Steroids 76 (2011) 1491–1504

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Synthesis of 2-substituted 17β-hydroxy/17-methylene estratrienes and their *in vitro* cytotoxicity in human cancer cell cultures

Ganapathy Panchapakesan [a,b], Vasudevan Dhayalan [a], Nachiappan Dhatchana Moorthy [b], Nidhyanandan Saranya [b], Arasambattu K. Mohanakrishnan [a,*]

[a] Department of Organic Chemistry, University of Madras, Guindy Campus, Chennai 600 025, India
[b] Research and Development Centre, Orchid Chemicals and Pharmaceuticals Ltd., 476/14 Old Mahabalipuram Road, Sholinganallur, Chennai 600 119, India

ARTICLE INFO

Article history:
Received 4 March 2011
Received in revised form 8 August 2011
Accepted 11 August 2011
Available online 22 August 2011

Keywords:
2-Methoxyestradiol
2-Substituted 17β-estradiols
2-Substituted 17-methylene estratrienes
In vitro cytotoxicity

ABSTRACT

Synthesis of various types of 2-(alkylaminomethyl) and 2-(aroyl) 17β-estradiol analogs are reported. The synthesis of similar types of 2-substituted 17-methylene estratriene analogs was also achieved. Synthesis of chalcone derivatives of 17β-estradiol and 17-methylene estratriene were also realized. All these 2-substituted estratrienes were tested for their antiproliferative activity by using four different cell lines from colon, lung, glioma and breast cancers. Among the various 2-substituted estratrienes, the compounds **10d**, **14a–h** and **17e** were found to have *in vitro* antiproliferative activity comparable to that of parent analogs **1–4**. Comparison of the SAR pattern of these 2-susbtituted estratriene derivatives confirmed that relatively, 17-methylene estratrienes are more active than that of 17β-estradiol analogs.

1. Introduction

Interest in the synthesis of various analogs of 2-methoxyestradiol have been stimulated by their cytotoxicity in cancer cell cultures [1]. During the last ten years, various analogs of 2-methoxyestradiol have been synthesized and studied for their antiproliferative activity against human cancer cells. It has been established that the anticancer activities of 2-substituted estratriene derivatives are independent of the estrogen receptors. These compounds are indeed thought to bind the colchicine binding-site of tubulin [2]. Based on the cytotoxicity profile displayed by a number of 2-substituted estradiol analogs, Cushman and coworkers established that the steric and electronic factors at the 2-position of estradiol are prominent contributor to the observed cytotoxicity, as well as the antitubulin activity [3].

The same group also carried out the synthesis of B-ring homologated analogs of estradiol, some of which resembled paclitaxel (Taxol) in their ability to inhibit tubulin polymerization [4]. Macdonald et al. synthesized a series of 2-methoxy estradiol analogs in which the A-ring of the steroid was replaced by substituted tropone rings [5]. In particular, Rao et al. have carried out extensive work on the synthesis and antimitotic activity of D-ring modified 2-methoxyestradiol analogs [6]. It has been shown that 2-methoxyestradiol is metabolized rapidly into inactive 2-methoxyestrone by 17β-hydroxy steroid dehydrogenase type II [7]. To enhance further the oral bio-availability of 2-methoxyestradiol, several 17β-hydroxy modified derivatives have been synthesized. The combination of inactivating cellular metabolism with rapid conjugative inactivation through the reaction of hydroxyl group emphasizes the need for 2-substituted estradiol analogs with improved pharmacokinetic profiles, as well as potency. The structure–activity relationship of several C-17-cyano substituted estratrienes has been reported [8]. Several of these compounds displayed high activity against the proliferation of cancer cells *in vitro*. Oral bioavailability of 2-substituted estratriene-3-sulfamate compounds was also confirmed [9]. Treston and coworkers recently reported the synthesis and antiproliferative structure–activity relationship of 2- and 17-substituted estrone analogs [10]. Liou et al. explored a wide variety of benzophenone derivatives and aroylindoles as potential antimitotic agents [11]. Recently, Boumendjel and coworkers reported antimitotic and antiproliferative activities of chalcones [12]. Hence, the synthesis of similar type of estradiol analogs possessing aroyl as well as chalcone units at the 2-position was planned. It is expected that the most useful analogs of 2-methoxyestradiol should retain its inhibitory effects on tubulin polymerization and cell growth, while addressing the metabolic stability of 17β-hydroxy unit. Some of the simplest 2-substituted estratrienes **1–4** that showed high cytotoxicity against human cancer cell lines are listed in Fig. 1. It should be noted that almost all these 2-substituted estratrienes also exhibited tubulin polymerase inhibitory activity.

* Corresponding author. Tel.: +91 44 22202813; fax: +91 44 22300488.
E-mail addresses: mohanakrishnan@unom.ac.in, mohan_67@hotmail.com (A.K. Mohanakrishnan).

0039-128X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2011.08.004

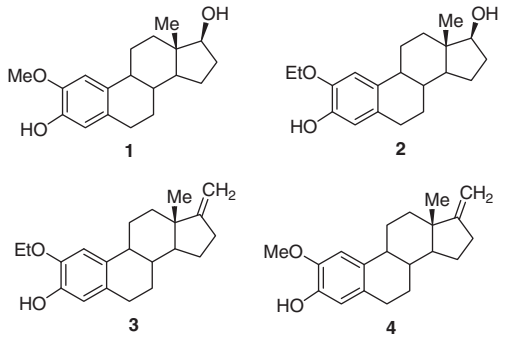


Fig. 1. Structures of representative 2-substituted estratrienes **1**–**4**.

2. Experimental

2.1. Proliferation assays

Human colon carcinoma HCT-116 cells, NCI-H460 non-small cell lung cancer cells, U251 glioblastoma cells, and MDA MB-435 breast cancer cells were purchased from the American Type Culture Collection. They were subcultured as per the instructions provided in the product information sheet. Cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% heat-inactivated fetal bovine serum (FBS, GIBCO), 100 units/ml penicillin and 100 μg/ml streptomycin (GIBCO). Cells were cultured at 37 °C in a humidified 5% CO_2 atmosphere and were passaged three times weekly. Cells were passaged by first washing with sterile PBS and then incubating with trypsin-EDTA. The resulting single-cell suspension was resuspended in fresh DMEM medium (complete), counted, and seeded at 3×10^3 cells/well in 96-well plates. Growth inhibition assays were performed in the presence of different drug concentrations ranging from 100 μM to 0.01 μM, in a logarithmic scale. After 48 h, at the end of the incubation, a Sulphorhodamine-B (SRB) assay [13,14] was performed to determine the cellular growth and viability. Briefly, cells attached to the bottom of the plate were fixed by addition of cold trichloroacetic acid (TCA, 4 °C) on the top of the growth medium (final TCA 10% w/v). The plate was placed at 4 °C for 1 h before being gently washed five times with tap water. It was allowed to dry in air, then 100 μl of 0.057% w/v SRB dissolved in 1% acetic acid in water was added to each well for 30 min. At the end of the staining period, unbound SRB was removed by washing three times with 1% acetic acid. The plate was air dried again, and 200 μl of 10 mM aqueous Tris base [tris (hydroxymethyl) aminomethane] was added into each well to solubilize the cell-bound dye. The plate was shaken for 15 min on a gyratory shaker followed by reading the optical density (OD) at 530 nm in a microplate spectrophotometer (Spectramax Plus, Molecular devices, Softmax Pro Ver. 5.2).

