), 2.40 (s, 3H, Ph-CH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.35, 14.55, 20.59, 21.48, 28.43, 29.35, 31.13, 31.44, 31.68, 34.72, 35.74, 36.87, 38.05, 44.85, 47.59, 49.57, 54.53, 60.43, 71.13, 129.44, 130.36, 132.85, 135.19, 139.55, 210.09. Anal. Calcd. for $C_{27}H_{36}O_2$: C 82.61, H 9.24; Found: C 82.34, H 9.31.

2.2.2. 16-(4-methoxy-benzylidene)-5α-androstan-3β-ol-17-one (2b)

White solid. yield 94%. m.p. 156–158 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 3.59–3.61 (m, 1H C_3-OH), 3.84 (s, 3H, OCH_3), 7.38 (s, 1H, =CH), 6.92 (d, 2H, J = 8.76 Hz), 7.49 (d, 2H, J = 8.76 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): 12.35, 14.58, 20.59, 28.44, 29.28, 31.15, 31.44, 31.69, 34.72, 35.74, 36.87, 38.05, 44.86, 47.53, 49.63, 54.55, 55.36, 71.13, 114.19, 128.36, 132.07, 132.76, 133.77, 160.41, 210.13. Anal. Calcd. for $C_{27}H_{36}O_3$: C 79.37, H 8.88; Found: C 79.43, H 9.03.

2.2.3. 16-(4-nitro-benzylidene)-5α-androstan-3β-ol-17-one (2c)

Yellow solid. Yield 93%. m.p. 258–260 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (s, 3H, CH_3-19), 0.99 (s, 3H, CH_3-18), 3.60–3.63 (m, 1H, C_3-OH), 7.45 (s, 1H, =CH), 7.67 (d, 2H, J = 8.80 Hz), 8.27 (d, 2H, J = 8.84 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): 12.33, 14.43, 20.51, 28.33, 29.38, 31.10, 31.39, 31.60, 34.72, 35.74, 36.86, 37.98, 44.81, 47.77, 49.24, 54.47, 71.06, 123.86, 130.07, 130.64, 140.18, 142.03, 147.45, 209.05. Anal. Calcd. for $C_{26}H_{33}NO_4$: C 73.73, H 7.85, N 3.31; Found: C 73.62, H 7.91, N 3.19.

2.2.4. 16-(3,4,5-trimethoxy-benzylidene)-5α-androstan-3β-ol-17-one (2d)

White solid. Yield 91%. m.p. 130–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 0.87 (s, 3H, CH_3-19), 0.97 (s, 3H, CH_3-18), 3.57–3.63 (m, 1H, C_3-OH), 3.89 (s, 9H), 7.35 (s, 1H, =CH), 6.77 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.32, 14.18, 20.54, 22.68, 28.37, 29.35, 31.12, 31.41, 31.64, 34.67, 35.73, 36.84, 38.02, 44.82, 47.60, 49.51, 54.48, 56.15, 60.96, 71.10, 107.62, 131.15, 133.10, 135.27, 139.23, 153.16, 209.75. Anal. Calcd. for $C_{29}H_{40}O_5$: C 74.33, H 8.60; Found: C 74.51, H 8.42.

2.2.5. 16-(4-chloro-benzylidene)-5α-androstan-3β-ol-17-one (2e)

White solid. Yield 91%. m.p.171–173 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 3.59–3.63 (m, 1H, C_3-OH), 7.39 (s, 1H, =CH), 7.38 (d, 2H, J = 8.56 Hz), 7.47 (d, 2H, J = 8.48 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.35, 14.49, 20.56, 21.06, 28.39, 29.27, 31.13, 31.45, 31.65, 34, 73, 35.75, 36.87, 38.05, 44.84, 47.64, 49.46, 54.52, 60.41, 71.15, 128.94, 131.42, 134.13, 135.08, 136.67, 209.64. Anal. Calcd. for $C_{26}H_{33}ClO_2$: C 75.61, H 8.05; Found: C 75.62, H 8.00.

2.2.6. 16-(4-bromo-benzylidene)-5α-androstan-3β-ol-17-one (2f)

White solid. Yield 91%. m.p. 182–184 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 3.59–3.65 (m, 1H, C_3-OH), 7.36 (s, 1H, =CH), 7.39 (d, 2H, J = 8.52 Hz), 7.54 (d, 2H, J = 8.44 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.35, 14.21, 14.49, 20.56, 21.09, 28.38, 29.29, 31.12, 31.45, 31.63, 34.72, 35.74, 36.86, 38.04, 44.83, 47.66, 49.44, 54.50, 60.43, 71.16, 123.44, 131.65, 134.54, 136.84, 209.66. Anal. Calcd. for $C_{26}H_{33}BrO_2$: C 68.27, H 7.27; Found: C 68.51, H 7.08.

2.2.7. 16-(4-fluoro-benzylidene)-5α-androstan-3β-ol-17-one (2g)

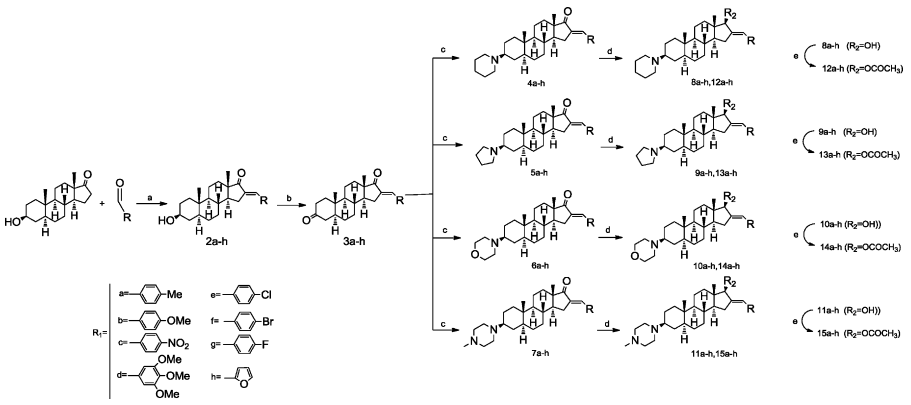
White solid. Yield 95%. m.p. 181–183 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 3.59–3.66 (m, 1H, C_3-OH), 7.40 (s, 1H, =CH), 7.12 (t, 2H), 7.54 (t, 3H). ^{3}C NMR (150 MHz, $CDCl_3$): δ 12.35, 14.52, 20.56, 28.40, 29.20, 31.14, 31.45, 31.64, 34.72, 35.74, 36.86, 38.04, 44.84, 47.62, 49.50, 54.52, 71.15, 115.93, 131.73, 132.21, 135.69, 161.76, 164.25, 209.80. Anal. Calcd. for $C_{26}H_{33}FO_2$: C 78.75, H 8.39; Found: C 78.61, H 8.22.

2.2.8. 16-(furan-2-ylmethylene)-5α-androstan-3β-ol-17-one (2h)

White solid. Yield 95%. m.p. 154–156 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (s, 3H, CH_3-19), 0.93 (s, 3H, CH_3-18), 3.60–3.65 (m, 1H, C_3-OH), 7.21 (s, 1H, =CH), 6.52 (q, 1H), 6.66 (d, 1H, J = 3.44 Hz), 7.57 (d, 1H, J = 1.68 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.35, 14.62, 20.59, 28.44, 28.91, 31.14, 31.44, 31.62, 34.69, 35.74, 36.87, 38.05, 44.87, 47.77, 49.11, 54.55, 71.15, 112.32, 115.51, 119.51, 133.68, 144.66, 152.27, 209.94. Anal. Calcd. for $C_{24}H_{32}O_3$: C 78.22, H 8.75; Found: C 78.19, H 8.81.

2.3. General procedure for the synthesis of compounds 3a–h

To a solution of condensation products (5 mmol) (2a–h) in acetone (20 ml), Jones reagent was added drop wise. The mixture was stirred at 0 °C until the reaction was completed (monitored by TLC). The reaction was quenched with ethanol (3–5 ml) and concentrated in reduced pressure. The crude product was dissolved in CH_2Cl_2, the organic layer was washed with brine and dried over anhydrous Na_2SO_4, filtered. The filtrate was concentrated in reduced pressure to afford a crude product. The products were purified by flash col-



Scheme 1. Preparation of target compounds (12a–h, 13a–h, 14a–h, 15a–h).

umn chromatography to yield compound 3a–h. The Jones reagent was prepared according to previous study [21–23].

2.3.1. 16-(4-methyl-benzylidene)-5α-androstan-3,17-dione (3a)

White solid. Yield 95%. m.p.165-167 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 (s, 3H, CH_3-19), 1.09 (s, 3H, CH_3-18), 2.40 (s, 3H, Ph-CH_3), 7.43 (s, 1H, =CH), 7.25 (d, 2H, *J* = 7.96 Hz), 7.46 (d, 2H, *J* = 8.20 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.50, 14.54, 20.77, 21.49, 28.64, 29.31, 30.76, 31.60, 34.60, 35.89, 38.07, 38.33, 44.58, 46.56, 47.49, 49.38, 53.94, 129.46, 130.37, 132.78, 133.16, 134.94, 209.65, 211.57. Anal. Calcd. for $C_{27}H_{34}O_2$: C 83.03, H 8.77; Found: C 83.31, H 8.62.

2.3.2. 16-(4-methoxy-benzylidene)-5α-androstan-3,17-dione (3b)

White solid. Yield 97%. m.p. 273–275 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (s, 3H, CH_3-19), 1.07 (s, 3H, CH_3-18), 3.84 (s, 3H, OCH_3), 7.39 (s, 1H, =CH), 6.94 (d, 2H, *J* = 8.76 Hz), 7.51 (d, 2H, *J* = 8.76 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.47, 14.56, 20.76, 28.63, 29.22, 30.74, 31.59, 34.57, 35.86, 38.05, 38.30, 44.56, 46.53, 47.39, 49.40, 53.93, 55.34, 114.21, 128.23, 132.07, 132.86, 133.49, 160.46, 209.61, 211.50. Anal. Calcd. for $C_{27}H_{34}O_3$: C 79.76, 8.43; Found: C 79.62, H 8.70.

2.3.3. 16-(4-nitro-benzylidene)-5α-androstan-3,17-dione (3c)

Yellow solid. Yield 96%. m.p. 236–238 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00 (s, 3H, CH_3-19), 1.08 (s, 3H, CH_3-18), 7.44 (s, 1H, =CH), 7.67 (d, 2H, *J* = 8.80 Hz), 8.27 (d, 2H, *J* = 8.80 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.46, 14.40, 20.68, 28.52, 29.31, 30.69, 31.50, 34.56, 35.86, 38.00, 38.28, 44.49, 46.49, 47.65, 49.02, 53.85, 123.83, 130.14, 130.66, 139.97, 141.94, 147.42, 208.62, 211.36. Anal. Calcd. for $C_{26}H_{31}NO_4$: C 74.08, H 7.41, N 3.32; Found: C 74.15, H 7.52, N 3.40.

2.3.4. 16-(3,4,5-trimethoxy-benzylidene)-5α-androstan-3,17-dione (3d)

White solid. Yield 98%. m.p. 211–213 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (s, 3H, CH_3-19), 1.07 (s, 3H, CH_3-18), 3.89 (s, 9H),7.36 (s, 1H, =CH), 6.77 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.45, 14.50, 20.72, 22.68, 29.12, 29.35, 29.69, 30.73, 31.55, 31.91, 34.54, 35.87, 38.03, 38.30, 44.53, 46.53, 47.49, 49.32, 53.89, 56.14, 60.93, 107.65, 131.06, 133.29, 134.99, 139.30, 153.17, 209.34, 211.48. Anal. Calcd. for $C_{29}H_{38}O_5$: C 74.65, H 8.21; Found: C 74.58, H 8.37.

2.3.5. 16-(4-chloro-benzylidene)-5α-androstan-3,17-dione (3e)

White solid. Yield 98%. m.p. 256–258 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (s, 3H, CH_3-19), 1.00 (s, 3H, CH_3-18), 7.31 (s, 1H, =CH), 7.29 (d, 2H, *J* = 8.44 Hz), 7.40 (d, 2H, *J* = 8.56 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.50, 14.48, 20.75, 28.60, 29.23, 30.76, 31.56, 34.61, 35.90, 38.06, 38.38, 44.56, 46.56, 47.55, 49.27, 53.94, 128.96, 131.43, 131.74, 134.05, 135.16, 136.41, 209.27, 211.51. Anal. Calcd. for $C_{26}H_{31}ClO_2$: C 75.98, H 7.60; Found: C 75.81, H 7.72.

2.3.6. 16-(4-bromo-benzylidene)-5α-androstan-3,17-dione (3f)

White solid. Yield 98%. m.p. 182–184 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 (s, 3H, CH_3-19), 1.09 (s, 3H, CH_3-18), 7.37 (s, 1H, =CH), 7.39 (d, 2H, *J* = 8.48 Hz), 7.40 (d, 2H, *J* = 8.44 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.51, 14.48, 20.74, 28.59, 29.26, 30.75, 31.55, 34.60, 35.89, 38.07, 38.33, 44.56, 46.55, 47.57, 49.25, 53.92, 123.52, 131.66, 131.81, 131.93, 134.46, 136.57, 209.30, 211.58. Anal. Calcd. for $C_{26}H_{31}BrO_2$: C 68.57, H 6.86; Found: C 68.44, H 6.94.

2.3.7. 16-(4-fluoro-benzylidene)-5α-androstan-3,17-dione (3g)

White solid. Yield 90%. m.p. 208–210 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 (s, 3H, CH_3-19), 1.09 (s, 3H, CH_3-18), 7.41 (s, 1H, =CH), 7.29 (d, 2H, *J* = 8.44 Hz), 7.12 (t, 2H), 7.54 (t, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.48, 14.49, 20.73, 28.59, 29.14, 30.74, 31.55, 34.58, 35.87, 38.05, 38.31, 44.55, 46.54, 47.51, 49.29, 53.91, 115.94, 132.22, 161.76, 164.26, 209.39, 211.56. Anal. Calcd. for $C_{26}H_{31}FO_2$: C 79.15, H 7.92; Found: C 79.28, H 7.88.

2.3.8. 16-(furan-2-ylmethylene)-5α-androstan-3,17-dione (3h)

Yellow solid. Yield 97%. m.p. 197–199 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (s, 3H, CH_3-19), 1.08 (s, 3H, CH_3-18), 7.21 (s, 1H, =CH), 6.52 (q, 1H), 6.66 (d, 1H, *J* = 3.40 Hz), 7.57 (d, 1H, *J* = 1.68 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.46, 14.58, 20.75, 28.63, 28.85, 30.73, 31.52, 34.53, 35.86, 38.05, 38.31, 44.56, 46.55, 47.64, 48.89, 53.93, 209.42, 211.54. Anal. Calcd. for $C_{24}H_{30}O_3$: C 78.65, H 8.25; Found: C 78.77, H 8.30.

2.4. General procedure for the synthesis of compounds 4a–h, 5a–h, 6a–h, 7a–h

To a suspension of dione 3a–h (1 mmol) in 20 ml of absolute methanol was added 0.5 ml of piperidine and 60 mg (1.2 mmol, 1.2 equive.) of $NaBH_3CN$, then the solution was acidified to pH ≈ 6 with acetic acid. The resulting mixture was stirred at 40 °C for 8 h. The methanol was removed under reduced pressure, 20 ml CH_2Cl_2 was added to the residue, the organic layer was washed with saturated $NaHCO_3$, brine and dried over anhydrous Na_2SO_4, filtered. The filtrate was concentrated in vacuum to afford a crude product. The product was purified by flash column chromatography to yield compound 4a–h. Compounds 5a–h, 6a–h and 7a–h were obtained with pyrrolidine, morpholine, N-methylpiperazine by using the same method that described above [24].