2.2. General

All melting points were uncorrected. Reagents were purchased from commercial sources and used as received without purification. Solvents were dried by standard procedures. Column chromatography was carried on silica gel (grade 60, mesh size 230–400, Merck). ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using TMS as an internal standard on a Bruker 300 MHz spectrometer. Chemical shift values were quoted in ppm and coupling constants were quoted in Hz. Chemical shift multiplicities were reported as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Mass spectra were recorded on an Agilent MSD1 SPC spectrometer. Elemental analyses were carried out on Perkin–Elmer series II 2400 equipment.

2.2.1. Synthesis of 2-formylestradiol (**6**) [15]

Interaction of freshly prepared ethylmagnesium bromide (2.1 g Mg, 13.1 g bromoethane) with estradiol (4 g, 14.7 mmol) followed by addition of paraformaldehyde (7 g, 233 mmol) and subsequent work up of the reaction led to the isolation of crude 2-formyl estradiol as light yellow viscous oil. The crude product upon trituration with diisopropyl ether furnished 2-formyl estradiol **6** (3 g, 70%) as light yellow solid. mp 219–221 °C; IR (KBr, cm^{-1}): 1675 (C=O).

2.2.2. Representative procedure for preparation of 2-(2′-N,N-dimethylethylenediamino)methyl estradiol **7a** (Procedure A)

To a solution of 2-formyl estradiol **6** (1 g, 3.33 mmol) in DMF (15 mL) at 5 °C was added sodium sulphate (2 g) followed by *N*,*N*-dimethylethylenediamine (0.59 g, 6.66 mmol) in DMF (5 mL), and the mixture was stirred for 1 h. To the resulting yellow suspension was added sodium borohydride (0.5 g, 13.2 mmol) followed by slow addition of methanol (2 mL) during 30 min. The reaction mixture was further stirred for 1 h at 5 °C. It was then poured in to water (40 mL) and washed with ethyl acetate (2×75 mL). The combined organics were washed with brine (2×40 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give light yellowish oil. The crude product was purified by silica gel column chromatography using (80:20 ethyl acetate/methanol) as an eluent to yield **7a** as yellow viscous oil (0.84 g, 60%); IR (KBr, cm^{-1}): 3402 (OH and NH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.13 (s, 1H), 6.93 (s, 1H), 6.36 (s, 1H), 3.75 (s, 2H), 2.68–2.67 (d, J = 3.6 Hz, 2H), 2.55–2.54 (d, J = 6.2 Hz, 1H), 2.33–2.30 (t, J = 6.3 Hz, 2H), 2.18–2.17 (d, J = 3.3 Hz, 1H), 2.14 (s, 1H), 2.11 (s, 6H), 1.35 (m, 2H), 1.28–1.04 (m, 13H), 0.65 (s, 3H); *m*/*z* (relative intensity) 373 (MH^+, 100%); Anal. Calcd. for ($C_{23}H_{36}N_2O_2$): C, 74.15; H, 9.74; N, 7.52. Found: C, 74.01; H, 9.98; N, 7.30%.

2.2.3. 2-(3′-N,N-Dimethylpropylenediamino)methyl estradiol (**7b**)

Condensation of 2-formyl estradiol **6** (1 g, 3.33 mmol) with *N*,*N*-dimethylpropylenediamine (0.68 g, 6.66 mmol) followed by sodium borohydride (0.5 g, 13.2 mmol) reduction using the above-mentioned procedure A and subsequent column chromatography purification using (60:40 ethyl acetate/methanol) as an eluent afforded compound **7b** as a light brown viscous liquid (810 mg, 63%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.13 (s, 1H), 7.01 (s, 1H), 6.34 (s, 1H), 3.78 (s, 2H), 2.67 (s, 2 H), 2.22–2.18 (m, 3H), 2.08–2.03 (m, 8H), 1.82 (m, 4H), 1.56–1.52 (m, 3H), 1.6 (m, 1H), 1.28–1.19 (m, 9H), 0.65 (s, 3H); *m*/*z* (relative intensity) 387 (MH^+, 100%); Anal. Calcd. for ($C_{24}H_{38}N_2O_2$): C, 74.57; H, 9.91; N, 7.25. Found: C, 74.85; H, 9.70; N, 7.06%.

2.2.4. 2-(Cyclopropylamino) methyl estradiol (**7c**)

Condensation of 2-formyl estradiol **6** (1 g, 3.33 mmol) with cyclopropylamine (0.38 g, 6.66 mmol) followed by sodium borohydride (0.5 g, 13.2 mmol) reduction using the above-mentioned procedure A and subsequent column chromatography purification using (30:70 ethyl acetate/hexane) as an eluent afforded compound **7c** as a colorless solid (0.95 g, 84%). mp 172–173 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.12 (s, 1H), 6.97 (s, 1H), 6.37 (s, 1H), 4.50 (m, 1H), 3.74 (s, 2H), 3.53–3.49 (t, J = 8.4 Hz, 1H), 2.67 (s, 2H), 2.07–2.04 (m, 2H), 1.97–1.75 (m, 3H), 1.6 (m, 1H), 1.28–1.17 (m, 9H), 0.65 (s, 3H), 0.38–0.36 (t, J = 3.3 Hz, 2H), 0.28–0.27 (d, J = 3.1 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.3, 135.3, 130.0, 125.3, 122.0, 115.0, 80.0, 50.3, 49.5, 43.5, 42.8, 36.6, 30.0, 29.9, 28.8, 27.0, 26.2, 22.8, 11.2, 5.6; *m*/*z* (relative intensity) 342 (MH^+, 100%); Anal. Calcd. for ($C_{22}H_{31}NO_2$): C, 77.38; H, 9.15; N, 4.10. Found: C, 77.18; H, 9.39; N, 4.35%.

*2.2.5. 2-(Ethylglycine) methyl estradiol (**7d**)*

Condensation of 2-formyl estradiol **6** (1 g, 3.33 mmol) with glycine ethyl ester (0.69 g, 6.66 mmol) in DMF for 3 h followed by sodium borohydride (0.5 g, 13.2 mmol) reduction using the above-mentioned procedure A and subsequent column chromatography purification using (22:78 ethyl acetate/hexane) as an eluent afforded compound **7d** as a pale yellow low melting solid (710 mg, 55%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.98 (s, 1H), 6.96 (s, 1H), 6.4 (s, 1H), 4.49 (s, 1H), 4.10–4.08 (d, J = 7.1 Hz, 1H), 3.68 (s, 1H), 3.51 (m, 3H), 2.68 (s, 2H), 2.25–2.15 (m, 2H), 1.95–2.05 (m, 1H), 1.84–1.81 (m, 3H), 1.5 (m, 1H), 1.32–1.10 (m, 11H), 0.85 (m, 1H), 0.65 (s, 3H); m/z (relative intensity) 388 (MH^+, 100%); Anal. Calcd. for ($C_{23}H_{33}NO_4$): C, 71.29; H, 8.58; N, 3.61. Found: C, 71.04; H, 8.36; N, 3.85%.