2.4.1. 16-(4-methyl-benzylidene)-3β-piperidino-5α-androstan-17-one (4a)

White solid. Yield 45%. m.p 165–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.81 (s, 3H, CH_3-19), 0.97 (s, 3H, CH_3-18), 2.40 (s, 3H, Ph-CH_3), 2.53 (s, 4H, NCH_2), 7.42 (s, 1H, =CH), 7.15 (d, 2H, *J* = 7.96 Hz), 7.27 (d, 2H, *J* = 8.20 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.40, 14.57, 20.43, 24.10, 24.77, 26.38, 28.77, 29.19, 30.68, 31.22, 31.66, 34.68, 36.44, 37.89, 46.11, 47.59, 49.52, 50.42, 54.60, 55.31, 64.79, 114.19, 128.46, 132.08, 132.47, 134.96, 209.76. Anal. Calcd. for $C_{32}H_{45}NO$: C 83.61, H 9.87, N 3.05; Found C 83.55, H 9.91, N 3.12.

2.4.2. 16-(4-methyl-benzylidene)-3β-pyrrolidino-5α-androstan-17-one (5a)

White solid. Yield 48%. m.p 206–208 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 2.40 (s, 3H, Ph-CH_3), 2.57 (br, 4H, NCH_2), 7.42 (s, 1H, =CH), 7.22 (d, 2H, *J* = 7.96 Hz), 7.44 (d, 2H, *J* = 8.08 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.07, 14.52, 20.43, 21.46, 23.26, 24.16, 28.22, 29.27, 30.67, 30.91, 31.57, 34.55, 35.75, 36.75, 45.18, 47.53, 49.49, 50.66, 54.22, 63.96, 129.44, 130.35, 132.76, 133.14, 135.01, 139.63, 176.31, 209.94. Anal. Calcd. for $C_{31}H_{43}NO$: C 83.54, H 9.72, N 3.14; Found C 83.49, H 9.88, N 3.22.

2.4.3. 16-(4-methyl-benzylidene)- 3β-morpholino-5α-androstan-17-one (6a)

White solid. Yield 42%. m.p. 234–236 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (s, 3H, CH_3-19), 0.87 (s, 3H, CH_3-18), 2.31 (s, 3H, Ph-CH_3), 2.49 (s, 4H, NCH_2), 3.63–3.65 (m, 4H, OCH_2), 7.33 (s, 1H, =CH), 7.13 (d, 2H, *J* = 7.96 Hz), 7.35 (d, 2H, *J* = 8.08 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.18, 14.52, 20.44, 21.46, 21.98, 22.38, 28.40, 28.88, 29.27, 30.92, 31.57, 34.59, 35.87, 37.01, 45.34, 47.54, 48.60, 49.46, 54.25, 64.70, 64.99, 129.44, 130.36, 132.78, 133.16, 135.02, 139.61, 176.08, 209.98. Anal. Calcd. for $C_{31}H_{43}NO_2$: C 80.65, H 9.39, N 3.03; Found C 80.51, H 9.47, N 3.22.

2.4.4. 16-(4-methyl-benzylidene)-3β-(4-Methylpiperazinyl)-5α-androstan-17-one (7a)

White solid. Yield 43%. m.p. 256–258 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (s, 3H, CH_3-19), 0.86 (s, 3H, CH_3-18), 2.31 (s, 3H, Ph-CH_3), 2.21 (s, 3H, N-CH_3), 2.39–2.53 (m, 8H, NCH_2), 7.32 (s, 1H, =CH), 7.13 (d, 2H, *J* = 7.96 Hz), 7.35 (d, 2H, *J* = 8.08 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.42, 14.54, 20.47, 21.47, 24.58, 28.73, 29.34, 31.19, 31.27, 31.69, 34.73, 36.12, 37.74, 45.86, 46.05, 47.60, 49.32, 49.59, 54.65, 55.53, 63.77, 129.42, 130.34, 132.88, 132.91, 135.25, 139.49, 210.08. Anal. Calcd. for $C_{32}H_{46}N_2O$: C 80.96, H 9.77, N 5.90; Found C 81.22, H 9.88, N 5.80.

2.4.5. 16-(4-methoxy-benzylidene)-3β-piperidino-5α-androstan-17-one (4b)

White solid. Yield 45%. m.p. 215–217 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (s, 3H, CH_3-19), 0.98 (s, 3H, CH_3-18), 2.53 (s, 4H, NCH_2), 3.86 (s, 3H, OCH_3), 7.40 (s, 1H, =CH), 6.94 (d, 2H, *J* = 8.72 Hz), 7.51 (d, 2H, *J* = 8.72 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.44, 14.59, 20.49, 24.12, 24.87, 26.43, 28.77, 29.29, 30.73, 31.22, 31.71, 34.73, 36.21, 37.94, 46.09, 47.57, 49.66, 50.37, 54.69, 55.36, 64.48, 114.16, 128.38, 132.05, 132.66, 133.85, 160.37, 209.83. Anal. Calcd. for $C_{32}H_{45}NO_2$: C 80.79, H 9.53, N 2.94; Found C 80.91, H 9.66, N 2.81.

2.4.6. 16-(4-methoxy-benzylidene)-3β-pyrrolidino-5α-androstan-17-one (5b)

White solid. Yield 47%. m.p. 224–226 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (s, 3H, CH_3-19), 0.93 (s, 3H, CH_3-18), 2.57 (br, 4H, NCH_2), 3.85 (s, 3H, OCH_3), 7.38 (s, 1H, =CH), 6.92 (d, 2H, *J* = 8.80 Hz), 7.49 (d, 2H, *J* = 8.80 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.36, 14.58, 20.43, 23.17, 27.98, 28.68, 29.29, 31.23, 31.71, 34.73, 34.76, 36.02, 37.35, 45.29, 47.56, 49.68, 51.83, 54.69, 55.35, 64.29, 114.16, 128.39, 132.06, 132.65, 133.86, 160.38, 210.21. Anal. Calcd. for $C_{31}H_{43}NO_2$: C 80.65, H 9.39, N 3.03; Found C 80.51, H 9.18, N 3.11.

2.4.7. 16-(4-methoxy-benzylidene)-3β-morpholino-5α-androstan-17-one (6b)

White solid. Yield 41%. m.p. 214–216 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (s, 3H, CH_3-19), 0.94 (s, 3H, CH_3-18), 2.57 (s, 4H, NCH_2), 3.72–3.75 (m, 4H, OCH_2), 3.84 (s, 3H, OCH_3), 7.38 (s, 1H, =CH), 6.92 (d, 2H, *J* = 8.76 Hz), 7.49 (d, 2H, *J* = 8.80 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.39, 14.58, 20.48, 24.49, 28.74, 29.27, 31.09, 31.18, 31.69, 34.71, 36.12, 37.62, 45.73, 47.54, 49.64, 50.05, 54.64, 55.35, 64.05, 67.34, 114.17, 128.36, 132.06, 132.71, 133.79, 160.40, 210.13. Anal. Calcd. for $C_{31}H_{43}NO_3$: C 77.95, H 9.07, N 2.93; Found C 77.88, H 9.16, N 2.88.

2.4.8. 16-(4-methoxy-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17-one (7b)

White solid. Yield 44%. m.p. 239–241 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (s, 3H, CH_3-19), 0.93 (s, 3H, CH_3-18), 2.28 (s, 3H, N-CH_3), 2.40–2.61 (m, 8H, NCH_2), 3.84 (s, 3H, OCH_3), 7.38 (s, 1H, =CH), 6.92 (d, 2H, *J* = 8.68 Hz), 7.49 (d, 2H, *J* = 8.68 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.41, 14.58, 20.47, 24.55, 28.73, 29.27, 31.19, 31.23, 31.69, 34.71, 36.11, 37.73, 45.85, 46.03, 47.54, 49.28, 49.63, 54.65, 55.35, 55.49, 63.75, 114.16, 128.36, 132.05, 132.67, 132.81, 160.38, 210.14. Anal. Calcd. for $C_{32}H_{46}N_2O_2$: C 78.32, H 9.45, N 5.71; Found C 78.21, H 9.58, N 5.66.

2.4.9. 16-(4-nitro-benzylidene)-3β-piperidino-5α-androstan-17-one (4c)

Yellow solid. Yield 44%. m.p. 243–245 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (s, 3H, CH_3-19), 0.98 (s, 3H, CH_3-18), 2.54 (s, 4H, NCH_2), 7.45 (s, 1H, =CH), 7.67 (d, 2H, *J* = 8.92 Hz), 8.26 (d, 2H, *J* = 8.80 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.42, 14.42, 20.41, 24.08, 24.82, 26.36, 28.64, 29.25, 29.39, 30.62, 31.17, 31.61, 34.72, 36.19, 37.90, 46.03, 47.79, 49.26, 50.35, 53.77, 54.59, 64.43, 123.85, 129.98, 130.62, 140.24, 142.06, 147.42, 209.09. Anal. Calcd. for $C_{31}H_{42}N_2O_3$: C 75.88, H 8.63, N 5.71; Found C 75.97, H 8.58, N 5.62.

2.4.10. 16-(4-nitro-benzylidene)-3β-pyrrolidino-5α-androstan-17-one (5c)

Yellow solid. Yield 41%. m.p. 225–227 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (s, 3H, CH_3-19), 0.98 (s, 3H, CH_3-18), 2.59 (br, 4H, NCH_2), 7.45 (s, 1H, =CH), 7.67 (d, 2H, *J* = 8.80 Hz), 8.26 (d, 2H, *J* = 8.84 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.35, 14.43, 20.35, 23.17, 27.99, 28.57, 29.40, 31.19, 31.62, 34.74, 36.02, 37.34, 45.25, 47.80, 49.30, 51.85, 54.61, 64.26, 123.85, 129.95, 130.62, 140.26, 142.06, 147.42, 209.08. Anal. Calcd. for $C_{30}H_{40}N_2O_3$: C 75.59, H 8.46, N 5.88; Found C 75.71, H 8.31, N 5.97.

2.4.11. 16-(4-nitro-benzylidene)-3β-morpholino-5α-androstan-17-one (6c)

Yellow solid. Yield 44%. m.p. 241–243 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (s, 3H, CH_3-19), 1.00 (s, 3H, CH_3-18), 2.59 (s, 4H, NCH_2), 3.73–3.76 (s, 4H, OCH_2), 7.45 (s, 1H, =CH), 7.67 (d, 2H, *J* = 8.76 Hz), 8.26 (d, 2H, *J* = 8.80 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.38, 14.43, 20.41, 24.47, 28.62, 29.38, 31.04, 31.14, 31.60, 34.72, 36.12, 37.61, 45.69, 47.79, 49.26, 50.04, 54.56, 64.00, 67.32, 123.86, 130.04, 130.63, 140.19, 142.04, 147.44, 209.07. Anal. Calcd. for $C_{30}H_{40}N_2O_4$: C 73.14, H 8.18, N 5.69; Found C 73.22, H 8.26, N 5.55.

2.4.12. 16-(4-nitro-benzylidene)-3β-(4-Methylpiperazinyl)-5α-androstan-17-one (7c)

Yellow solid. Yield 44%. m.p. 259–261 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (s, 3H, CH_3-19), 0.98 (s, 3H, CH_3-18), 2.30 (s, 3H, N-CH_3), 2.42–2.62 (m, 8H, NCH_2), 7.45 (s, 1H, =CH), 7.67 (d, 2H, *J* = 8.76 Hz), 8.26 (d, 2H, *J* = 8.76 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ

12.40, 14.43, 20.40, 24.53, 28.62, 29.38, 31.15, 31.19, 31.60, 34.72, 36.11, 37.72, 45.81, 46.01, 47.79, 49.28, 54.57, 55.47, 63.72, 123.85, 130.00, 130.62, 140.22, 142.05, 147.43, 209.07. Anal. Calcd. for $C_{31}H_{43}N_3O_3$: C 73.63, H 8.57, N 8.31; Found C 73.81, H 8.42, N 8.26.

2.4.13. 16-(3,4,5-trimethoxy-benzylidene)-3β-piperidino-5α-androstan-17-one (4d)

White solid. Yield 47%. m.p. 197–199 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (s, 3H, CH_3-19), 0.95 (s, 3H, CH_3-18), 2.51 (s, 4H, NCH_2), 3.89 (s, 9H, OCH_3), 7.34 (s, 1H, =CH), 6.77 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.41, 14.53, 20.45, 24.10, 24.86, 26.41, 28.72, 29.18, 30.71, 31.20, 31.67, 34.70, 36.20, 37.91, 46.06, 47.64, 49.55, 50.36, 54.63, 56.15, 60.97, 64.45, 107.62, 131.19, 133.02, 135.37, 139.21, 153.17, 209.83. Anal. Calcd. for $C_{34}H_{49}NO_4$: C 76.22, H 9.22, N 2.61; Found C 76.41, H 9.31, N 2.48.

2.4.14. 16-(3,4,5-trimethoxy-benzylidene)-3β-pyrrolidino-5α-androstan-17-one (5d)

White solid. Yield 48%. m.p. 162–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (s, 3H, CH_3-19), 0.95 (s, 3H, CH_3-18), 2.56 (br, 4H, NCH_2), 3.89 (s, 9H, OCH_3), 7.34 (s, 1H, =CH), 6.77 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 10.73, 18.98, 19.61, 22.82, 27.13, 29.28, 29.47, 30.07, 33.21, 34.37, 34.86, 36.00, 41.35, 44.02, 47.23, 48.41, 52.71, 54.46, 59.29, 62.45, 65.71, 82.93, 103.93, 122.00, 131.76, 135.22, 138.82, 151.34, 169.62. Anal. Calcd. for $C_{33}H_{47}NO_4$: C 75.97, H 9.08, N 2.68; Found C 75.88, H 9.12, N 2.79.

2.4.15. 16-(3,4,5-trimethoxy-benzylidene)-3β-morpholino-5α-androstan-17-one (6d)

White solid. Yield 48%. m.p. 168–170 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (s, 3H, CH_3-19), 0.95 (s, 3H, CH_3-18), 2.56 (s, 4H, NCH_2), 3.73–3.75 (m, 4H, OCH_2), 3.89 (s, 9H, OCH_3), 7.35 (s, 1H, =CH), 6.77 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.37, 14.52, 20.43, 24.50, 28.68, 29.92, 31.10, 31.16, 31.64, 34.66, 35.64, 36.11, 37.60, 45.70,47.53, 49.52, 50.05, 53.78, 54.57, 56.14, 60.97, 67.34, 107.58, 131.16, 133.04, 133.27, 135.04, 135.31, 139.19, 153.16, 209.75. Anal. Calcd. for $C_{33}H_{47}NO_5$: C 73.71, H 8.81, N 2.60; Found C 73.52, H 8.93, N 2.46.

2.4.16. 16-(3,4,5-trimethoxy-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17-one (7d)

White solid. Yield 49%. m.p. 185–187 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 2.31 (s, 3H, N-CH_3), 2.48–2.65 (m, 8H, NCH_2), 3.89 (s, 9H, OCH_3), 7.35 (s, 1H, =CH), 6.78 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.36,14.51, 20.41, 24.44, 28.65, 29.14, 31.15, 31.62, 34.64, 36.07, 37.66, 45.78, 47.59, 49.14, 49.50, 54.55, 55.31, 56.12, 60.93, 63.70, 107.57, 131.15, 133.00, 135.30, 139.17, 153.13, 209.71. Anal. Calcd. for $C_{34}H_{50}N_2O_4$: C 74.14, H 9.15, N 5.09; Found C 74.26, H 9.22, N 5.01.

2.4.17. 16-(4-chloro-benzylidene)-3β-piperidino-5α-androstan-17-one (4e)

White solid. Yield 49%. m.p. 163–165 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 2.51 (s, 4H, NCH_2), 7.39 (s, 1H, =CH), 7.40 (d, 2H, J = 8.44 Hz), 7.48 (d, 2H, J = 8.52 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.33, 14.49, 20.45, 22.13, 22.75, 23.03, 28.20, 28.79, 29.17, 30.85, 31.50, 34.55, 35.84, 36.99, 45.43, 47.56, 49.31, 49.93, 54.12, 65.45, 128.95, 131.43, 131.72, 134.03, 135.14, 136.43, 209.43. Anal. Calcd. for $C_{31}H_{42}ClNO$: C 77.55, H 8.82, N 2.92; Found C 77.71, H 8.76, N 2.89.

2.4.18. 16-(4-chloro-benzylidene)-3β-pyrrolidino-5α-androstan-17-one (5e)

White solid. Yield 49%. m.p. 232–234 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 2.57 (br, 4H, NCH_2), 7.39 (s, 1H, =CH), 7.40 (d, 2H, J = 8.56 Hz), 7.49 (d, 2H, J = 8.48 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.35, 14.48, 20.38, 23.18, 23.66, 28.01, 28.63, 29.26, 31.20, 31.66, 34.72, 34.77, 36.01, 37.34, 45.26, 47.64, 49.48, 51.85, 54.63, 64.27, 128.91, 131.42, 134.14, 135.02, 136.74, 209.63. Anal. Calcd. for $C_{30}H_{40}ClNO$: C 77.31, H 8.65, N 3.01; Found C 77.44, H 8.78, N 2.99.

2.4.19. 16-(4-chloro-benzylidene)-3β-morpholino-5α-androstan-17-one (6e)

White solid. Yield 49%. m.p. 220–222 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 2.57 (s, 4H, NCH_2), 3.74 (s, 4H, OCH_2), 7.39 (s, 1H, =CH), 7.40 (d, 2H, J = 8.56 Hz), 7.49 (d, 2H, J = 8.52 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.39, 14.49, 20.44, 24.49, 28.68, 29.25, 31.08, 31.16, 31.63, 34.70, 36.11, 37.61, 45.71, 47.65, 49.45, 50.05, 54.59, 64.03, 67.34, 128.93, 131.42, 131.51, 134.12, 135.05, 136.68, 209.67. Anal. Calcd. for $C_{30}H_{40}ClNO_2$: C 74.74, H 8.36, N 2.91; Found C 74.68, H 8.43, 21, N 2.88.

2.4.20. 16-(4-chloro-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17-one (7e)

White solid. Yield 47%. m.p. 265–267 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (s, 3H, CH_3-19), 0.87 (s, 3H, CH_3-18), 2.21 (s, 3H, N-CH_3), 2.39–2.55 (m, 8H, NCH_2), 7.29 (s, 1H, =CH), 7.31 (d, 2H, J = 8.60 Hz), 7.39 (d, 2H, J = 8.48 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.41, 14.49, 20.44, 24.53, 28.68, 29.26, 31.17, 31.22, 31.65, 34.72, 36.12, 37.73, 45.84, 46.03, 47.66, 49.31, 49.47, 54.62, 55.47, 63.79, 128.93, 131.42, 131.48, 134.14, 135.04, 136.72, 209.68. Anal. Calcd. for $C_{31}H_{43}ClN_2O$: C 75.20, H 8.75, N 5.66; Found C 75.11, H 8.99, N 5.49.

2.4.21. 16-(4-bromo-benzylidene)-3β-piperidino-5α-androstan-17-one (4f)

White solid. Yield 45%. m.p. 207–209 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (s, 3H, CH_3-19), 0.97 (s, 3H, CH_3-18), 2.55 (s, 4H, NCH_2), 7.37 (s, 1H, =CH), 7.40 (d, 2H, J = 8.52 Hz), 7.54 (d, 2H, J = 8.61 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.34, 14.49, 20.55, 22.66, 28.38, 29.29, 29.71, 31.12, 31.44, 34.71, 35.74, 36.86, 38.04, 44.83, 45.99, 47.66, 49.44, 51.63, 54.50, 71.14, 123.44, 131.65, 131.19, 134.54, 136.84, 209.66. Anal. Calcd. for $C_{31}H_{42}BrNO$: C 70.98, H 8.07, N 2.67; Found C 70.77, H 7.98, N 2.71.

2.4.22. 16-(4-bromo-benzylidene)-3β-pyrrolidino-5α-androstan-17-one (5f)

White solid. Yield 41%. m.p. 242–244 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 2.59 (br, 4H, NCH_2), 7.36 (s, 1H, =CH), 7.40 (d, 2H, J = 8.52 Hz), 7.54 (d, 2H, J = 8.61 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.36, 14.49, 20.39, 23.18, 27.91, 28.62, 29.29, 31.20, 31.66, 34.66, 34.72, 36.02, 37.33, 45.26, 47.68, 49.48, 51.83, 54.62, 64.30, 123.38, 131.50, 131.63, 131.87, 134.56, 136.91, 209.71. Anal. Calcd. for $C_{30}H_{40}BrNO$: C 70.58, H 7.90, N 2.74; Found C 70.44, H 7.81, N 2.88.

2.4.23. 16-(4-bromo-benzylidene)-3β-morpholino-5α-androstan-17-one (6f)

White solid. Yield 47%. m.p. 247–249 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 2.58 (s, 4H, NCH_2), 3.73–3.75 (m, 4H, OCH_2), 7.36 (s, 1H, =CH), 7.39 (d, 2H, J = 8.52 Hz), 7.54 (d, 2H, J = 8.48 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.38, 14.49, 20.34, 24.50, 28.66, 29.29, 31.02, 31.11, 31.61, 34.73, 36.11, 37.58, 45.73, 47.66, 49.35, 50.15, 54.49, 64.00, 67.27, 128.88, 131.42, 131.49, 134.11, 135.03, 136.71, 209.77. Anal. Calcd. for $C_{30}H_{40}BrNO_2$: C 68.43, H 7.66, N 2.66; Found C 68.51, H 7.59, N 2.71.

2.4.24. 16-(4-bromo-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17-one (7f)

White solid. Yield 40%. m.p. 290–292 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 2.30 (s, 3H, N-CH_3), 2.49–2.62 (m, 8H, NCH_2), 7.36 (s, 1H, =CH), 7.39 (d, 2H, *J* = 8.32 Hz), 7.54 (d, 2H, *J* = 8.24 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.38, 14.55, 20.51, 24.49, 28.73, 29.22, 31.27, 31.19, 31.58, 34.66, 36.33, 37.73, 45.91, 46.13, 47.72, 49.41, 49.48, 54.64, 55.43, 63.81, 128.98, 131.44, 131.54, 134.26, 135.10, 136.77, 209.71. Anal. Calcd. for $C_{31}H_{43}BrN_2O$: C 69.00, H 8.03, N 5.19; Found C 69.13, H 8.01, N 5.27.

2.4.25. 16-(4-fluoro-benzylidene)-3β-piperidino-5α-androstan-17-one (4g)

White solid. Yield 43%. m.p. 228–230 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (s, 3H, CH_3-19), 0.95 (s, 3H, CH_3-18), 2.52 (s, 4H, NCH_2), 7.39 (s, 1H, =CH), 7.11 (t, 2H), 7.54 (t, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.41, 14.48, 20.44, 24.14, 24.85, 26.42, 28.71, 29.16, 30.69, 31.18, 31.65, 34.70, 36.18, 37.91, 46.05, 47.59, 49.50, 50.37, 54.63, 64.47, 115.66, 115.88, 131.57, 132.08, 132.17, 135.73, 135.75, 161.70, 164.19, 209.67. Anal. Calcd. for $C_{31}H_{42}FNO$: C 80.30, H 9.13, N 3.02; Found C 80.11, H 9.08, N 2.88.

2.4.26. 16-(4-fluoro-benzylidene)-3β-pyrrolidino-5α-androstan-17-one (5g)

White solid. Yield 46%. m.p. 265–267 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3-19), 0.87 (s, 3H, CH_3-18), 2.49 (br, 4H, NCH_2), 7.31 (s, 1H, =CH), 7.02 (t, 2H), 7.45 (t, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.35, 14.51, 20.39, 23.17, 27.91, 28.63, 29.20, 31.21, 31.66, 34.68, 34.73, 36.02, 37.33, 45.27, 47.64, 49.55, 51.83, 54.64, 64.30, 115.69, 115.91, 131.63, 131.91, 132.11, 132.19, 135.80, 161.74, 164.23, 209.87. Anal. Calcd. for $C_{30}H_{40}FNO$: C 80.13, H 8.97, N 3.12; Found C 80.22, H 8.77, N 3.25.

2.4.27. 16-(4-fluoro-benzylidene)-3β-morpholino-5α-androstan-17-one (6g)

White solid. Yield 45%. m.p. 236–238 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 2.58 (s, 4H, NCH_2), 3.72–3.74 (m, 4H, OCH_2), 7.40 (s, 1H, =CH), 7.12 (t, 2H), 7.54 (t, 2H).^{13}C NMR (150 MHz, $CDCl_3$): δ 12.39, 14.51, 20.45, 24.51, 28.70, 29.18, 31.09, 31.17, 31.64, 34.71, 36.12, 37.62, 45.72, 47.63, 49.51, 50.06, 54.60, 64.04, 67.35, 115.70, 115.92, 131.68, 131.86, 131.89, 132.11, 132.19, 161.74, 164.24, 209.80. Anal. Calcd. for $C_{30}H_{40}FNO_2$: C 77.38, H 8.66, N 3.01; Found C 77.47, H 8.51, N 2.92.

2.4.28. 16-(4-fluoro-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17-one (7g)

White solid. Yield 48%. m.p. 245–247 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (s, 3H, CH_3-19), 0.96 (s, 3H, CH_3-18), 2.30 (s, 3H, N-CH_3), 2.49–2.63 (m, 8H, NCH_2), 7.39 (s, 1H, =CH), 7.11 (t, 2H), 7.54 (t, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.40, 14.49, 20.43, 24.55, 28.68, 29.17, 31.17, 31.22, 31.64, 34.71, 36.10, 37.72, 45.83, 46.00, 47.61, 49.26, 49.50, 54.61, 55.46, 63.74, 115.68, 115.89, 131.61, 131.86, 131.89, 132.09, 132.17, 135.75, 161.72, 164.21, 209.74. Anal. Calcd. for $C_{31}H_{43}FN_2O$: C 77.78, H 9.05, N 5.85; Found C 77.91, H 8.99, N 5.72.

2.4.29. 16-(furan-2-ylmethylene)-3β-piperidino-5α-androstan-17-one (4h)

White solid. Yield 49%. m.p. 198–200 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (s, 3H, CH_3-19), 0.92 (s, 3H, CH_3-18), 2.53 (s, 4H, NCH_2), 7.20 (s, 1H, =CH), 6.51 (q, 1H), 6.64 (d, 2H, *J* = 3.44 Hz), 7.56 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.44, 14.62, 20.50, 24.17, 24.90, 26.49, 28.78, 28.92, 30.77, 31.23, 31.65, 34.72, 36.22, 37.96, 46.13, 47.80, 49.15, 50.39, 54.71, 64.51, 112.30, 115.42, 119.43, 133.81, 144.62, 152.32, 209.97. Anal. Calcd. for $C_{29}H_{41}NO_2$: C 79.95, H 9.49, N 3.22; Found C 79.80, H 9.61, N 3.08.

2.4.30. 16-(furan-2-ylmethylene)-3β-pyrrolidino-5α-androstan-17-one (5h)

White solid. Yield 48%. m.p. 228–230 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (s, 3H, CH_3-19), 0.93 (s, 3H, CH_3-18), 2.58 (br, 4H, NCH_2), 7.21 (s, 1H, =CH), 6.52 (q, 1H), 6.65 (d, 2H, *J* = 3.52 Hz), 7.57 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.35, 14.61, 20.42, 23.17, 27.95, 28.68, 28.91, 31.22, 31.64, 34.70, 34.73, 36.01, 37.35, 45.30, 47.79, 49.15, 51.82, 54.68, 64.30, 112.29, 115.40, 119.41, 133.78, 144.60, 152.30, 209.93. Anal. Calcd. for $C_{28}H_{39}NO_2$: C 79.76, H 9.32, N 3.32; Found C 79.52, H 9.44, N 3.19.

2.4.31. 16-(furan-2-ylmethylene)-3β-morpholino-5α-androstan-17-one (6h)

White solid. Yield 42%. m.p.232–234 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (s, 3H, CH_3-19), 0.92 (s, 3H, CH_3-18), 2.58 (s, 4H, NCH_2), 3.73–3.75 (m, 4H, OCH_2), 7.20 (s, 1H, =CH), 6.52 (q, 1H), 6.65 (d, 2H, *J* = 3.40 Hz), 7.57 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.39, 14.61, 20.48, 24.47, 28.74, 28.90, 31.08, 31.17, 31.62, 34.68, 36.11, 37.62, 45.74, 47.77, 49.11, 50.04, 54.64, 64.08, 67.30, 112.31, 115.46, 119.45, 133.70, 144.64, 152.27, 209.87. Anal. Calcd. for $C_{28}H_{39}NO_3$: C 76.85, H 8.98, N 3.20; Found C 76.62, H 8.76, N 3.39.

2.4.32. 16-(furan-2-ylmethylene)-3β-(4-methylpiperazinyl)-5α-androstan-17-one (7h)

White solid. Yield 49%. m.p. 218–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (s, 3H, CH_3-19), 0.92 (s, 3H, CH_3-18), 2.30 (s, 3H, N-CH_3), 2.46–2.62 (m, 8H, NCH_2), 7.21 (s, 1H, =CH), 6.52 (q, 1H), 6.65 (d, 2H, *J* = 3.42 Hz), 7.57 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.41, 14.61, 20.47, 24.56, 28.74, 28.90, 31.19, 31.27, 31.62, 34.69, 36.11, 37.74, 45.87, 46.05, 47.79, 49.11, 49.31, 54.66, 55.51, 63.77, 112.30, 115.44, 119.44, 133.74, 144.62, 152.28, 209.95. Anal. Calcd. for $C_{29}H_{42}N_2O_2$: C 77.29, H 9.39, N 6.22; Found C 77.41, H 9.51, N 6.08.

2.5. General procedure for the synthesis of compounds 8a–h,9a–h, 10a–h, 11a–h

To the suspension of compounds 4a–h, 5a–h, 6a–h, 7a–h (1 mmol) in 20 ml of absolute methanol at 0 °C, sodium borohydride (1.2 equiv.) was added in small amounts over a period of 3 h. The methanol was removed under reduced pressure, 20 ml CH_2Cl_2 was added to the residue, the organic layer was washed with saturated $NaHCO_3$, brine and dried over anhydrous Na_2SO_4, filtered. The filtrate was concentrated in vacuum to afford a crude product. The product was purified by flash column chromatography to yield compound 8a–h, 9a–h, 10a–h, 11a–h.