*2.2.6. 2-(Ethyl 2-amino butyrate)methyl estradiol (**7e**)*

Condensation of 2-formyl estradiol **6** (1 g, 3.33 mmol) with ethyl 2-aminobutyrate (0.87 g, 6.66 mmol) followed by sodium borohydride (0.5 g, 13.2 mmol) reduction using the above-mentioned procedure A and subsequent column chromatography purification using (12:88 ethyl acetate/hexane) as an eluent afforded compound **7e** as a yellow solid (0.91 g, 66%). IR (KBr, cm^{-1}): 3433 (OH and NH), 1734 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.9 (s, 1H), 6.96 (s, 1H), 6.39 (s, 1H), 4.50–4.49 (d, J = 4.0 Hz, 1H), 4.10–4.09 (t, J = 4.2 Hz, 2H), 3.7–3.6 (d, J = 4.0 Hz, 1H), 3.57–3.53 (m, 2H), 3.11 (s, 1H), 2.67 (s, 2H), 2.25–2.15 (m, 1H), 2.05–1.95 (m, 1H), 1.84 (m, 3H), 1.59–1.56 (m, 3H), 1.28–1.17 (m, 11H), 0.87–0.86 (t, J = 7.4 Hz, 3H), 0.65 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.9, 154.1, 135.6, 130.1, 125.5, 121.5, 115.0, 80.0, 61.0, 60.0, 49.5, 48.2, 43.5, 42.8, 36.6, 29.9, 28.8, 27.0, 26.1, 25.5, 22.7, 14.1, 11.2, 10.0; HRMS Calcd. for $C_{25}H_{37}NO_4$ (M): 415.2723. Found: 415.2720.

*2.2.7. 2-(2′-Hydroxyethylamino)methyl estradiol (**7f**)*

Condensation of 2-formyl estradiol **6** (1 g, 3.33 mmol) with ethanolamine (406 mg, 6.66 mmol) followed by sodium borohydride (0.5 g, 13.2 mmol) reduction using the above-mentioned procedure A and subsequent column chromatography purification using ethyl acetate as an eluent afforded compound **7f** as a light brown low melting solid (0.9 g, 77%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.95 (s, 1H), 6.95 (s, 1H), 6.37 (s, 1H), 4.03–4.02 (d, J = 7.1 Hz, 2H), 3.77 (s, 1H), 3.53–3.53 (m, 3H), 3.11 (s, 1H), 2.67 (s, 2H), 2.58–2.57 (t, J = 5.6 Hz, 2H), 2.25 (m, 1H), 1.98 (s, 1H), 1.88–1.75 (m, 3H), 1.7–1.5 (m, 1H), 1.42–1.15 (m, 8H), 0.65 (s, 3H); m/z (relative intensity) 346 (MH^+, 100%); Anal. Calcd. for ($C_{21}H_{31}NO_3$): C, 73.01; H, 9.04; N, 4.05. Found: C, 73.28; H, 9.19; N, 4.25%.

*2.2.8. 2-(1′-Hydroxybutyl-2′-amino) methyl estradiol (**7g**)*

Condensation of 2-formyl estradiol **6** (1 g, 3.33 mmol) with 2-amino butanol (0.59 g, 6.66 mmol) followed by sodium borohydride (0.5 g, 13.2 mmol) reduction using the above-mentioned procedure A afforded compound **7g** as a pale yellow solid (0.91 g, 73%). mp 171–172 °C; IR (KBr, cm^{-1}): 3432 (OH and NH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.93 (s, 1H), 6.93 (s, 1H), 6.35 (s, 1H), 4.58 (bs, 1H), 3.77 (s, 2H), 3.51–3.33 (m, 3H), 2.67 (s, 2H), 2.4–2.3 (m, 1H), 2.0–2.1 (m, 1H), 1.98–1.96 (m, 1H), 1.9–1.7 (m, 3H), 1.65–1.50 (m, 1H), 1.32–1.17 (m, 11H), 0.86–0.83 (t, J = 7.4 Hz, 3H), 0.65 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.0, 135.5, 129.9, 125.2, 121.5, 115.2, 80.1, 61.5, 59.1, 49.5, 48.3, 43.5, 42.8, 38.6, 36.6, 29.9, 28.8, 27.0, 26.2, 23.1, 22.8, 11.2, 10.0; m/z (relative intensity) 374 (MH^+, 100%); Anal. Calcd. for ($C_{23}H_{35}NO_3$): C, 73.96; H, 9.44; N, 3.75. Found: C, 73.74; H, 9.28; N, 3.99%.

*2.2.9. (3,17-Bis-tert-butyldimethylsilyl)-2-formyl estradiol (**8**)*

To the solution of 2-formyl estradiol **6** (10 g, 33.3 mmol) in DMF (150 mL) at 10 °C was added triethylamine (18.8 g, 187 mmol) and stirred for 10 min, a solution of *tert*-butyldimethylsilyl chloride (20 g, 132.7 mmol) in DMF (100 mL) was added slowly. The resulting mixture was further stirred for another 3 h at 10 °C, and the reaction mixture was poured into sodiumbicarbonate solution (500 mL, 5% w/v) and washed with hexane (2 × 500 mL). The combined organics were washed with brine solution (2 × 200 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give light yellow viscous oil. The crude product was purified by silica gel column chromatography using (98:2 hexane/ethyl acetate) as an eluent to yield **8** as white solid (14.2 g, 81%). mp 181–182 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.38 (s, 1H), 7.72 (s, 1H), 7.26 (s, 1H), 6.56 (s, 1H), 3.65–3.61 (t, J = 8.3 Hz, 1H), 2.85–2.82 (q, J = 4.1 Hz, 1H), 2.47–2.43 (d, J = 4.2 Hz 2H), 2.3 (m, 1H), 1.90–1.85 (m, 3H), 1.58–1.07 (m, 7H), 1.01 (s, 9H), 0.89 (s, 9H), 0.75 (s, 3H), 0.25 (s, 6H), 0.03–0.02 (d, J = 6.4 Hz, 6H); m/z (relative intensity) 529 (MH^+, 100%); Anal. Calcd. for ($C_{31}H_{52}O_3Si_2$): C, 70.40; H, 9.91. Found: C, 70.62; H, 9.72%.

*2.2.10. Representative procedure for preparation of 2-benzoyl estradiol **9a** (Procedure B)*

A freshly prepared solution of phenylmagnesium bromide (magnesium turnings (0.2 g, 8.23 mmol) and bromobenzene (2 g, 12.7 mmol)) in dry THF (10 mL) was slowly added to the disilylated aldehyde **8** (1.5 g, 2.84 mmol) in 10 mL of anhydrous THF solution at 0 °C. After complete addition of Grignard solution, the reaction mixture was allowed to stir at room temperature for another 1 h. it was then poured in to 30 mL saturated ammonium chloride solution and washed with diisopropyl ether (2 × 50 mL). The combined organics were washed with brine (2 × 30 mL), dried (sodium sulfate), filtered, and concentrated via rotary evaporation to give 1.8 g of the alcohol as a pale yellow oil [m/z (relative intensity) 607 (MH^+, 100%)]. The crude product was dissolved in MDC (50 mL) and stirred with manganese dioxide (2.5 g, 28.7 mmol) containing powdered molecular sieves 5 Å (2.5 g) for 36 h at room temperature. The mixture was filtered through a pad of celite, the filtrate was concentrated in vacuo, to afford 1.7 g of ketone (m/z (relative intensity) 605 (MH^+, 100%)). It was then dissolved in anhydrous THF (25 mL) stirred with tetrabutylammonium fluoride trihydrate (9 g, 28.5 mmol) and powdered molecular sieves 5 Å (9 g) for 40 h at room temperature. The mixture was filtered through a pad of celite, the filtrate was poured in to 40 mL water and extracted with ethyl acetate (2 × 50 mL). The combined organics were washed with brine (2 × 30 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give dark yellow viscous oil. The crude product was purified by silica gel column chromatography using (10:90 ethyl acetate/hexane) as an eluent to yield 2-benzoyl estradiol **9a** as a light yellow solid (0.67 g, 63%). mp 120–122 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.63 (s, 1H), 7.69–7.51 (m, 5H), 7.28 (s, 1H), 6.7 (s, 1H), 4.51–4.50 (d, J = 4.8 Hz, 1H), 3.52–3.50 (m, 1H), 2.84–2.82 (d, J = 8.0 Hz, 2H), 2.1–2.0 (m, 2H), 1.9–1.8 (m, 3H), 1.7–1.5 (m, 1H), 1.39–1.13 (m, 7H), 0.65 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 201.2, 160.9, 147.3, 138.2, 131.8, 131.4, 130.4, 129.1, 128.3, 117.6, 117.1, 81.7, 50.0, 43.6, 43.2, 38.6, 36.5, 30.5, 30.0, 26.8, 26.1, 23.1, 11.1; m/z (relative intensity) 375 (MH^+, 100%); Anal. Calcd. for ($C_{25}H_{28}O_3$): C, 79.75; H, 7.50. Found: C, 79.98; H, 7.23%.

*2.2.11. 2-(4′-Methylbenzoyl)estradiol (**9b**)*

Addition of freshly prepared 4-methylphenylmagnesium bromide (magnesium turnings (0.2 g, 8.52 mmol) and 4-bromotoluene (2.17 g, 12.7 mmol)) in dry THF (10 mL) to disilylated aldehyde **8** (1.5 g, 2.84 mmol) followed by manganese dioxide (2.5 g, 28.7 mmol) oxidation, tetrabutylammonium fluoride trihydrate (9 g, 28.5 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography

purification (12:88 ethyl acetate/hexane) afforded compound **9b** as light yellow solid (0.66 g, 60%). mp 108–110 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.65 (s, 1H), 7.63–7.61 (d, J = 7.8 Hz, 2H), 7.37–7.25 (m, 3H), 6.7 (s, 1H), 4.55–4.54 (d, J = 4.4 Hz, 1H), 3.52 (m, 1H), 2.83 (s, 2H), 2.42 (s, 3H), 2.15–2.0 (m, 2H),1.83–1.8 (m, 3H), 1.7–1.5 (m, 1H), 1.36–1.15 (m, 6H), 0.86 (m, 1H), 0.68 (s, 3H); HRMS Calcd. for $C_{26}H_{30}O_3$ (M): 390.2195. Found: 390.2199.

*2.2.12. 2-(4′-Fluorobenzoyl)estradiol (**9c**)*

Addition of freshly prepared 4-fluorophenylmagnesium bromide [magnesium turnings (0.2 g, 8.52 mmol) and 1-bromo-4-fluorobenzene (2.22 g, 12.7 mmol] in dry THF (10 mL) to disilylated aldehyde **8** (1.5 g, 2.84 mmol) followed by manganese dioxide (2.5 g, 28.7 mmol) oxidation, tetrabutylammonium fluoride trihydrate (9 g, 28.5 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (12:88 ethyl acetate/hexane) gave compound **9c** as light yellow solid (0.71 g, 64%). mp 150–152 °C; IR (KBr, cm^{-1}): 3514 (OH), 1631 (CO), 1596 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.44 (s, 1H), 7.81–7.77 (m, 2H), 7.40–7.35 (m, 2H), 7.28 (s, 1H), 6.71(s, 1H), 4.62 (s, 1H), 3.52 (m, 1H), 2.83 (s, 2H), 2.11 (m, 2H), 1.84–1.81 (m, 3H), 1.70–1.50 (m, 1H), 1.36–1.13 (m, 6H), 0.85 (m, 1H), 0.68 (s, 3H); m/z (relative intensity) 393 (MH$^+$, 100%); Anal. Calcd. for ($C_{25}H_{27}FO_3$): C, 76.12; H, 6.90. Found: C, 76.36; H, 6.70%.

*2.2.13. 2-(4′-Methoxybenzoyl)estradiol (**9d**)*

Addition of freshly prepared 4-methoxyphenylmagnesium bromide [magnesium turnings (0.2 g, 8.52 mmol) and 4-bromoanisole (2.37 g, 12.7 mmol] in dry THF (10 mL) to disilylated aldehyde **8** (1.5 g, 2.84 mmol) followed by manganese dioxide (2.5 g, 28.7 mmol) oxidation, tetrabutylammonium fluoride trihydrate (9 g, 28.5 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (15:85 ethyl acetate/hexane) yielded compound **9d** as light yellow solid (0.74 g, 65%). mp 90–92 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.41 (s, 1H), 7.7–7.68 (d, J = 8.6 Hz, 2H), 7.25 (s, 1H), 7.07–7.05 (d, J = 8.6 Hz, 2H), 6.67 (s, 1H), 4.5–4.49 (d, J = 4.6 Hz, 1H), 3.85 (s, 3H), 3.5 (m, 1H), 2.81 (s, 2H), 2.11–2.08 (m, 2H), 1.82–1.79 (m, 3H), 1.7–1.5 (m, 1H), 1.39–1.11 (m, 6H), 0.85 (m, 1H), 0.68 (s, 3H); m/z (relative intensity) 405 (MH$^+$, 100%); Anal. Calcd. for ($C_{26}H_{30}O_4$): C, 76.82; H, 7.44. Found: C, 76.60; H, 7.59%.

*2.2.14. 2-(4′-Ethoxybenzoyl)estradiol (**9e**)*

Addition of freshly prepared 4-ethoxyphenylmagnesium bromide [magnesium turnings (0.2 g, 8.52 mmol) and 1-bromo-4-ethoxybenzene (2.62 g, 12.7 mmol] in dry THF (10 mL) to disilylated aldehyde **8** (1.5 g, 2.84 mmol) followed by manganese dioxide (2.5 g, 28.7 mmol) oxidation, tetrabutylammonium fluoride trihydrate (9 g, 28.5 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (15:85 ethyl acetate/hexane) furnished compound **9e** as light yellow solid (0.75 g, 67%). mp 82–84 °C; IR (KBr, cm^{-1}): 1632 (CO), 1598 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.41 (s, 1H), 7.68–7.66 (d, J = 8.5 Hz, 2H), 7.25 (s, 1H), 7.05–7.03 (d, J = 8.5 Hz, 2H), 6.66 (s, 1H), 4.52–4.5 (d, J = 4.6 Hz, 1H), 4.13–4.11 (d, J = 6.9 Hz, 2H), 3.5 (m, 1H), 2.81 (s, 2H), 2.1–2.08 (m, 2H),1.81–1.78 (m, 3H), 1.7–1.5 (m, 1H), 1.37–1.11 (m, 9H), 0.85 (s, 1H), 0.68 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 196.9, 162.0, 156.3, 143.5, 131.6, 130.9, 130.0, 127.8, 120.6, 116.5, 114.0, 79.9, 63.5, 49.5, 43.0, 42.7, 38.3, 36.3, 29.8, 29.2, 26.5, 25.8, 22.7, 14.4, 11.1; m/z (relative intensity) 419 (MH$^+$, 100%); Anal. Calcd. for ($C_{27}H_{32}O_4$): C, 77.11; H, 7.67. Found: C, 77.38; H, 7.50%.