2.5.1. 16-(4-methyl-benzylidene)-3β-piperidino-5α-androstan-17β-ol (8a)

White solid. Yield 42%. m.p. 116–118 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.60 (s, 3H, CH_3-19), 0.73 (s, 3H, CH_3-18), 2.40 (s, 3H, Ph-CH_3), 2.59 (s, 4H, NCH_2), 3.90 (s, 1H, C_{17}-αH), 6.40 (s, 1H, =CH), 7.07 (d, 2H, *J* = 7.92 Hz), 7.20 (d, 2H, *J* = 7.96 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.26, 12.27, 20.79, 21.14, 21.81, 22.75, 22.96, 23.11, 28.14, 28.44, 30.83, 31.47, 34.86, 35.79, 36.38, 37.05, 42.91, 45.38, 48.51, 48.98, 54.31, 64.44, 84.60, 122.53, 128.01, 129.00, 135.24, 135.82, 145.06, 176.90. Anal. Calcd. for $C_{32}H_{47}NO$: C 83.24, H 10.26, N 3.03; Found C 83.42, H 10.19, N 2.99.

2.5.2. 16-(4-methyl-benzylidene)-3β-pyrrolidino-5α-androstan-17β-ol (9a)

White solid. Yield 42%. m.p. 202–204 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-19), 0.84 (s, 3H, CH_3-18), 2.35 (s, 3H,

Ph-CH_3), 2.59 (br, 4H, NCH_2), 3.90 (d, 1H, C_{17}-αH, J = 8.72 Hz), 6.49 (s, 1H, =CH), 7.16 (d, 2H, J = 7.96 Hz), 7.30 (d, 2H, J = 8.31 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.17, 12.38, 20.73, 21.17, 23.19, 27.83, 28.78, 30.96, 31.84, 34.59, 35.04, 35.94, 36.45, 37.43, 43.02, 45.34, 48.59, 51.80, 54.71, 64.40, 85.00, 122.61, 128.13, 129.04, 135.12, 35.99, 145.34. Anal. Calcd. for $C_{31}H_{45}NO$: C 83.17, H 10.13, N 3.13; Found C 83.27, H 10.25, N 3.01.

2.5.3. 16-(4-methyl-benzylidene)-3β-morpholino-5α-androstan-17β-ol (10a)

White solid. Yield 47%. m.p. 196–198 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70 (s, 3H, CH_3-19), 0.83 (s, 3H, CH_3-18), 2.36 (s, 3H, Ph-CH_3), 2.58 (s, 4H, NCH_2), 3.72–3.76 (s, 4H, OCH_2), 4.04 (d, 1H, C_{17}-αH, J = 8.12 Hz), 6.49 (s, 1H, =CH), 7.16 (d, 2H, J = 7.96 Hz), 7.30 (d, 2H, J = 8.33 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.19, 12.26, 20.77, 21.16, 22.20, 22.85, 28.62, 29.37, 30.90, 31.62, 34.95, 35.86, 36.33, 37.26, 42.99, 45.49, 48.47, 48.82, 54.41, 64.45, 65.54, 84.93, 122.70, 128.13, 129.06, 135.07, 136.05, 145.04. Anal. Calcd. for $C_{31}H_{45}NO_2$: C 80.30, H 9.78, N 3.02; Found C 80.12, H 9.69, N 3.11.

2.5.4. 16-(4-methyl-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-ol (11a)

White solid. Yield 41%. m.p. 205–207 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.39 (s, 3H, Ph-CH_3), 2.43–2.63 (m, 8H, NCH_2), 3.92 (d, 1H, C_{17}-αH, J = 8.72 Hz), 6.49 (s, 1H, =CH), 7.17 (d, 2H, J = 7.96 Hz), 7.28 (d, 2H, J = 8.30 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.24, 12.28, 20.76, 21.15, 22.67, 23.35, 28.63, 29.93, 30.88, 31.63, 34.93, 35.84, 37.38, 42.97, 44.51, 45.58, 47.38, 48.50, 53.08, 54.44, 63.75, 84.77, 122.57, 128.11, 129.02, 135.16, 135.90, 145.12. Anal. Calcd. for $C_{32}H_{48}N_2O$: C 80.62, H 10.15, N 5.88; Found C 80.39, H 10.01, N 5.99.

2.5.5. 16-(4-methoxy-benzylidene)-3β-piperidino-5α-androstan-17β-ol (8b)

White solid. Yield 41%. m.p. 204–206 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.53 (s, 4H, NCH_2), 3.83 (s, 3H, OCH_3), 4.06 (d, 1H, C_{17}-αH, J = 8.52 Hz), 6.46 (s, 1H, =CH), 6.90 (d, 2H, J = 8.80 Hz), 7.35 (d, 2H, J = 8.76 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.17, 12.46, 20.80, 24.12, 24.88, 26.42, 28.88, 30.68, 30.90, 31.85, 35.06, 36.13, 36.43, 38.02, 43.03, 46.13, 48.58, 50.36, 54.70, 55.26, 64.54, 85.02, 113.77, 122.16, 129.39, 130.80, 143.93, 158.01. Anal. Calcd. for $C_{32}H_{47}NO_2$: C 80.45, H 9.92, N 2.93; Found C 80.27, H 9.88, N 3.02.

2.5.6. 16-(4-methoxy-benzylidene)-3β-pyrrolidino-5α-androstan-17β-ol (9b)

White solid. Yield 41%. m.p. 178–180 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, CH_3-19), 0.82 (s, 3H, CH_3-18), 2.30 (s, 3H, N-CH_3), 2.57 (br, 4H, NCH_2), 3.83 (s, 3H, OCH_3), 4.03 (d, 1H, C_{17}-αH, J = 8.52 Hz), 6.44 (s, 1H, =CH), 6.88 (d, 2H, J = 8.72 Hz), 7.33 (d, 2H, J = 8.72 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.17, 12.37, 20.73, 23.18, 27.67, 28.75, 30.88, 31.82, 34.42, 35.03, 35.92, 36.43, 37.38, 43.01, 45.32, 48.60, 51.73, 54.67, 55.25, 64.36, 84.98, 113.76, 122.44, 129.39, 130.82, 143.93, 157.99. Anal. Calcd. for $C_{31}H_{45}NO_2$: C 80.30, H 9.78, N 3.02; Found C 80.22, H 9.91, N 2.89.

2.5.7. 16-(4-methoxy-benzylidene)-3β-morpholino-5α-androstan-17β-ol (10b)

White solid. Yield 47%. m.p. 173–175 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-19), 0.86 (s, 3H, CH_3-18), 2.59 (s, 4H, NCH_2), 3.73–3.75 (m, 4H, OCH_2), 3.83 (s, 3H, OCH_3), 4.06 (d, 1H, C_{17}-αH, J = 8.88 Hz), 6.47 (s, 1H, =CH), 6.90 (d, 2H, J = 8.72 Hz), 7.35 (d, 2H, J = 8.72 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.18, 12.41, 20.78, 24.50, 28.85, 30.89, 31.12, 31.81, 35.03, 36.04, 37.70, 43.02, 45.76, 46.14, 48.55, 50.05, 54.65, 55.26, 64.12, 67.35, 85.00, 113.76, 122.18, 129.39, 130.78, 143.84, 158.01. Anal. Calcd. for $C_{31}H_{45}NO_3$: C 77.62, H 9.46, N 2.92; Found C 77.43, H 9.59, N 2.71.

2.5.8. 16-(4-methoxy-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-ol (11b)

White solid. Yield 44%. m.p. 147–149 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, CH_3-19), 0.80 (s, 3H, CH_3-18), 2.29 (s, 3H, N-CH_3), 2.45–2.62 (m, 8H, NCH_2), 3.83 (s, 3H, OCH_3), 4.02 (s, 1H, C_{17}-αH), 6.44 (d, 1H, =CH, J = 2.12 Hz), 6.88 (d, 2H, J = 8.76 Hz), 7.33 (d, 2H, J = 8.72 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.20, 11.38, 12.42, 20.78, 24.52, 28.84, 30.87, 31.25, 31.81, 35.03, 36.02, 36.45, 37.80, 43.01, 45.87, 46.02, 46.11, 48.58, 49.22, 54.67, 55.24, 55.45, 63.81, 84.90, 113.75, 122.13, 129.38, 130.83, 143.91, 157.97. Anal. Calcd. for $C_{32}H_{48}N_2O_2$: C 78.00, H 9.82, N 5.69; Found C 77.82, H 9.93, N 5.43.

2.5.9. 16-(4-nitro-benzylidene)-3β-piperidino-5α-androstan-17β-ol (8c)

Yellow solid. Yield 47%. m.p. 227–229 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71 (s, 3H, CH_3-19), 0.82 (s, 3H, CH_3-18), 2.54 (s, 4H, NCH_2), 4.09 (s, 1H, C_{17}-αH), 6.62 (d, 1H, =CH, J = 2.32 Hz), 7.52 (d, 2H, J = 8.88 Hz), 8.21 (d, 2H, J = 8.84 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.23, 12.43, 20.71, 24.05, 24.79, 26.29, 28.76, 30.52, 31.23, 31.81, 34.98, 36.12, 37.97, 43.16, 46.09, 48.48, 50.31, 54.65, 64.50, 85.05, 121.42, 123.75, 128.54, 144.51, 145.65, 152.09. Anal. Calcd. for $C_{31}H_{44}N_2O_3$: C 75.57, H 9.00, N 5.69; Found C 75.64, H 8.93, N 5.80.

2.5.10. 16-(4-nitro-benzylidene)-3β-pyrrolidino-5α-androstan-17β-ol (9c)

Yellow solid. Yield 45%. m.p. 221–223 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72 (s, 3H, CH_3-19), 0.85 (s, 3H, CH_3-18), 2.59 (br, 4H, NCH_2), 4.10 (s, 1H, C_{17}-αH), 6.62 (d, 1H, =CH, J = 2.28 Hz), 7.52 (d, 2H, J = 8.88 Hz), 8.21 (d, 2H, J = 8.88 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.22, 12.38, 20.66, 23.18, 27.93, 28.69, 31.23, 31.83, 34.67, 34.99, 35.95, 36.36, 37.42, 43.17, 45.32, 48.51, 51.84, 54.67, 64.35, 85.08, 121.40, 123.75, 128.53, 144.51, 145.65, 152.15. Anal. Calcd. for $C_{30}H_{42}N_2O_3$: C 75.28, H 8.84, N 5.85; Found C 75.31, H 8.77, N 5.93.

2.5.11. 16-(4-nitro-benzylidene)-3β-morpholino-5α-androstan-17β-ol (10c)

Yellow solid. Yield 41%. m.p. 212–214 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71 (s, 3H, CH_3-19), 0.87 (s, 3H, CH_3-18), 2.42 (s, 4H, NCH_2), 3.72–3.74 (m, 4H, OCH_2), 4.09 (s, 1H, C_{17}-αH), 6.62 (d, 1H, =CH, J = 2.28 Hz), 7.52 (d, 2H, J = 8.88 Hz), 8.21 (d, 2H, J = 8.88 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.23, 12.24, 20.39, 23.74, 28.41, 30.62, 31.23, 31.73, 32.84, 34.97, 36.28, 36.39, 39.42, 43.19, 48.48, 50.84, 54.55, 59.35, 67.41, 85.16, 121.39, 123.75, 128.54, 144.50, 145.65, 152.10. Anal. Calcd. for $C_{30}H_{42}N_2O_4$: C 72.84, H 8.56, N 5.66; Found C 72.66, H 8.41, N 5.39.

2.5.12. 16-(4-nitro-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-ol (11c)

Yellow solid. Yield 40%. m.p. 244–246 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.71 (s, 3H, CH_3-19), 0.87 (s, 3H, CH_3-18), 2.30 (s, 3H, N-CH_3), 2.48–2.65 (m, 8H, NCH_2), 4.09 (s, 1H, C_{17}-αH), 6.62 (d, 1H, =CH, J = 2.00 Hz), 7.52 (d, 2H, J = 8.80 Hz), 8.21 (d, 2H, J = 8.80 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.24, 12.42, 20.70, 24.51, 28.74, 31.22, 31.79, 34.97, 36.03, 37.78, 43.17, 45.87, 46.01, 48.47, 49.24, 54.63, 55.44, 63.79, 85.06, 121.43, 123.75, 128.54, 144.48, 145.66, 152.06. Anal. Calcd. for $C_{31}H_{45}N_3O_3$: C 73.34, H 8.93, N 8.28; Found C 73.17, H 8.76, N 8.51.

2.5.13. 16-(3,4,5-trimethoxy-benzylidene)-3β-piperidino-5α-androstan-17β-ol (8d)

White solid. Yield 49%. m.p. 217–219 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, CH_3-19), 0.80 (s, 3H, CH_3-18), 2.51 (s, 4H,

NCH_2), 3.86 (s, 9H, OCH_3), 4.09 (d, 1H, C_{17}-αH, J = 9.00 Hz), 6.44 (d, 1H, =CH, J = 2.00 Hz), 6.62 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.20, 12.44, 20.77, 24.08, 24.86, 26.38, 28.84, 30.64, 30.78, 31.82, 35.00, 36.12, 36.40, 38.00, 43.01, 46.10, 48.53, 50.34, 54.68, 56.05, 60.91, 64.51, 84.93, 105.48, 122.83, 133.72, 136.70, 145.63, 152.99. Anal. Calcd. for $C_{34}H_{51}NO_4$: C 75.94, H 9.56, N 2.60; Found C 75.88, H 9.41, N 2.39.

2.5.14. 16-(3,4,5-trimethoxy-benzylidene)-3β-pyrrolidino-5α-androstan-17β-ol (9d)

White solid. Yield 48%. m.p. 186–188 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-19), 0.83 (s, 3H, CH_3-18), 2.56 (br, 4H, NCH_2), 3.87 (s, 9H, OCH_3), 4.04 (d, 1H, C_{17}-αH, J = 8.28 Hz), 6.43 (d, 1H, =CH, J = 2.00 Hz), 6.62 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 9.77, 10.96, 19.29, 21.77, 26.58, 27.35, 29.38, 30.44, 33.35, 33.60, 34.54, 34.99, 36.02, 41.61, 43.91, 47.15, 50.43, 53.29, 54.68, 59.52, 62.93, 83.64, 104.09, 121.47, 132.27, 135.36, 144.23, 151.61. Anal. Calcd. for $C_{33}H_{49}NO_4$: C 75.68, H 9.43, N 2.67; Found C 75.51, H 9.19, N 2.59.

2.5.15. 16-(3,4,5-trimethoxy-benzylidene)-3β-morpholino-5α-androstan-17β-ol (10d)

White solid. Yield 48%. m.p. 241–243 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-19), 0.82 (s, 3H, CH_3-18), 2.56 (s, 4H, NCH_2), 3.72–3.75 (m, 4H, OCH_2), 3.84 (s, 9H, OCH_3), 4.03 (d, 1H, C_{17}-αH, J = 8.28 Hz), 6.43 (d, 1H, =CH, J = 1.60 Hz), 6.62 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.19, 12.38, 20.73, 24.44, 28.79, 30.75, 31.08, 31.76, 34.96, 36.01, 36.36, 37.66, 42.98, 45.72, 48.49, 50.01, 54.61, 56.04, 60.89, 64.07, 67.30, 84.91, 105.48, 122.85, 133.68, 136.70, 145.50, 152.97. Anal. Calcd. for $C_{33}H_{49}NO_5$: C 73.43, H 9.15, N 2.60; Found C 73.21, H 9.01, N 2.81.

2.5.16. 16-(3,4,5-trimethoxy-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-ol (11d)

White solid. Yield 48%. m.p. 210–212 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, CH_3-19), 0.80 (s, 3H, CH_3-18), 2.28 (s, 3H, N-CH_3), 2.47–2.62 (m, 8H, NCH_2), 3.86 (s, 9H, OCH_3), 4.04 (d, 1H, C_{17}-αH, J = 8.28 Hz), 6.44 (d, 1H, =CH, J = 1.60 Hz), 6.62 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 10.31, 11.55, 19.88, 23.67, 27.94, 29.92, 30.50, 30.94, 34.14, 35.18, 35.51, 36.94, 42.15, 45.02, 45.19, 47.65, 48.46, 53.80, 54.67, 55.21, 60.06, 62.95, 84.16, 104.63, 122.02, 132.79, 135.91, 144.72, 152.15. Anal. Calcd. for $C_{34}H_{52}N_2O_4$: C 73.87, H 9.48, N 5.07; Found C 73.77, H 9.62, N 4.91.

2.5.17. 16-(4-chloro-benzylidene)-3β-piperidino-5α-androstan-17β-ol (8e)

White solid. Yield 48%. m.p. 225–227 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.53 (s, 4H, NCH_2), 4.06 (d, 1H, C_{17}-αH, J = 8.88 Hz), 6.48 (d, 1H, =CH, J = 2.28 Hz), 7.30 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.88, 12.45, 20.76, 24.17, 24.90, 26.46, 28.84, 30.68, 30.91, 31.84, 35.03, 36.13, 38.03, 43.06, 46.13, 48.52, 50.38, 54.69, 64.55, 84.98, 121.75, 128.46, 129.39, 131.84, 136.39, 147.12. Anal. Calcd. for $C_{31}H_{44}ClNO$: C 77.22, H 9.20, N 2.91; Found C 77.03, H 9.01, N 3.03.

2.5.18. 16-(4-chloro-benzylidene)-3β-pyrrolidino-5α-androstan-17β-ol (9e)

White solid. Yield 41%. m.p. 224–226 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70 (s, 3H, CH_3-19), 0.84 (s, 3H, CH_3-18), 2.58 (br, 4H, NCH_2), 4.06 (d, 1H, C_{17}-αH, J = 8.64 Hz), 6.48 (d, 1H, =CH, J = 2.24 Hz), 7.30 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.18, 12.38, 20.70, 23.19, 27.99, 28.75, 30.92, 31.84, 34.75, 35.02, 35.95, 36.40, 37.44, 43.06, 45.33, 48.55, 51.86, 54.70, 64.39, 85.00, 121.74, 128.46, 129.39, 131.83, 136.39, 147.15. Anal. Calcd. for $C_{30}H_{42}ClNO$: C 76.97, H 9.04, N 2.99; Found C 77.11, H 8.89, N 2.74.

2.5.19. 16-(4-chloro-benzylidene)-3β-morpholino-5α-androstan-17β-ol (10e)

White solid. Yield 41%. m.p. 224–226 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70 (s, 3H, CH_3-19), 0.84 (s, 3H, CH_3-18), 2.58 (s, 4H, NCH_2), 3.82–3.84 (m, 4H, OCH_2), 4.06 (d, 1H, C_{17}-αH, J = 8.64 Hz), 6.48 (d, 1H, =CH, J = 2.24 Hz), 7.30 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.20, 12.43, 20.66, 28.13, 28.77, 30.88, 31.79, 34.75, 35.13, 35.97, 36.29, 37.51, 43.12, 45.29, 48.71, 51.78, 54.66, 64.21, 85.12, 121.71, 128.55, 129.24, 131.79, 136.28, 147.22. Anal. Calcd. for $C_{30}H_{42}ClNO_2$: C 74.43, H 8.74, N 2.89; Found C 74.51, H 8.82, N 2.97.

2.5.20. 16-(4-chloro-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-ol (11e)

White solid. Yield 41%. m.p. 229–231 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-19), 0.82 (s, 3H, CH_3-18), 2.31 (s, 3H, N-CH_3), 2.51–2.63 (m, 8H, NCH_2), 4.05 (s, 1H, C_{17}-αH), 6.48 (d, 1H, =CH, J = 2.26 Hz), 7.30 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.19, 12.42, 20.74, 24.47, 28.78, 30.90, 31.18, 31.79, 35.00, 36.02, 37.77, 43.05, 45.87, 46.00, 48.50, 49.22, 54.64, 55.35, 63.88, 84.94, 121.76, 128.46, 129.39, 131.84, 136.38, 147.08. Anal. Calcd. for $C_{31}H_{45}ClN_2O$: C 74.89, H 9.12, N 5.63; Found C 74.69, H 9.03, N 5.51.

2.5.21. 16-(4-bromo-benzylidene)-3β-piperidino-5α-androstan-17β-ol (8f)

White solid. Yield 44%. m.p. 210–212 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.54 (s, 4H, NCH_2), 4.05 (d, 1H, C_{17}-αH, J = 8.80 Hz), 6.46 (s, 1H, =CH), 7.26 (d, 2H, J = 8.44 Hz), 7.46 (d, 2H, J = 8.44 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.20, 12.45, 20.75, 24.07, 24.81, 26.31, 28.81, 30.57, 30.92, 31.81, 35.00, 36.11, 37.98, 43.04, 46.09, 48.50, 50.34, 54.65, 64.57, 84.93, 119.96, 121.77, 129.72, 131.39, 136.83, 147.34. Anal. Calcd. for $C_{31}H_{44}BrNO$: C 70.71, H 8.42, N 2.66; Found C 70.59, H 8.31, N 2.88.

2.5.22. 16-(4-bromo-benzylidene)-3β-pyrrolidino-5α-androstan-17β-ol (9f)

White solid. Yield 46%. m.p. 203–205 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-19), 0.84 (s, 3H, CH_3-18), 2.58 (br, 4H, NCH_2), 4.04 (s, 1H, C_{17}-αH), 6.46 (s, 1H, =CH), 7.26 (d, 2H, J = 8.60 Hz), 7.46 (d, 2H, J = 8.62 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.18, 12.37, 20.69, 23.19, 27.83, 28.73, 30.92, 31.82, 35.00, 35.93, 37.40, 43.04, 45.31, 46.17, 48.53, 51.80, 54.67, 64.36, 85.00, 119.98, 121.78, 129.72, 131.39, 136.81, 147.35. Anal. Calcd. for $C_{30}H_{42}BrNO$: C 70.30, H 8.26, N 2.73; Found C 70.08, H 8.41, N 2.59.

2.5.23. 16-(4-bromo-benzylidene)-3β-morpholino-5α-androstan-17β-ol (10f)

White solid. Yield 46%. m.p. 241–243 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, CH_3-19), 0.82 (s, 3H, CH_3-18), 2.57 (s, 4H, NCH_2), 3.82–3.85 (m, 4H, OCH_2), 4.04 (s, 1H, C_{17}-αH), 6.46 (s, 1H, =CH), 7.26 (d, 2H, J = 8.60 Hz), 7.46 (d, 2H, J = 8.62 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.19, 12.51, 20.77, 20.11, 24.81, 26.09, 28.91, 30.58, 30.99, 31.77, 35.02, 36.15, 37.99, 43.01, 46.10, 48.48, 50.31, 54.62, 64.55, 84.90, 119.98, 121.73, 129.68, 131.41, 136.79, 147.37. Anal. Calcd. for $C_{30}H_{42}BrNO_2$: C 68.17, H 8.01, N 2.65; Found C 68.03, H 7.96, N 2.78.

2.5.24. 16-(4-bromo-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-ol (11f)

White solid. Yield 46%. m.p. 235–237 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.60 (s, 3H, CH_3-19), 0.73 (s, 3H, CH_3-18), 2.22 (s, 3H, N-CH_3), 2.43–2.54 (m, 8H, NCH_2), 3.94 (s, 1H, C_{17}-αH), 6.37 (s, 1H,

=CH), 7.17 (d, 2H, J = 8.48 Hz), 7.37 (d, 2H, J = 8.44 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.22, 12.42, 20.74, 24.47, 28.78, 30.90, 31.18, 31.79, 34.99, 36.02, 37.77, 43.04, 45.85, 46.00, 48.50, 49.20, 54.63, 55.35, 63.85, 84.90, 119.97, 121.79, 129.73, 131.40, 136.84, 147.34. Anal. Calcd. for $C_{31}H_{45}BrN_2O$: C 68.75, H 8.37, N 5.17; Found C 68.79, H 8.51, N 5.23.

2.5.25. 16-(4-fluoro-benzylidene)-3β-piperidino-5α-androstan-17β-ol (8g)

White solid. Yield 46%. m.p. 188–190 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.57 (s, 4H, NCH_2), 4.04 (s, 1H, C_{17}-αH), 6.48 (d, 1H, =CH, J = 2.08 Hz), 7.02 (t, 2H), 7.35 (q, 2h). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.24, 12.41, 20.77, 23.82, 24.65, 26.00, 28.80, 30.31, 31.78, 34.99, 36.07, 37.91, 43.01, 45.97, 46.03, 48.55, 50.14, 54.65, 64.46, 84.63, 115.01, 115.22, 121.62, 129.59, 134.24, 145.87, 159.92, 162.37. Anal. Calcd. for $C_{31}H_{44}FNO$: C 79.95, H 9.52, N 3.01; Found C 79.80, H 9.38, N 3.22.

2.5.26. 16-(4-fluoro-benzylidene)-3β-pyrrolidino-5α-androstan-17β-ol (9g)

White solid. Yield 46%. m.p. 240–242 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.60 (s, 3H, CH_3-19), 0.76 (s, 3H, CH_3-18), 2.37–2.39 (m, 4H, NCH_2), 3.96 (s, 1H, C_{17}-αH, J = 8.64 Hz), 6.39 (d, 1H, =CH), 6.94 (t, 2H), 7.27 (q, 2 h). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.26, 12.45, 20.87, 23.90, 26.11, 28.91, 30.13, 31.82, 34.96, 36.10, 37.92, 43.05, 45.88, 46.12, 48.50,50.12, 54.66, 64.45, 84.60, 115.04, 115.21, 121.56, 129.60, 134.31, 145.93, 159.95, 162.40. Anal. Calcd. for $C_{30}H_{42}FNO$: C 79.78, H 9.37, N 3.10; Found C 79.61, H 9.45, N 3.01.

2.5.27. 16-(4-fluoro-benzylidene)-3β-morpholino-5α-androstan-17β-ol (10g)

White solid. Yield 46%. m.p. 183–185 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61 (s, 3H, CH_3-19), 0.73 (s, 3H, CH_3-18), 2.46–2.49 (m, 4H, NCH_2), 3.64–3.66 (m, 4H, OCH_2), 3.96 (s, 1H, C_{17}-αH), 6.40 (d, 1H, =CH), 6.94 (t, 2H), 7.26 (q, 2h). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.31, 12.52, 20.79, 23.88, 26.13, 29.09, 30.15, 31.88, 34.95, 36.01, 37.99, 43.03, 45.99, 36.15, 48.01, 50.11, 54.84, 64.44, 84.55, 115.01, 115.22, 121.55, 129.49, 134.13, 145.90, 159.99, 162.38. Anal. Calcd. for $C_{30}H_{42}FNO_2$: C 77.05, H 9.05, N 3.00; Found C 76.88, H 8.91, N 3.21.

2.5.28. 16-(4-fluoro-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-ol (11g)

White solid. Yield 46%. m.p. 213–215 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61 (s, 3H, CH_3-19), 0.73 (s, 3H, CH_3-18), 2.21 (s, 3H, N-CH_3), 2.40–2.55 (m, 8H, NCH_2), 3.96 (s, 1H, C_{17}-αH), 6.40 (d, 1H, =CH), 6.94 (t, 2H), 7.26 (q, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.20, 12.42, 14.19, 20.75, 24.54, 28.80, 30.77, 31.24, 31.81, 35.01, 36.03, 37.81, 43.03, 45.88, 46.03, 48.54, 49.23, 54.67, 55.47, 60.40, 63.82, 84.81, 115.27, 121.70, 129.68, 134.13, 145.83, 159.99, 162.43. Anal. Calcd. for $C_{31}H_{45}FN_2O$: C 77.46, H 9.44, N 5.83; Found C 77.63, H 9.19, N 5.77.

2.5.29. 16-(furan-2-ylmethylene)-3β-piperidino-5α-androstan-17β-ol (8h)

White solid. Yield 41%. m.p. 267–269 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.54 (s, 4H, NCH_2), 4.06 (s, 1H, C_{17}-αH), 6.23 (d, 1H, =CH, J = 3.24 Hz), 6.42 (s, 2H), 7.39 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.15, 12.43, 20.79, 24.51, 28.89, 30.97, 31.12, 31.77, 35.10, 36.08, 36.33, 37.69, 43.39, 45.77, 48.35, 50.00, 54.69, 64.13, 67.33, 84.55, 107.49, 111.29, 111.66, 141.14, 145.22, 153.80. Anal. Calcd. for $C_{29}H_{43}NO_2$: C 79.59, H 9.90, N 3.20; Found C 79.67, H 9.79, N 3.08.

2.5.30. 16-(furan-2-ylmethylene)-3β-pyrrolidino-5α-androstan-17β-ol (9h)

White solid. Yield 44%. m.p. 220–222 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, CH_3-19), 0.84 (s, 3H, CH_3-18), 2.58 (br, 4H, NCH_2), 4.07 (d, 1H, C_{17}-αH, J = 8.60 Hz), 6.22 (d, 1H, =CH, J = 3.24 Hz), 6.42 (s, 2H), 7.39 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.15, 12.38, 20.72, 23.17, 27.90, 28.78, 30.97, 31.83, 34.68, 35.02, 35.93, 36.41, 37.44, 43.38, 45.34, 48.37, 51.80, 54.71, 64.36, 84.52, 107.43, 111.21, 111.62, 141.14, 145.42, 153.82. Anal. Calcd. for $C_{28}H_{41}NO_2$: C 79.39, H 9.76, N 3.31; Found C 79.55, H 9.59, N 3.43.

2.5.31. 16-(furan-2-ylmethylene)-3β-morpholino-5α-androstan-17β-ol (10h)

White solid. Yield 49%. m.p. 220–222 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, CH_3-19), 0.82 (s, 3H, CH_3-18), 2.58 (s, 4H, NCH_2), 3.73–3.76 (m, 4H, OCH_2), 4.06 (s, 1H, C_{17}-αH), 6.23 (d, 1H, =CH, J = 3.20 Hz), 6.42 (s, 2H), 7.39 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.15, 12.41, 20.77, 24.48, 28.84, 30.96, 31.11, 31.78, 35.01, 36.03, 37.70, 43.38, 45.78, 48.31, 50.04, 54.66, 64.11, 67.33, 84.53, 107.47, 111.22, 111.67, 141.18, 145.27, 153.80. Anal. Calcd. for $C_{28}H_{41}NO_3$: C 76.50, H 9.40, N 3.19; Found C 76.22, H 9.51, N 3.29.

2.5.32. 16-(furan-2-ylmethylene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-ol (11h)

White solid. Yield 40%. m.p. 239–241 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, CH_3-19), 0.82 (s, 3H, CH_3-18), 2.30 (s, 3H, N-CH_3), 2.48–2.62 (m, 8H, NCH_2), 4.06 (s, 1H, C_{17}-αH), 6.22 (d, 1H, =CH, J = 3.20 Hz), 6.42 (s, 2H), 7.39 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.14, 12.43, 14.20, 20.77, 21.06, 24.55, 28.84, 30.97, 31.32, 31.81, 35.03, 36.04, 37.83, 43.39, 45.93, 46.06, 48.33, 49.30, 54.70, 55.53, 60.40, 63.84, 84.57, 107.49, 111.23, 111.67, 141.20, 145.36, 153.83. Anal. Calcd. for $C_{29}H_{44}N_2O_2$: C 76.95, H 9.80, N 6.19; Found C 76.80, H 9.91, N 6.11.