*2.2.15. 2-(3′,4′-Dimethoxybenzoyl)estradiol (**9f**)*

Addition of freshly prepared 3,4-dimethoxyphenylmagnesium bromide [magnesium turnings (0.2 g, 8.52 mmol) and 1-bromo-3,4-dimethoxybenzne (2.75 g, 12.7 mmol] in dry THF (10 mL) to disilylated aldehyde **8** (1.5 g, 2.84 mmol) followed by manganese dioxide (2.5 g, 28.7 mmol) oxidation, tetrabutylammonium fluoride trihydrate (9 g, 28.5 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (20:80 ethyl acetate/hexane) afforded compound **9f** as light yellow solid (0.65 g, 53%). mp 83–85 °C; IR (KBr, cm^{-1}): 1632 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.51 (s, 1H), 7.33–7.26 (m, 3H), 7.09 (s, 1H), 6.68 (s, 1H), 4.5–4.49 (d, J = 4.7 Hz, 1H), 3.85 (s, 3H), 3.80 (s, 3H), 3.5 (m, 1H), 2.81 (s, 2H), 2.11–2.08 (m, 2H), 1.82–1.79 (m, 3H), 1.7–1.5 (m, 1H), 1.39–1.11 (m, 6H), 0.85 (m, 1H), 0.68 (s, 3H); m/z (relative intensity) 437 (MH$^+$, 100%). Anal. Calcd. for ($C_{27}H_{32}O_5$): C, 74.29; H, 7.39. Found: C, 74.04; H, 7.64%.

*2.2.16. 2-(3′,5′-Dimethoxybenzoyl)estradiol (**9g**)*

Addition of freshly prepared 3,5-dimethoxyphenylmagnesium bromide [magnesium turnings (0.2 g, 8.52 mmol) and 1-bromo-3,5-dimethoxybenzne (2.75 g, 12.7 mmol] in dry THF (10 mL) to disilylated aldehyde **8** (1.5 g, 2.84 mmol) followed by manganese dioxide (2.5 g, 28.7 mmol) oxidation, tetrabutylammonium fluoride trihydrate (9 g, 28.5 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (20:80 ethyl acetate/hexane) gave compound **9g** as bright yellow solid (0.68 g, 55%). mp 68–70 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.76 (s, 1H), 7.36 (s, 1H), 6.82–6.8 (m, 3H), 6.74 (s, 1H), 4.56–4.54 (d, J = 6.4 Hz, 1H), 3.82 (s, 6H), 3.55 (m, 1H), 2.86 (s, 2H), 2.11–2.08 (m, 2H), 1.84–1.82 (m, 3H), 1.7–1.5 (m, 1H), 1.39–1.11 (m, 6H), 0.85 (m, 1H), 0.69 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 198.4, 160.2, 157.6, 145.1, 139.6, 131.1, 128.6, 119.1, 116.7, 106.8, 104.0, 79.9, 55.4, 49.4, 42.9, 42.7, 38.2, 36.3, 29.8, 29.2, 26.4, 25.9, 25.7, 22.7, 11.1; m/z (relative intensity) 437 (MH$^+$, 100%); Anal. Calcd. for ($C_{27}H_{32}O_5$): C, 74.29; H, 7.39. Found: C, 74.02; H, 7.60%.

*2.2.17. 2-(3′,4′,5′-Trimethoxybenzoyl)estradiol (**9h**)*

Addition of freshly prepared 3,4,5-trimethoxyphenylmagnesium bromide [magnesium turnings (0.20 g, 8.52 mmol) and 1-bromo-3,4,5-trimethoxybenzene (3.10 g, 12.70 mmol] in dry THF (10 mL) to disilylated aldehyde **8** (1.50 g, 2.84 mmol) followed by manganese dioxide (2.50 g, 28.70 mmol) oxidation, tetrabutylammonium fluoride trihydrate (9.0 g, 28.50 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (30:70 ethyl acetate/hexane) yielded compound **9h** as light yellow solid (0.67 g, 51%). mp 59–61 °C; IR (KBr, cm^{-1}): 3312 (OH), 1630 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.7 (s, 1H), 7.38 (s, 1H), 7.01 (s, 2H), 6.7 (s, 1H), 4.52–4.51 (d, J = 4.6 Hz, 1H), 3.80–3.76 (m, 9H), 3.50 (m, 1H), 2.82 (s, 2H), 2.11 (m, 2H),1.82–1.79 (m, 3H), 1.6–1.5 (m, 1H), 1.38–1.11 (m, 6H), 0.86 (m, 1H), 0.69 (s, 3H); HRMS Calcd. for $C_{28}H_{34}O_6$ (M): 466.2355. Found: 466.2352.

*2.2.18. Representative procedure for preparation of (2E)-3-(3,17β-dihydroxy-1,3,5(10)-estratrienyl)-1-(3′-methoxyphenyl)prop-2-en-1-one **10a** (Procedure C)*

To a stirred solution of 2-formyl estradiol **6** (0.75 g, 2.5 mmol), and 3′-methoxy acetophenone (0.5 g, 3.33 mmol) in methanol (30 mL) was added 6 mL of 50% potassium hydroxide solution, the reaction mixture was then heated at 70 °C for 6 h. The solvent was evaporated, and the residue was dissolved in ethyl acetate/water (100 mL, 3:1), and acidified with 5 N HCl to pH 6.0. The organic layer was washed with brine (2 × 25 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give

dark yellow viscous oil. The crude product was purified by silica gel column chromatography using (10:90 ethyl acetate/hexane) as an eluent to yield *trans*-isomer **10a** as a light yellow solid (0.54 g, 50%). mp 189–190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.04–8.00 (d, J = 15.7 Hz, 1H), 7.8–7.68 (m, 3H), 7.54 (s, 1H), 7.5–7.46 (t, J = 8.0 Hz, 1H), 7.23–7.21 (d, J = 8.1 Hz, 1H), 6.61 (s, 1H), 4.52 (s, 1H), 3.84 (s, 3H), 3.56–3.51 (m, 1H), 2.74 (s, 2H), 2.12–2.08 (t, J = 6.9 Hz, 1H), 1.89–1.86 (m, 2H), 1.80–1.77 (m, 1H), 1.60–1.58 (m, 1H), 1.38–1.23 (m, 8H), 0.68 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 194.4, 164.7, 160.4, 146.6, 145.6, 144.8, 136.8, 135.0, 130.7, 126.0, 124.8, 124.2, 123.7, 121.0, 118.1, 85.3, 65.0, 60.5, 54.7, 48.6, 48.0, 41.8, 35.1, 34.5, 31.9, 31.1, 28.0, 16.4; HRMS Calcd. for $C_{28}H_{32}O_4$ (M): 432.2301. Found: 432.2307.

*2.2.19. (2E)-3-(3, 17β-Dihydroxy-1,3,5(10)-estratrienyl)-1-(2′-trifluoromethylphenyl)prop-2-en-1-one (**10b**)*

Condensation of 2-formyl estradiol **6** (0.75 g, 2.5 mmol), and 2′-trifluoromethylacetophenone (0.62 g, 3.33 mmol) using the above-mentioned procedure C followed by silica gel column chromatographic purification (25:75 ethyl acetate/hexane) afforded **10b** as pale yellow solid (0.61 g, 52%). mp 221–222 °C; IR (KBr, cm^{-1}): 3463 (OH), 1637 (C=O), 1615 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.98 (s, 1H), 7.88–7.86 (d, J = 7.8 Hz, 1H), 7.81–7.78 (t, J = 7.4 Hz, 2H), 7.75–7.71 (t, J = 7.6 Hz, 1H), 7.61–7.59 (d, J = 7.4 Hz, 1H), 7.53 (s, 1H), 7.19 (s, 1H), 6.58 (s, 1H), 4.5–4.49 (d, J = 4.6 Hz, 1H), 3.54–3.51 (m, 1H), 2.72 (s, 2H), 2.08–2.03 (m, 1H), 1.85–1.75 (m, 3H), 1.58–1.56 (m, 1H), 1.38–1.22 (m, 8H), 0.68 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 194.5, 155.1, 143.7, 142.1, 139.1, 132.3, 131.7, 130.0, 128.2, 126.5, 125.7, 124.5, 121.9, 118.2, 115.8, 80.0, 67.3, 49.5, 43.2, 42.7, 38.3, 36.4, 29.8, 29.2, 26.5, 25.8, 22.7, 11.1; m/z (relative intensity) 469 (MH$^-$, 100%); Anal. Calcd. for ($C_{28}H_{29}F_3O_3$): C, 71.47; H, 6.21. Found: C, 71.20; H, 6.01%.

*2.2.20. (2E)-3-(3, 17β-Dihydroxy-1,3,5(10)-estratrienyl)-1-(2′,4′,6′-trimethoxyphenyl)prop-2-en-1-one (**10c**)*

Condensation of 2-formyl estradiol **6** (0.75 g, 2.5 mmol), and 2′,4′,6′-trimethoxyphenylacetophenone (0.7 g, 3.33 mmol) using the above-mentioned procedure C followed by silica gel column chromatographic purification (40:60 ethyl acetate/hexane) furnished **10c** as pale yellow solid (0.88 g, 72%). mp 184–186 °C; IR (KBr, cm^{-1}): 3242 (OH), 1630 (C=O), 1603 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.81 (s, 1H), 7.43–7.38 (d, J = 16.3 Hz, 1H), 7.38 (s, 1H), 6.91–6.87 (d, J = 16.2 Hz, 1H), 6.55 (s, 1H), 6.29 (s, 2H), 4.51–4.50 (d, J = 4.7 Hz, 1H), 4.05 (s, 3H), 3.82 (s, 3H), 3.69 (s, 3H), 2.71 (s, 2H), 2.30 (s, 1H), 2.08 (m, 1H),1.85–1.82 (m, 3H), 1.58–1.56 (m, 1H), 1.38–1.22 (m, 8H), 0.68 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 193.8, 161.6, 157.9, 154.5, 141.0, 140.3, 131.5, 127.4, 125.2, 118.6, 115.7, 111.5, 91.0, 80.0, 67.3, 55.7, 55.3, 49.5, 43.3, 42.7, 38.4, 36.5, 29.8, 29.1, 26.6, 25.9, 22.7, 11.2; m/z (relative intensity) 491 (MH$^+$, 100%); Anal. Calcd. for ($C_{30}H_{36}O_6$): C, 73.15; H, 7.37. Found: C, 73.43; H, 7.15%.

*2.2.21. (2E)-3-(3, 17β-Dihydroxy-1,3,5(10)-estratrienyl)-1-(3′,4′,5′-trimethoxyphenyl)prop-2-en-1-one (**10d**)*

Condensation of 2-formyl estradiol **6** (0.75 g, 2.5 mmol), and 3′,4′,5′-trimethoxyphenylacetophenone (0.7 g, 3.33 mmol) using the above-mentioned procedure C followed by silica gel column chromatographic purification (40:60 ethyl acetate/hexane) gave **10d** as pale yellow solid (763 mg, 62%). mp 166–168 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.03–7.99 (d, J = 15.7 Hz, 1H), 7.79–7.75 (d, J = 15.7 Hz, 1H), 7.66 (s, 1H), 7.35 (s, 2H), 6.61(s, 1H), 4.51–4.49 (d, J = 4.6 Hz, 1H), 3.94 (s, 6H), 3.76 (s, 3H), 3.56 (m, 1H), 2.74 (s, 2H), 2.12–2.08 (m, 1H),1.88–1.85 (m, 2H), 1.80–1.77 (m, 1H), 1.6–1.58 (m, 1H), 1.38–1.22 (m, 8H), 0.68 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 188.5, 155.1, 152.8, 141.7, 141.2, 140.3, 133.6, 131.4, 125.7, 119.8, 119.1, 115.8, 106.1, 80.0, 60.1, 56.1, 49.5, 43.4, 42.7, 38.5, 36.5, 29.9, 29.2, 26.6, 25.9, 22.7, 11.2; m/z (relative intensity) 491 (MH$^+$, 100%); Anal. Calcd. for ($C_{30}H_{36}O_6$): C, 73.15; H, 7.37. Found: C, 73.01; H, 7.59%.

*2.2.22. Preparation of 17-methylene estratriene (**11**)*

The suspension of sodium hydride (7.5 g, (60%) 187 mmol) in anhydrous DMSO (200 mL) was heated to 60 °C under inert atmosphere for 1 h. To the resulting green coloured solution at RT was added solution of methyltriphenylphosphonium iodide (75 g, 187 mmol) in DMSO (150 mL) and stirred for 30 min. The resulting red coloured solution was mixed with the solution of estrone (10 g, 37 mmol) in DMSO (150 mL) and stirred for another 20 h at RT. The resulting mixture was poured in to water (1 L) and extracted with diisopropyl ether (2 × 750 mL). The combined organics were washed with brine (2 × 250 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give dark yellow viscous oil. The crude product was purified by silica gel column chromatography using (4:96 ethyl acetate/hexane) as an eluent to yield the title compound **11** as a dull white solid (9 g, 91%). mp 135–136 °C ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.99 (s, 1H), 7.06–7.04 (d, J = 8.5 Hz, 1H), 6.65–6.49 (m, 1H), 6.44 (s, 1H), 4.65–4.63 (d, J = 8.7 Hz, 2H), 2.72–2.71 (m, 2H), 2.45 (m, 1H), 2.3 (m, 1H), 1.9–1.84 (m, 4H), 1.39–1.28 (m, 7H), 0.77 (s, 3H); m/z (relative intensity) 267 (MH$^+$, 100%); Anal. Calcd. for ($C_{19}H_{24}O$): C, 85.03; H, 9.01. Found: C, 85.19; H, 9.26%.