2.6. General procedure for the synthesis of compounds 12a–h, 13a–h, 14a–h, 15a–h

To a solution of compounds 8a–h, 9a–h, 10a–h, 11a–h (5 mmol) in CH_2Cl_2 was added acetic anhydride (10 mmol, 2 equiv) and 4-dimethylaminopyridine (5 mmol, 1 equiv). The mixture was stirred at 40 °C until the reaction was completed (monitored by TLC). The reaction was concentrated in reduced pressure. The crude product was dissolved in CH_2Cl_2, the organic layer was washed with brine and dried over anhydrous Na_2SO_4, filtered. The filtrate was concentrated in reduced pressure to afford a crude product. The products were purified by flash column chromatography to yield compound 12a–h, 13a–h, 14a–h, 15a–h.

2.6.1. 16-(4-methyl-benzylidene)-3β-piperidino-5α-androstan-17β-yl acetate (12a)

White solid. Yield 95%. m.p. 171–173 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.80 (s, 3H, CH_3-18), 2.21 (s, 3H, $OCOCH_3$), 2.35 (s, 3H, Ph-CH_3), 2.52 (s, 4H, NCH_2), 5.37 (s, 1H, C_{17}-αH), 6.18 (d, 1H, =CH, J = 2.12 Hz), 7.15 (d, 2H, J = 8.00 Hz), 7.20 (d, 2H, J = 8.08 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.30, 12.42, 20.66, 21.20, 24.20, 24.94, 26.52, 28.86, 30.75, 31.00, 31.74, 34.89, 36.07, 37.97, 42.97, 46.03, 48.88, 50.40, 54.40, 64.53, 84.63, 123.36, 128.21, 129.00, 134.85, 136.08, 139.94, 171.10. Anal. Calcd. for $C_{34}H_{49}NO_2$: C 81.06, H 9.80, N 2.78; Found C 81.22, H 9.71, N 2.90. MS (m/z): 504.7$[M+1]^+$.

2.6.2. 16-(4-methyl-benzylidene)-3β-pyrrolidino-5α-androstan-17β-yl acetate (13a)

White solid. Yield 92%. m.p. 188–190 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.83 (s, 3H, CH_3-18), 2.21 (s, 3H,

$OCOCH_3$), 2.35 (s, 3H, Ph-CH_3), 2.57 (br, 4H, NCH_2), 5.37 (s, 1H, C_{17}-αH), 6.18 (d, 1H, =CH, J = 2.00 Hz), 7.15 (d, 2H, J = 8.00 Hz), 7.26 (d, 2H, J = 8.04 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.28, 12.33, 20.58, 21.17, 21.21, 23.18, 27.93, 28.74, 30.98, 31.74, 34.70, 34.89, 35.89, 36.55, 37.37, 42.97, 45.23, 48.92, 51.83, 54.42, 64.35, 84.65, 123.35, 128.19, 128.99, 134.83, 136.13, 139.96, 171.19. Anal. Calcd. for $C_{33}H_{47}NO_2$: C 80.93, H 9.67, N 2.86; Found C 80.77, H 9.58, N 2.99. MS (m/z): 490.6[M+1]$^+$.

2.6.3. 16-(4-methyl-benzylidene)-3β-morpholino-5α-androstan-17β-yl acetate (14a)

White solid. Yield 96%. m.p. 96–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.83 (s, 3H, CH_3-18), 2.18 (s, 3H, $OCOCH_3$), 2.27 (s, 3H, NCH_3), 2.32 (s, 3H, Ph-CH_3), 2.57 (s, 4H, NCH_2), 3.73–3.75 (m, 4H, OCH_2) 5.37 (s, 1H, C_{17}-αH), 6.18 (d, 1H, =CH, J = 2.02 Hz), 7.15 (d, 2H, J = 7.88 Hz), 7.26 (d, 2H, J = 8.00 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.55, 12.29, 12.37, 20.64, 21.17, 21.21, 24.50, 28.81, 30.97, 31.12, 31.71, 34.88, 36.00, 37.65, 42.97, 45.68, 46.21, 48.88, 50.06, 54.37, 64.08, 67.37, 84.62, 123.38, 128.19, 129.00, 134.82, 136.15, 139.91, 171.19. Anal. Calcd. for $C_{33}H_{47}NO_3$: C 78.37, H 9.37, N 2.77; Found C 78.52, H 9.45, N 2.59. MS (m/z): 506.6[M+1]$^+$.

2.6.4. 16-(4-methyl-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-yl acetate (15a)

White solid. Yield 95%. m.p. 180–182 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-18), 2.21 (s, 3H, $OCOCH_3$), 2.35 (s, 3H, Ph-CH_3), 2.46–2.60 (m, 8H, NCH_2), 5.34 (s, 1H, C_{17}-αH), 6.16 (d, 1H, =CH, J = 2.06 Hz), 7.13 (d, 2H, J = 7.96 Hz), 7.24 (d, 2H, J = 8.12 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.27, 12.37, 20.62, 21.17, 24.52, 28.79, 30.94, 31.27, 31.68, 34.85, 35.95, 37.73, 42.93, 45.76, 46.03, 48.83, 49.27, 54.34, 55.51, 63.75, 84.58, 123.34, 128.18, 128.97, 134.81, 136.05, 139.88, 171.05. Anal. Calcd. for $C_{34}H_{50}N_2O_2$: C 78.72, H 9.71, N 5.40; Found C 78.88, H 9.60, N 5.19. MS (m/z): 519.6[M+1]$^+$.

2.6.5. 16-(4-methoxy-benzylidene)-3β-piperidino-5α-androstan-17β-yl acetate (12b)

White solid. Yield 95%. m.p. 190–192 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.80 (s, 3H, CH_3-18), 2.21 (s, 3H, $OCOCH_3$), 2.53 (s, 4H, NCH_2), 3.81 (s, 3H, OCH_3), 5.36 (s, 1H, C_{17}-αH), 6.15 (d, 1H, =CH, J = 2.08 Hz), 6.89 (d, 2H, J = 8.44 Hz), 7.31 (d, 2H, J = 8.76 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.30, 12.41, 20.65, 21.24, 24.05, 24.79, 26.29, 28.81, 30.61, 30.93, 31.74, 34.89, 36.07, 37.91, 42.98, 46.00, 48.92, 50.34, 54.39, 55.24, 64.55, 84.68, 113.69, 122.88, 129.45, 130.48, 138.60, 158.08, 171.23. Anal. Calcd. for $C_{34}H_{49}NO_3$: C 78.57, H 9.50, N 2.69; Found C 78.44, H 9.37, N 2.81. MS (m/z): 520.6[M+1]$^+$.

2.6.6. 16-(4-methoxy-benzylidene)-3β-pyrrolidino-5α-androstan-17β-yl acetate (13b)

White solid. Yield 92%. m.p. 205–207 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.19 (s, 3H, $OCOCH_3$), 2.62 (br, 4H, NCH_2), 3.81 (s, 3H, OCH_3), 5.34 (s, 1H, C_{17}-αH), 6.13 (d, 1H, =CH, J = 2.08 Hz), 6.87 (d, 2H, J = 8.56 Hz), 7.30 (d, 2H, J = 8.76 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.29, 12.42, 20.59, 21.23, 23.18, 26.88, 28.58, 30.90, 31.65, 33.57, 34.82, 36.51, 37.17, 42.95, 45.20, 48.92, 51.56, 54.27, 55.23, 64.41, 84.65, 113.70, 113.75, 122.93, 129.68, 130.45, 138.50, 158.11, 171.26. Anal. Calcd. for $C_{33}H_{47}NO_3$: C 78.37, H 9.37, N 2.77; Found C 78.61, H 9.53, N 2.59. MS (m/z): 506.6[M+1]$^+$.

2.6.7. 16-(4-methoxy-benzylidene)-3β-morpholino-5α androstan-17β-yl acetate (14b)

White solid. Yield 88%. m.p. 217–219 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.82 (s, 3H, CH_3-18), 2.21 (s, 3H, $OCOCH_3$), 2.59 (s, 4H, NCH_2), 3.74–3.77 (m, 4H, OCH_2), 3.83 (s, 3H, OCH_3), 5.36 (s, 1H, C_{17}-αH), 6.16 (d, 1H, =CH, J = 2.16 Hz), 6.89 (d, 2H, J = 8.80 Hz), 7.31 (d, 2H, J = 8.80 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.31, 12.38, 20.65, 21.25, 24.48, 28.80, 30.92, 31.09, 31.72, 34.88, 36.00, 37.64, 42.98, 45.67, 48.91, 50.05, 54.36, 55.25, 64.11, 67.33, 84.67, 113.71, 122.93, 129.48, 130.48, 138.56, 158.11, 171.27. Anal. Calcd. for $C_{33}H_{47}NO_4$: C 75.97, H 9.08, N 2.68; Found C 75.88, H 8.97, N 2.56. MS (m/z): 522.6[M+1]$^+$.

2.6.8. 16-(4-methoxy-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-yl acetate (15b)

White solid. Yield 91%. m.p. 186–188 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.21 (s, 3H, $OCOCH_3$), 2.30 (s, 3H, Ph-CH_3), 2.50–2.67 (m, 8H, NCH_2), 3.82 (s, 3H, OCH_3), 5.36 (s, 1H, C_{17}-αH), 6.15 (d, 1H, =CH, J = 2.16 Hz), 6.89 (d, 2H, J = 8.80 Hz), 7.31 (d, 2H, J = 8.76 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.30, 12.39, 20.63, 21.23, 24.53, 28.79, 30.91, 31.27, 31.72, 34.88, 35.98, 36.52, 37.75, 42.96, 45.79, 46.05, 48.90, 49.30, 54.37, 55.22, 55.52, 63.80, 84.67, 113.69, 122.89, 129.47, 130.48, 138.58, 158.09, 171.21. Anal. Calcd. for $C_{34}H_{50}N_2O_3$: C 76.36, H 9.42, N 5.24; Found C 76.47, H 9.60, N 5.11. MS (m/z): 535.7[M+1]$^+$.

2.6.9. 16-(4-nitro-benzylidene)-3β-piperidino-5α-androstan-17β-yl acetate (12c)

Yellow solid. Yield 90%. m.p. 190–192 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.23 (s, 3H, $OCOCH_3$), 2.52 (s, 4H, NCH_2), 5.39 (s, 1H, C_{17}-αH), 6.28 (d, 1H, =CH, J = 2.20 Hz), 7.49 (d, 2H, J = 8.84 Hz), 8.21 (d, 2H, J = 8.84 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.27, 12.38, 20.56, 21.11, 24.11, 24.86, 26.42, 28.72, 30.63, 31.28, 31.72, 34.84, 36.06, 37.92, 43.09, 45.99, 48.75, 50.35, 54.36, 64.44, 84.52, 121.88, 123.69, 128.65, 144.09, 145.81, 146.98, 170.97. Anal. Calcd. for $C_{33}H_{46}N_2O_4$: C 74.12, H 8.67, N 5.24; Found C 74.29, H 8.88, N 5.09. MS (m/z): 535.6[M+1]$^+$.

2.6.10. 16-(4-nitro-benzylidene)-3β-pyrrolidino-5α-androstan-17β-yl acetate (13c)

Yellow solid. Yield 90%. m.p. 143–145 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3-19), 0.83 (s, 3H, CH_3-18), 2.22 (s, 3H, $OCOCH_3$), 2.57 (br, 4H, NCH_2), 5.38 (s, 1H, C_{17}-αH), 6.28 (d, 1H, =CH, J = 2.20 Hz), 7.49 (d, 2H, J = 8.80 Hz), 8.20 (d, 2H, J = 8.76 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.25, 12.30, 20.49, 21.10, 23.15, 27.88, 28.62, 31.27, 31.71, 34.64, 34.82, 35.86, 37.34, 43.08, 45.18, 48.76, 51.77, 54.36, 64.23, 84.52, 121.89, 123.69, 128.66, 144.09, 145.80, 146.98, 171.00. Anal. Calcd. for $C_{32}H_{44}N_2O_4$: C 73.81, H 8.52, N 5.38; Found C 73.99, H 8.49, N 5.17. MS (m/z): 521.6[M+1]$^+$.

2.6.11. 16-(4-nitro-benzylidene)-3β-morpholino-5α-androstan-17β-yl acetate (14c)

Yellow solid. Yield 95%. m.p. 122–124 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3-19), 0.86 (s, 3H, CH_3-18), 2.23 (s, 3H, $OCOCH_3$), 2.42 (s, 4H, NCH_2), 3.72–3.74 (m, 4H, OCH_2), 5.39 (s, 1H, C_{17}-αH), 6.29 (d, 1H, =CH, J = 2.16 Hz), 7.50 (d, 2H, J = 8.80 Hz), 8.21 (d, 2H, J = 8.76 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.19, 12.29, 20.25, 21.13, 23.70, 28.37, 30.69, 31.28, 31.63, 32.80, 34.84, 36.36, 39.34, 43.12, 48.73, 50.86, 54.22, 59.36, 67.38, 84.59, 121.87, 123.71, 128.67, 144.12, 145.81, 147.06, 171.03. Anal. Calcd. for $C_{32}H_{44}N_2O_5$: C 71.61, H 8.26, N 5.22; Found C 71.48, H 8.44, N 5.06. MS (m/z): 537.6[M+1]$^+$.

2.6.12. 16-(4-nitro-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-yl acetate (15c)

Yellow solid. Yield 98%. m.p. 177–179 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-18), 2.21 (s, 3H, $OCOCH_3$), 2.28 (s, 3H, Ph-CH_3), 2.48–2.61 (m, 8H, NCH_2), 5.37 (s, 1H, C_{17}-αH), 6.27 (d, 1H, =CH, J = 2.08 Hz), 7.47 (d, 2H, J = 8.84 Hz), 8.18 (d, 2H, J = 8.84 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.22, 12.33, 20.52,

21.05, 24.45, 28.66, 31.17, 31.22, 31.65, 34.78, 35.93, 37.69, 43.04, 45.73, 45.95, 48.70, 49.19, 54.31, 55.41, 63.69, 84.44, 121.86, 123.64, 128.63, 144.03, 145.78, 146.92, 170.87. Anal. Calcd. for $C_{33}H_{47}N_3O_4$: C 72.10, H 8.62, N 7.64; Found C 72.31, H 8.47, N 7.49. MS (m/z): 550.6[M+1]$^+$.

2.6.13. 16-(3,4,5-trimethoxy-benzylidene)-3β-piperidino-5α-androstan-17β-yl acetate (12d)

White solid. Yield 91%. m.p. 161–163 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-18), 2.20 (s, 3H, $OCOCH_3$), 2.51 (br, 4H, NCH_2), 3.86 (s, 9H, OCH_3), 5.35 (s, 1H, C_{17}-αH), 6.13 (d, 1H, =CH, *J* = 2.20 Hz), 6.57 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.33, 12.39, 20.62, 21.24, 24.03, 24.82, 26.34, 28.78, 30.63, 30.93, 31.73, 34.84, 36.07, 36.50, 37.91, 42.98, 45.98, 48.87, 50.32, 54.36, 56.08, 60.92, 64.46, 84.60, 105.53, 123.58, 133.41, 136.80, 140.51, 152.95, 171.25. Anal. Calcd. for $C_{36}H_{53}NO_5$: C 74.57, H 9.21, N 2.42; Found C 74.66, H 9.08, N 2.19. MS (m/z): 580.6[M+1]$^+$.