*2.2.23. 2-Formyl 17-methylene estratriene (**12**)*

To the suspension of magnesium turnings (2.1 g, 86.4 mmol) in THF (20 mL) was added solution of bromoethane (13.1 g, 120 mmol) in THF (10 mL) slowly at RT during 20 min and stirred for another 30 min at RT. To the resulting clear solution was added the solution of methylene compound **11** (4.0 g, 14.5 mmol) in THF (40 mL) at RT and further stirred for 30 min. To the resulting suspension was added hexamethylphosphoramide (7.4 g, 41.3 mmol) followed by paraformaldehyde (7.0 g, 233 mmol) and heated to 60 °C for 20 h. The resulting yellow reaction mixture was poured in to water (100 mL) and washed with ethyl acetate (2 × 100 mL) at pH 4.0. The combined organics were washed with brine (2 × 25 mL) and concentrated via rotary evaporation to give yellowish oil. The crude product was suspended in methanol (80 mL) at 10 °C and added sodium hydroxide solution (20% w/v, 8 mL) and stirred for 1 h. The resulting yellow clear solution was poured into the water (160 mL) and washed with ethyl acetate (2 × 150 mL) at pH 4.0. The combined organics were washed with brine (2 × 50 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give light yellow viscous oil. The crude product was purified by silica gel column chromatography using hexane as an eluent to yield 2-formyl methylene compound **12** as a light yellow solid (3.65 g, 83%). mp 128–130 °C ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.44 (s, 1H), 10.14 (s, 1H), 7.58 (s, 1H), 6.68 (s, 1H), 4.66–4.64 (d, J = 10.0 Hz, 2H), 3.5 (m, 1H), 2.84–2.82 (s, J = 7.4 Hz, 2H), 2.34–2.3 (d, 1H), 2.29–2.1 (s, 2H), 1.94–1.81 (m, 3H), 1.5–1.13 (m, 6H), 0.77 (s, 3H); m/z (relative intensity) 295 (MH$^+$, 100%); Anal. Calcd. for ($C_{20}H_{24}O_2$): C, 81.04; H, 8.16. Found: C, 81.18; H, 8.40%.

*2.2.24. Representative procedure for preparation of 2-(N,N-dimethylhydrazinimino)methyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol **13a** (Procedure D)*

To the solution of 2-formyl-17-methylene estratriene **12** (0.2 g, 0.675 mmol) in DMF (5 mL) at 5 °C was added sodium sulphate (400 mg) and stirred for 5 min. To this, *N*,*N*-dimethylhydrazine (81 mg, 1.35 mmol) in DMF (2 mL) was then added and the mixture was stirred for 1 h. It was then poured in to water (15 mL) and washed with ethyl acetate (2 × 25 mL). The combined organics

were washed with brine (2 × 15 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give light yellow semi solid. Triturated of the crude product with hexane (10 mL), followed by filtration afforded compound **13a** as a white solid (0.19 g, 82%). mp 179–181 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.0 (s, 1H), 7.6 (s, 1H), 7.2 (s, 1H), 6.5 (s, 1H), 4.66–4.64 (d, J = 10.4 Hz, 2H), 2.85 (s, 6H), 2.75 (s, 2H), 2.4–2.30 (m, 1H), 2.25–2.0 (m, 2H), 1.95–1.7 (m, 3H), 1.39–1.32 (m, 6H), 1.2–1.1 (s, 1H), 0.72 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.7, 154.1, 138.0, 137.1, 130.6, 125.6, 117.3, 115.3, 100.8, 52.9, 43.8, 43.3, 42.5, 38.2, 35.2, 29.0, 28.9, 27.0, 26.2, 23.4, 18.2; m/z (relative intensity) 337 (MH$^+$, 100%); Anal. Calcd. for ($C_{22}H_{30}N_2O$): C, 78.06; H, 8.93; N, 8.28. Found: C, 78.32; H, 8.80; N, 8.05%.

2.2.25. 2-(2′-(N,N-Dimethylaminoethylimino)methyl-17-(1′-methylene) estra-1,3,5(10)-triene-3-ol (**13b**)

Condensation of 2-formyl-17-methylene estratriene **12** (0.2 g, 0.675 mmol) with *N,N*-dimethylethylenediamine (0.12 g, 1.35 mmol) using the above-mentioned procedure D followed by trituration of the crude product with hexane afforded compound **13b** as a light yellow solid (0.19 g; 79%). mp 101–103 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.1 (s, 1H), 8.32 (s, 1H), 7.26 (s, 1H), 7.15 (s, 1H), 6.67 (s, 1H), 4.68–4.67 (d, J = 6.8 Hz, 2H), 3.7–3.67 (t, J = 6.8 Hz, 2H), 2.88–2.85 (m, 2H), 2.63–2.6 (m, 2H), 2.53–2.51 (m, 1H), 2.36–2.35 (m, 1H), 2.31 (s, 6H), 2.29–2.20 (m, 1H), 1.98–1.83 (m, 2H), 1.81–1.8 (m, 1H), 1.4–1.35 (m, 6H), 0.76 (s, 3H); HRMS Calcd. for $C_{24}H_{34}N_2O$ (M): 366.2671. Found: 366.2665.

2.2.26. 2-(Cyclopropylimino) methyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (^{13}C)

Condensation of 2-formyl-17-methylene estratriene **12** (0.2 g, 0.675 mmol) with cyclopropylamine (0.8 g, 1.35 mmol) using the above-mentioned procedure D followed by trituration of the crude product with hexane yielded compound ^{13}C as a light yellow solid (0.18 g, 83%). mp 124–126 °C; IR (KBr, cm^{-1}): 3448 (OH), 1624 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.3 (s, 1H), 8.6 (s, 1H), 7.3 (s, 1H), 6.5 (s, 1H), 4.67–4.64 (d, J = 10.4 Hz, 2H), 3.1 (s, 1H), 2.78 (s, 2H), 2.4–2.3 (m, 1H), 2.2–2.1 (m, 2H), 1.98–1.7 (m, 3H), 1.4–1.35 (m, 6H), 1.2 (m, 1H), 0.96–0.95 (d, J = 4.8 Hz, 2H), 0.82–0.81 (s, 2H), 0.76 (s, 3H); m/z (relative intensity) 336 (MH$^+$, 100%); Anal. Calcd. for ($C_{23}H_{29}NO$): C, 82.34; H, 8.71; N, 4.18. Found: C, 82.20; H, 8.96; N, 4.02%.