2.6.14. 16-(3,4,5-trimethoxy-benzylidene)-3β-pyrrolidino-5α-androstan-17β-yl acetate (13d)

White solid. Yield 96%. m.p. 117–119 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.20 (s, 3H, $OCOCH_3$), 2.53 (br, 4H, NCH_2), 3.86 (s, 9H, OCH_3), 5.35 (s, 1H, C_{17}-αH), 6.13 (d, 1H, =CH, *J* = 2.20 Hz), 6.57 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.31, 14.19, 20.55, 21.22, 23.16, 27.93, 28.70, 30.91, 31.74, 34.70, 34.84, 35.88, 36.51, 37.35, 42.97, 45.20, 48.90, 51.81, 54.38, 56.09, 60.38, 60.90, 64.30, 84.59, 105.56, 123.59, 133.40, 136.84, 140.50, 152.96, 171.21. Anal. Calcd. for $C_{35}H_{51}NO_5$: C 74.30, H 9.09, N 2.48; Found C 74.08, H 8.89, N 2.57. MS (m/z): 566.7[M+1]$^+$.

2.6.15. 16-(3,4,5-trimethoxy-benzylidene)-3β-morpholino-5α-androstan-17β-yl acetate (14d)

White solid. Yield 93%. m.p. 176–178 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.80 (s, 3H, CH_3-18), 2.20 (s, 3H, $OCOCH_3$), 2.56 (s, 4H, NCH_2), 3.72–3.74 (m, 4H, OCH_2), 3.86 (s, 9H, OCH_3), 5.35 (s, 1H, C_{17}-αH), 6.13 (d, 1H, =CH, *J* = 2.12 Hz), 6.57 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.18, 12.31, 20.57, 21.17, 24.42, 28.72, 30.86, 31.08, 31.65, 34.78, 35.94, 36.45, 37.58, 42.92, 45.59, 46.14, 48.80, 50.01, 54.28, 56.02, 60.83, 63.99, 67.29, 84.50, 105.51, 123.57, 133.33, 136.79, 140.35, 152.91, 171.10. Anal. Calcd. for $C_{35}H_{51}NO_6$: C 72.26, H 8.84, N 2.41; Found C 72.09, H 8.77, N 2.56. MS (m/z): 582.6[M+1]$^+$.

2.6.16. 16-(3,4,5-trimethoxy-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-yl acetate (15d)

White solid. Yield 92%. m.p. 159–161 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-18), 2.20 (s, 3H, $OCOCH_3$), 2.28 (s, 3H, Ph-CH_3), 2.46–2.62 (m, 8H, NCH_2), 3.86 (s, 9H, OCH_3), 5.35 (s, 1H, C_{17}-αH), 6.13 (d, 1H, =CH, *J* = 2.12 Hz), 6.57 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.31, 12.34, 20.59, 21.19, 24.39, 28.73, 30.88, 31.17, 31.67, 34.82, 35.95, 37.69, 42.95, 45.74, 45.90, 48.84, 49.13, 54.32, 55.31, 56.07, 60.87, 63.74, 84.55, 105.58, 123.58, 133.37, 136.85, 140.42, 152.94, 171.17. Anal. Calcd. for $C_{36}H_{54}N_2O_5$: C 72.69, H 9.15, N 4.71; Found C 72.55, H 9.37, N 4.51. MS (m/z): 595.7[M+1]$^+$.

2.6.17. 16-(4-chloro-benzylidene)-3β-piperidino-5α-androstan-17β-yl acetate (12e)

White solid. Yield 92%. m.p. 99–101 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.80 (s, 3H, CH_3-18), 2.21 (s, 3H, $OCOCH_3$), 2.51 (s, 4H, NCH_2), 5.35 (s, 1H, C_{17}-αH), 6.16 (d, 1H, =CH, *J* = 2.28 Hz), 7.28 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.62, 12.28, 12.40, 20.61, 21.18, 24.17, 24.89, 26.47, 28.79, 30.69, 30.95, 31.74, 34.87, 36.08, 37.95, 43.00, 46.02, 46.24, 48.83, 50.39, 54.39, 64.51, 84.56, 122.37, 128.43, 129.46, 132.03, 136.08, 141.96, 171.13. Anal. Calcd. for $C_{33}H_{46}ClNO_2$: C 75.61, H 8.85, N 2.67; Found C 75.44, H 8.67, N 2.59. MS (m/z): 524.5[M+1]$^+$.

2.6.18. 16-(4-chloro-benzylidene)-3β-pyrrolidino-5α-androstan-17β-yl acetate (13e)

White solid. Yield 95%. m.p. 185–187 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, CH_3-19), 0.74 (s, 3H, CH_3-18), 2.12 (s, 3H, $OCOCH_3$), 2.48 (br, 4H, NCH_2), 5.27 (s, 1H, C_{17}-αH), 6.07 (d, 1H, =CH, *J* = 2.36 Hz), 7.20 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.27, 12.32, 20.54, 21.18, 23.18, 28.01, 28.70, 30.95, 31.74, 34.77, 34.86, 35.88, 36.49, 37.37, 42.99, 45.21, 48.84, 51.86, 54.39, 64.33, 84.55, 122.36, 128.43, 129.47, 132.02, 136.08, 141.96, 171.12. Anal. Calcd. for $C_{32}H_{44}ClNO_2$: C 75.34, H 8.69, N 2.75; Found C 75.09, H 8.51, N 2.58. MS (m/z): 510.4[M+1]$^+$.

2.6.19. 16-(4-chloro-benzylidene)-3β-morpholino-5α-androstan-17β-yl acetate (14e)

White solid. Yield 95%. m.p. 86–88 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.21 (s, 3H, $OCOCH_3$), 2.58 (s, 4H, NCH_2), 3.73–3.75 (m, 4H, OCH_2), 5.35 (s, 1H, C_{17}-αH), 6.16 (d, 1H, =CH, *J* = 2.28 Hz), 7.29 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.58, 12.27, 12.36, 20.60, 21.17, 24.50, 28.76, 30.93, 31.10, 31.70, 34.85, 35.99, 37.64, 42.99, 45.66, 46.21, 48.81, 50.06, 54.34, 64.07, 67.36, 84.52, 122.40, 128.43, 129.47, 132.04, 136.06, 141.89, 171.12. Anal. Calcd. for $C_{32}H_{44}ClNO_3$: C 73.05, H 8.43, N 2.66; Found C 73.33, H 8.51, N 2.47. MS (m/z): 526.6[M+1]$^+$.

2.6.20. 16-(4-chloro-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-yl acetate (15e)

White solid. Yield 95%. m.p. 124–126 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-18), 2.19 (s, 3H, $OCOCH_3$), 2.27 (s, 3H, Ph-CH_3), 2.46–2.61 (m, 8H, NCH_2), 5.35 (s, 1H, C_{17}-αH), 6.14 (d, 1H, =CH, *J* = 2.24 Hz), 7.28 (s, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.25, 12.36, 20.58, 21.14, 24.50, 28.74, 30.91, 31.23, 31.68, 34.82, 35.95, 37.72, 42.96, 45.76, 46.01, 48.78, 49.26, 54.33, 55.48, 63.75, 84.49, 122.35, 128.41, 129.45, 132.00, 136.06, 141.89, 171.02. Anal. Calcd. for $C_{33}H_{47}ClN_2O_2$: C 73.51, H 8.79, N 5.20; Found C 73.42, H 8.66, N 5.03. MS (m/z): 539.6[M+1]$^+$.

2.6.21. 16-(4-bromo-benzylidene)-3β-piperidino-5α-androstan-17β-yl acetate (12f)

White solid. Yield 94%. m.p. 157–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H, CH_3-19), 0.71 (s, 3H, CH_3-18), 2.12 (s, 3H, $OCOCH_3$), 2.45 (s, 4H, NCH_2), 5.25 (s, 1H, C_{17}-αH), 6.05 (d, 1H, =CH, *J* = 2.00 Hz), 7.14 (d, 2H, *J* = 8.44 Hz), 7.37 (d, 2H, *J* = 8.48 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.28, 12.39, 20.61, 21.19, 23.99, 24.75, 26.21, 28.76, 30.53, 30.96, 31.71, 34.85, 36.05, 37.88, 42.99, 45.96, 48.80, 50.30, 54.34, 64.50, 84.55, 120.20, 122.42, 129.80, 131.38, 136.51, 142.17, 171.12. Anal. Calcd. for $C_{33}H_{46}BrNO_2$: C 69.70, H 8.15, N 2.46; Found C 69.55, H 8.31, N 2.29. MS (m/z): 569.6[M+1]$^+$.

2.6.22. 16-(4-bromo-benzylidene)-3β-pyrrolidino-5α-androstan-17β-yl acetate (13f)

White solid. Yield 94%. m.p. 184–186 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (s, 3H, CH_3-19), 0.82 (s, 3H, CH_3-18), 2.21 (s, 3H, $OCOCH_3$), 2.57 (br, 4H, NCH_2), 5.34 (s, 1H, C_{17}-αH), 6.14 (d, 1H, =CH, *J* = 2.04 Hz), 7.22 (d, 2H, *J* = 8.44 Hz), 7.45 (d, 2H, *J* = 8.48 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.26, 12.32, 20.54, 21.17, 23.17, 27.93, 28.69, 30.96, 31.72, 34.69, 34.84, 35.87, 36.47, 37.35, 42.98, 45.20, 48.82, 51.82, 54.37, 64.31, 84.54, 120.19, 122.40, 129.80, 131.37, 136.50, 142.17, 171.08. Anal. Calcd. for $C_{32}H_{44}BrNO_2$: C 69.30, H 8.00, N 2.53; Found C 69.06, H 7.83, N 2.41. MS (m/z): 554.5[M+1]$^+$.

2.6.23. 16-(4-bromo-benzylidene)-3β-morpholino-5α-androstan-17β-yl acetate (14f)

White solid. Yield 90%. m.p. 184–186 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.21 (s, 3H, $OCOCH_3$), 2.58 (s, 4H, NCH_2), 3.72–3.76 (m, 4H, OCH_2), 5.35 (s, 1H, C_{17}-αH), 6.15 (d, 1H, =CH, *J* = 2.04 Hz), 7.23 (d, 2H, *J* = 8.44 Hz), 7.46 (d, 2H, *J* = 8.48 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.26, 12.37, 20.65, 21.21, 23.97, 24.68, 26.22, 28.77, 30.55, 30.98, 31.70, 34.83, 36.03, 37.90, 42.92, 45.95, 48.84, 50.31, 54.35, 64.53, 84.60, 120.24, 122.48, 129.81, 131.37, 136.53, 142.20, 171.09. Anal. Calcd. for $C_{32}H_{44}BrNO_3$: C 67.36, H 7.77, N 2.45; Found C 67.21, H 7.59, N 2.28. MS (m/z): 571.6[M+1]$^+$.

2.6.24. 16-(4-bromo-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-yl acetate (15f)

White solid. Yield 98%. m.p. 172–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.74 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-18), 2.19 (s, 3H, $OCOCH_3$), 2.27 (s, 3H, Ph-CH_3), 2.46–2.61 (m, 8H, NCH_2), 5.32 (s, 1H, C_{17}-αH), 6.13 (d, 1H, =CH, *J* = 2.16 Hz), 7.20 (d, 2H, *J* = 8.52 Hz), 7.43 (d, 2H, *J* = 8.44 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.26, 12.37, 20.59, 21.15, 24.52, 28.75, 30.93, 31.25, 31.69, 34.84, 35.97, 37.74, 42.97, 45.78, 46.04, 48.79, 49.30, 54.35, 55.52, 63.77, 84.52, 120.18, 122.42, 129.79, 131.36, 136.50, 142.14, 171.04. Anal. Calcd. for $C_{33}H_{47}BrN_2O_2$: C 67.91, H 8.12, N 4.80; Found C 67.88, H 8.29, N 4.61. MS (m/z): 583.6[M+1]$^+$.

2.6.25. 16-(4-fluoro-benzylidene)-3β-piperidino-5α-androstan-17β-yl acetate (12g)

White solid. Yield 90%. m.p. 165–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, CH_3-19), 0.71 (s, 3H, CH_3-18), 2.12 (s, 3H, $OCOCH_3$), 2.47 (s, 4H, NCH_2), 5.26 (s, 1H, C_{17}-αH), 6.08 (d, 1H, =CH, *J* = 1.96 Hz), 6.93 (t, 2H), 7.23 (q, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 11.35, 12.26, 12.37, 20.61, 21.16, 23.96, 24.75, 26.20, 28.76, 30.51, 30.83, 31.70, 34.85, 36.03, 37.87, 42.97, 45.95, 46.07, 48.82, 50.25, 54.35, 64.44, 84.52, 115.04, 115.25, 122.38, 129.71, 129.79, 133.74, 133.77, 140.63, 160.07, 162.52, 171.11. Anal. Calcd. for $C_{33}H_{46}FNO_2$: C 78.06, H 9.13, N 2.76; Found C 78.31, H 9.01, N 2.55. MS (m/z): 508.5[M+1]$^+$.

2.6.26. 16-(4-fluoro-benzylidene)-3β-pyrrolidino-5α-androstan-17β-yl acetate (13g)

White solid. Yield 93%. m.p. 107–109 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, CH_3-19), 0.75 (s, 3H, CH_3-18), 2.12 (s, 3H, $OCOCH_3$), 2.72 (br, 4H, NCH_2), 5.26 (s, 1H, C_{17}-αH), 6.08 (d, 1H, =CH, *J* = 2.00 Hz), 6.93 (t, 2H), 7.23 (q, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 10.32, 12.21, 12.26, 20.56, 21.18, 23.23, 26.36, 28.54, 30.81, 31.63, 33.04, 34.80, 35.79, 36.45, 37.13, 42.96, 45.20, 48.82, 51.13, 54.22, 64.07, 84.51, 115.05, 115.26, 122.43, 129.71, 129.79, 133.72, 133.75, 140.58, 160.08, 162.54, 171.20. Anal. Calcd. for $C_{32}H_{44}FNO_2$: C 77.85, H 8.98, N 2.84; Found C 77.69, H 8.79, N 2.77. MS (m/z): 494.5[M+1]$^+$.

2.6.27. 16-(4-fluoro-benzylidene)-3β-morpholino-5α-androstan-17β-yl acetate (14g)

White solid. Yield 94%. m.p. 141–143 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (s, 3H, CH_3-19), 0.72 (s, 3H, CH_3-18), 2.12 (s, 3H, $OCOCH_3$), 2.57 (s, 4H, NCH_2), 3.68–3.71 (m, 4H, OCH_2), 5.27 (s, 1H, C_{17}-αH), 6.08 (d, 1H, =CH, *J* = 2.00 Hz), 6.93 (t, 2H), 7.23 (q, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.28, 20.60, 21.18, 23.80, 28.68, 30.39, 30.81, 31.64, 34.83, 35.92, 37.47, 42.97, 45.56, 48.81, 49.55, 54.25, 64.15, 66.61, 84.51, 115.06, 115.27, 122.45, 129.72, 129.79, 133.72, 133.76, 140.54, 160.10, 162.55, 171.18. Anal. Calcd. for $C_{32}H_{44}FNO_3$: C 75.41, H 8.70, N 2.75; Found C 75.27, H 8.58, N 2.91. MS (m/z): 510.4[M+1]$^+$.

2.6.28. 16-(4-fluoro-benzylidene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-yl acetate (15g)

White solid. Yield 94%. m.p. 178–180 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-18), 2.19 (s, 3H, $OCOCH_3$), 2.27 (s, 3H, Ph-CH_3), 2.46–2.61 (m, 8H, NCH_2), 5.34 (s, 1H, C_{17}-αH), 6.15 (d, 1H, =CH, *J* = 2.00 Hz), 7.00 (t, 2H), 7.30 (q, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.24, 12.35, 20.58, 21.12, 24.52, 28.74, 30.79, 31.25, 31.68, 34.83, 35.94, 37.72, 42.94, 45.76, 46.03, 48.80, 49.28, 54.33, 55.51, 63.74, 84.47, 115.01, 115.23, 122.37, 129.69, 129.77, 133.72, 133.75, 140.58, 160.04, 162.49, 171.00. Anal. Calcd. for $C_{33}H_{47}FN_2O_2$: C 75.82, H 9.06, N 5.36; Found C 75.69, H 8.89, N 5.18. MS (m/z): 523.5[M+1]$^+$.

2.6.29. 16-(furan-2-ylmethylene)-3β-piperidino-5α-androstan-17β-yl acetate (12h)

White solid. Yield 94%. m.p. 184–186 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.74 (s, 3H, CH_3-19), 0.80 (s, 3H, CH_3-18), 2.19 (s, 3H, $OCOCH_3$), 2.53 (s, 4H, NCH_2), 5.34 (s, 1H, C_{17}-αH), 6.13 (d, 1H, =CH, *J* = 2.32 Hz), 6.22 (d, 1H, *J* = 2.32 Hz), 6.41 (t, 1H), 7.38 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.24, 12.41, 20.65, 21.13, 24.13, 24.88, 26.45, 28.83, 30.71, 31.08, 31.74, 34.89, 36.08, 37.96, 43.36, 46.05, 48.64, 50.37, 54.43, 64.52, 84.27, 107.83, 111.26, 112.16, 140.01, 141.29, 153.42, 171.08. Anal. Calcd. for $C_{31}H_{45}NO_3$: C 77.62, H 9.46, N 2.92; Found C 77.47, H 9.33, N 3.09. MS (m/z): 480.4[M+1]$^+$.

2.6.30. 16-(furan-2-ylmethylene)-3β-pyrrolidino-5α-androstan-17β-yl acetate (13h)

White solid. Yield 94%. m.p. 155–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.74 (s, 3H, CH_3-19), 0.83 (s, 3H, CH_3-18), 2.19 (s, 3H, $OCOCH_3$), 2.57 (br, 4H, NCH_2), 5.35 (s, 1H, C_{17}-αH), 6.13 (d, 1H, =CH, *J* = 2.26 Hz), 6.22 (d, 1H, *J* = 2.30 Hz), 6.41 (t, 1H), 7.38 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.22, 12.32, 20.57, 21.12, 23.17, 27.97, 28.73, 31.06, 31.72, 34.75, 34.86, 35.87, 36.51, 37.37, 43.34, 45.24, 48.65, 51.82, 54.42, 64.32, 84.24, 107.82, 111.24, 112.14, 139.96, 141.26, 153.39, 171.03. Anal. Calcd. for $C_{30}H_{43}NO_3$: C 77.38, H 9.31, N 3.01; Found C 77.51, H 9.09, N 3.12. MS (m/z): 466.4[M+1]$^+$.

2.6.31. 16-(furan-2-ylmethylene)-3β-morpholino-5α-androstan-17β-yl acetate (14h)

White solid. Yield 94%. m.p. 194–196 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.74 (s, 3H, CH_3-19), 0.81 (s, 3H, CH_3-18), 2.19 (s, 3H, $OCOCH_3$), 2.58 (s, 4H, NCH_2), 3.70–3.73 (m, 4H, OCH_2), 5.35 (s, 1H, C_{17}-αH), 6.13 (d, 1H, =CH, *J* = 2.36 Hz), 6.22 (d, 1H, *J* = 3.32 Hz), 6.41 (t, 1H), 7.38 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.23, 12.37, 20.64, 21.13, 24.50, 28.80, 31.06, 31.12, 31.70, 34.86, 36.00, 37.65, 43.35, 45.69, 48.62, 50.06, 54.38, 64.08, 67.31, 84.23, 107.85, 111.26, 112.17, 139.91, 141.30, 153.39, 171.07. Anal. Calcd. for $C_{30}H_{43}NO_4$: C 74.81, H 9.00, N 2.91; Found C 74.66, H 8.83, N 3.12. MS (m/z): 482.4[M+1]$^+$.

2.6.32. 16-(furan-2-ylmethylene)-3β-(4-methylpiperazinyl)-5α-androstan-17β-yl acetate (15h)

White solid. Yield 94%. m.p. 145–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72 (s, 3H, CH_3-19), 0.79 (s, 3H, CH_3-18), 2.17 (s, 3H, $OCOCH_3$), 2.28 (s, 3H, Ph-CH_3), 2.46–2.61 (m, 8H, NCH_2), 5.32 (s, 1H, C_{17}-αH), 6.11 (d, 1H, =CH, *J* = 2.28 Hz), 6.20 (d, 1H, *J* = 3.28 Hz), 6.38 (t, 1H), 7.36 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.18, 12.34, 20.58, 21.02, 24.49, 28.76, 31.00, 31.26, 31.64, 34.80, 35.91, 37.70, 43.27, 45.74, 46.02, 48.56, 49.26, 54.34, 55.51, 63.71, 84.12, 107.80, 111.21, 112.14, 139.83, 141.21, 153.33, 170.81. Anal. Calcd. for $C_{31}H_{46}N_2O_3$: C 75.26, H 9.37, N 5.66; Found C 75.08, H 9.51, N 5.44. MS (m/z): 495.4[M+1]$^+$.

2.7. Biology

2.7.1. Cell lines and culture conditions

Human cancer cell lines SW480 [25], A549 [26], HepG2 [27] and HeLa [28,29] used in this work, were purchased from China Centre for Type Culture Collection (Wuhan, China). SW480, A549 and HepG2 cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 10 U penicillin and 100 μg/ml streptomycin at 37 °C with 5% CO_2 in a humidified atmosphere. HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplanted with FCS and aitibiotics as described above for RPMI 1640. Fresh medium was given every second day and on the day before the experiments were done. Cells were passaged at preconfluent densities, using a solution containing 0.05% trypsin and 0.5 mM EDTA.

2.7.2. Cell viability assay (MTT)

The anticancer activity in vitro was measured using the MTT assay. The assay was carried out according to previous study [30,31]. Exponentially growing cells were harvested and plated in 96-well plates at a concentration of 1×10^4 cells/well. After 24 h incubation at 37 °C under a humidified 5% CO_2 to allow cell attachment, the cells in the wells were respectively treated with target compounds at various concentrations for 48 h. The concentration of DMSO was always kept below 1.25%, which was found to be non-toxic to the cells. A solution of 3-(4,5-dimethylthizao1-2-y1)-2,5-diphenyltetrazolium bromide (MTT), was prepared at 5 mg/ml in phosphate buffered saline (PBS; 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mM NaCI, 2.7 mM KCI; pH 7.4). Of this solution 20 μl was added to each well. After incubation for 4 h at 37 °C in a humidified incubator with 5% CO_2, the medium/MTT mixtures were removed, and the formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in 100 μl of DMSO per well. The absorbance of the wells was read with a microplate reader (Bio-Rad Instruments) at 570 nm. Effects of the drug cell viability were calculated using cell treated with DMSO as control.

2.7.3. Data analysis

Cell survival was calculated using the formula: Survival (%) = [(absorbance of treated cells − absorbance of culture medium)/(absorbance of untreated cells − absorbance of culture medium)] × 100 [32,33]. The experiment was done in triplicate and the inhibitory concentration (IC) values were calculated from a dose response curve. IC_{50} is the concentration in μM required for 50% inhibition of cell growth as compared to that of untreated control. IC_{50} values were determined from the linear portion of the curve by calculating the concentration of agent that reduced absorbance in treated cells, compared to control cells, by 50%. Evaluation is based on mean values from three independent experiments, each comprising at least six microcultures per concentration level.

3. Results and discussion

3.1. Chemistry

We obtained an X-ray crystal structure of 8e that is presented in Fig. 2. The configuration at C-16 has been assigned E which was same with the earlier report [34,35]. This structure confirms the β-stereochemistry of the heterocycles at C-3 and the hydroxyl group at C-17. After the acetylation of 17β-hydroxy derivatives, the β configuration of acetoxy group at C-17 was maintained. Crystallographic data for 8e have been deposited with the Cambridge Crystallographic Data Centre with the deposition number CCDC 796339. These data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax (+44) 1223 336033, e-mail: deposit@ccdc.cam.ac.uk].

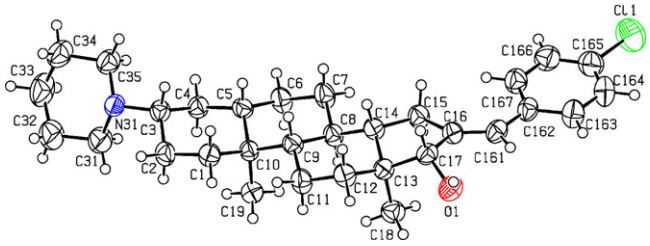


Fig. 2. X-ray crystal structure of 8e.

3.2. Cytotoxic activity

The newly synthesized compounds 12a–h, 13a–h, 14a–h, 15a–h were evaluated for their anticancer activity towards human tumor cell lines derived from various human cancer types: SW480 (human colon adenocarcinoma cells), HeLa (human cervical cancer cells), A549 (human lung carcinoma cells), HepG2 (human hepatic carcinoma cells). In vitro evaluation of anticancer activity of the synthesized compounds was carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Anticancer potency of the compounds was indicated by IC_{50} values that were calculated by linear regression analysis of the concentration–response curves obtained for each compound. Data were reported in Table 1.

Table 1
Cytotoxic activity data for compounds 12a–h, 13a–h, 14a–h, 15a–h against SW480, A549, HepG2, and HeLa.

Compounds	IC_{50} (μmol l^{-1})[a]			
	SW480	A549	HepG2	HeLa
12a	21.68	6.18	12.33	20.21
13a	15.54	13.88	22.47	28.51
14a	40.56	38.97	>50	>50
15a	>50	>50	>50	40.58
12b	12.86	9.03	6.54	17.26
13b	30.18	17.76	10.55	23.89
14b	>50	>50	39.48	>50
15b	>50	>50	>50	>50
12c	>50	>50	38.21	>50
13c	>50	>50	>50	>50
14c	>50	>50	>50	>50
15c	>50	>50	>50	>50
12d	>50	11.56	>50	23.10
13d	41.73	49.18	>50	19.89
14d	31.42	>50	13.37	44.51
15d	>50	>50	>50	>50
12e	>50	>50	38.77	>50
13e	>50	>50	>50	>50
14e	>50	>50	>50	>50
15e	>50	>50	>50	>50
12f	21.58	>50	>50	41.03
13f	>50	>50	38.83	29.86
14f	>50	>50	>50	>50
15f	>50	>50	>50	>50
12g	17.92	4.07	6.78	35.46
13g	6.45	6.14	23.61	19.20
14g	11.71	16.58	33.54	37.52
15g	>50	>50	>50	>50
12h	21.19	13.83	30.79	15.53
13h	>50	23.18	15.52	30.19
14h	>50	27.23	37.71	42.41
15h	>50	>50	>50	>50

Exponentially growing cells were treated with the compounds at different concentrations for 48 h. Cell-growth inhibition was analysed by the MTT assay.

[a] IC_{50} is the concentration of compound that inhibits 50% of cell growth.

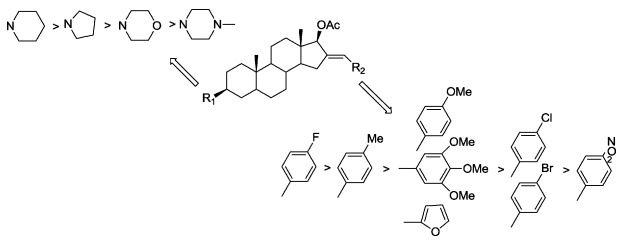


Fig. 3. General SAR of compounds for anticancer activity in vitro based on the IC_{50} results on the four cancer cells lines.

As shown in Table 1, compounds 12–15a, 12–14b, 12–14d, 12–13e, 12–13f, 12–15g, 12–15h exhibited different levels of anticancer properties; namely, the introduction of methyl and methoxy at the para position of the phenyl ring lead to potent anticancer activity. For A549, among the 12–15a, 12–15b bearing methyl and methoxy group, the IC_{50} values obtained are 6.18 μM, 13.88 μM, 38.97 μM, >50 μM, 9.03 μM, 17.76 μM, >50 μM and >50 μM, respectively. It was noteworthy that compound 12b which has methoxy at the 4-position of the phenyl ring exhibited potent activity (12b for A549 9.03 μM, for HepG2 6.54 μM). Compared to 12–15b, the inclusion of more methoxy groups (12–14d) to the phenyl ring enabled maintenance of anticancer activity. On the other hand, substitution with a strong electron-withdrawing group like nitro (12–15c) at the para position of the phenyl ring resulted in a complete loss of anticancer activity (IC_{50} > 50 μM). Comparison of the results obtained from p-methyl, p-methoxy, and p-nitro derivatives indicated that the electron-donating groups were relatively beneficial for anticancer activity. However, there was an exception, the IC_{50} value of compound 12c against HepG2 was 38.21 μM. When the alkyl groups were replaced by a halogen (12–15e, 12–15f) such as chlorine or bromine at the para position of the phenyl ring, decreased cytotoxicity was observed (>50 μM). Among the tested compounds, it was determined that 12-15 g exhibited potent activity against all four cancer cell lines. The p-fluorophenyl derivative 12g exhibited remarkable anticancer activity against A549 (4.07 μM) and HepG2 (6.78 μM). When the phenyl rings were replaced by a furan ring, 12–15h exhibited moderate activity against the A549 from 13.83 to 27.23 μM, suggesting that the phenyl ring at the C-16 position may be unnecessary for the activity. Furthermore, it appears that an appropriately substituted group of the phenyl ring at C-16 could serve as a promising launch point for the further design of this type of steroidal anticancer agent. A similar tendency was observed concerning the SW480, HepG2, and HeLa cell lines.

Most derivatives with a saturated heterocyclic ring moiety at C-3 position resulted in high activity, especially piperidine. For HepG2, as demonstrated in Table 1, the most highly active compound was 12g with a piperidine moiety, having a IC_{50} value of 6.78 μM, followed by 13g with a pyrrolidine moiety, exhibiting a IC_{50} value of 23.61 μM, the derivative 14g with a morpholine moiety, which exhibited a lower activity (33.54 μM), and finally the derivative 15g with a methyl piperazine moiety exhibited no activity (>50 μM). General SAR of compounds for anticancer activity in vitro based on the IC_{50} results on the four cancer cells lines is presened schematically in Fig. 3.

In the present study, 32 novel 16E-arylidene-3β-aminosteroid derivatives were synthesized and evaluated for their anticancer activity against four human cancer cell lines (SW480, A549, HepG2, and HeLa) in vitro. Most of the compounds showed moderate to good anticancer activity. Among the target derivatives, compounds 12a, 12b, 12g and 13g were found to have significant activity against all four cancer cell lines. In particular, compounds 12b and 12g were the most promising derivatives.

In conclusion, we have described a facile synthesis of novel promising anti-cancer steroid derivatives. This study may provide valuable information for the further design and development of more potent anticancer agents.

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