2.2.27. 2-(2′-Hydroxyethylimino)methyl-17-(1′-methylene)estra-1,3,5 (10)-triene-3-ol (**13d**)

Condensation of 2-formyl-17-methylene estratriene **12** (0.2 g, 0.675 mmol) with ethanolamine (0.82 g, 1.35 mmol) using the above-mentioned procedure D followed by trituration of the crude product with diisopropyl ether gave compound **13d** as light yellow solid (0.19 g, 85%). mp 151–153 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.93 (s, 1H), 8.36 (s, 1H), 7.17 (s, 1H), 6.69 (s, 1H), 4.68–4.67 (d, J = 4.2 Hz, 2H), 3.92–3.89 (t, J = 5.1 Hz, 2H), 3.75–3.72 (t, J = 5.1 Hz, 2H), 2.88–2.85 (m, 2H), 2.6–2.45 (m, 1H), 2.4–2.15 (m, 3H), 1.98–1.93 (m, 2H), 1.85–1.75 (m, 1H), 1.6–1.35 (m, 6H), 1.26–1.24 (m, 1H), 0.82 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.5, 160.9, 158.3, 141.2, 130.3, 128.2, 116.7, 115.7, 101.0, 61.2, 60.7, 52.9, 43.8, 43.1, 38.1, 35.1, 29.2, 29.0, 26.8, 26.1, 23.4, 18.2; m/z (relative intensity) 340 (MH$^+$, 100%); Anal. Calcd. for ($C_{22}H_{29}NO_2$): C, 77.84; H, 8.61; N, 4.13. Found: C, 77.60; H, 8.38; N, 4.41%.

2.2.28. 2-(2′-N,N-dimethylthylenediamino)methyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**14a**)

Condensation of 2-formyl-17-methylene estratriene **12** (0.4 g, 1.35 mmol) with *N,N*-dimethylethylenediamine (0.24 g, 2.7 mmol) followed by sodium borohydride (0.2 g, 5.26 mmol) reduction using the above-mentioned procedure A and subsequent silica gel column chromatography purification (methanol) afforded compound **14a** as yellow viscous liquid (0.3 g, 60%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.94 (s, 1H), 6.94 (s, 1H), 6.37 (s, 1H), 4.65–4.63 (d, J = 9.1 Hz, 2H), 3.75 (s, 2H), 2.7–2.69 (m, 2H), 2.55–2.50 (m, 1H), 2.33–2.29 (t, J = 6.3 Hz, 3H), 2.14–2.13 (m, 1H), 2.11 (s, 6H), 1.93–1.88 (m, 2H), 1.77–1.73 (m, 2H), 1.63 (s, 1H), 1.39–1.31 (m, 8H), 0.75 (s, 3H); m/z (relative intensity) 369 (MH$^+$, 100%); Anal. Calcd. for ($C_{24}H_{36}N_2O$): C, 78.21; H, 9.85; N, 7.60. Found: C, 78.01; H, 9.71; N, 7.86%.

2.2.29. 2-(2′-N,N-Dimethylpropylenediamino)methyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**14b**)

Condensation of 2-formyl-17-methylene estratriene **12** (0.4 g, 1.35 mmol) with *N,N*-dimethylpropylenediamine (0.28 g, 2.7 mmol) followed by sodium borohydride (0.2 g, 5.26 mmol) reduction using the above-mentioned procedure A and subsequent silica gel column chromatography purification, methanol as eluent yielded compound **14b** as yellow viscous oil (0.22 g, 43%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.01 (s, 1H), 6.39 (s, 1H), 4.65–4.63 (d, J = 9.6 Hz, 2H), 3.75 (s, 2H), 2.73–2.70 (m, 2H), 2.51 (m, 1H), 2.33–2.29 (m, 3H), 2.14–2.13 (m, 1H), 2.11 (s, 6H), 1.93–1.88 (m, 2H), 1.77–1.73 (m, 2H), 1.63 (s, 1H), 1.39–1.31 (m, 8H), 1.04–1.02 (m, 2H), 0.98 (s, 1H), 0.75 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.0, 155.1, 135.6, 135.5, 125.4, 125.3, 115.1, 101.0, 61.9, 57.1, 53.0, 50.7, 45.1, 45.0, 43.8, 43.5, 35.3, 29.0, 28.8, 27.2, 26.3, 23.4, 18.3; m/z (relative intensity) 383 (MH$^+$, 100%); Anal. Calcd. for ($C_{25}H_{38}N_2O$): C, 78.48; H, 10.01; N, 7.32. Found: C, 78.69; H, 10.26; N, 7.15%.

2.2.30. 2-(Cyclopropylamino) methyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**14c**)

Condensation of 2-formyl-17-methylene estratriene **12** (0.4 g, 1.35 mmol) with cyclopropylamine (0.15 g, 2.7 mmol) followed by sodium borohydride (0.2 g, 5.26 mmol) reduction using the above-mentioned procedure A and subsequent silica gel column chromatography purification (10:90 ethyl acetate/hexane) furnished compound **14c** as light yellow solid (0.34 g, 76%). mp 114–116 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.98 (s, 1H), 6.38 (s, 1H), 4.65–4.63 (d, J = 9.2 Hz, 2H), 3.75 (s, 2H), 2.7 (s, 2H), 2.5–2.47 (m, 1H), 2.34–2.32 (m, 1H), 2.24–2.2 (m, 1H), 2.09–2.05 (m, 2H), 1.91–1.75 (m, 3H), 1.4–1.37 (m, 7H), 1.20–1.10 (m, 1H), 0.75 (s, 3H), 0.38–0.36 (m, 2H), 0.28–0.27 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.9, 154.4, 135.3, 129.8, 125.3, 122.0, 115.0, 101.0, 52.9, 43.8, 43.5, 38.5, 35.3, 29.0, 28.8, 27.2, 26.3, 23.4, 18.2, 5.6; HRMS Calcd. for $C_{23}H_{31}NO$ (M): 337.2406. Found: 337.2402.

2.2.31. 2-(2′-Hydroxyethylamino) methyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**14d**)

Condensation of 2-formyl-17-methylene estratriene **12** (0.4 g, 1.35 mmol) with ethanolamine (0.16 g, 2.7 mmol) followed by sodium borohydride (0.2 g, 5.26 mmol) reduction using the above-mentioned procedure A and subsequent silica gel column chromatography purification (10:90 methanol/ethyl acetate) gave compound **14d** as yellow solid (0.36 g, 79%). mp 144–146 °C; IR (KBr, cm^{-1}): 3238 (OH & NH), 1623 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.0 (s, 1H), 6.41 (s, 1H), 4.66–4.63 (d, J = 9.3 Hz, 2H), 3.81 (s, 2H), 3.50–3.48 (t, J = 5.6 Hz, 2H), 2.71–2.7 (m, 2H), 2.62–2.59 (t, J = 5.6 Hz, 2H), 2.45–2.4 (m, 1H), 2.39–2.32 (m, 1H), 2.25–2.05 (m, 2H), 1.91–1.9 (s, 3H), 1.85–1.75 (m, 2H), 1.38–1.17 (m, 7H), 0.77 (s, 3H); m/z (relative intensity) 340 (MH$^+$, 100%); Anal. Calcd. for ($C_{22}H_{31}NO_2$): C, 77.38; H, 9.15; N, 4.10. Found: C, 77.62; H, 9.40; N, 4.38%.

*2.2.32. 2-(1′-Hydroxy-2′-methyl-propyl-2′-amino) methyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**14e**)*

Condensation of 2-formyl-17-methylene estratriene **12** (0.4 g, 1.35 mmol) with 2-amino-2-methyl-1-propanol (0.24 g, 2.7 mmol) followed by sodium borohydride (0.2 g, 5.26 mmol) reduction using the above-mentioned procedure A and subsequent silica gel column chromatography purification (35:65 ethyl acetate/hexane) afforded compound **14e** as a white solid (0.33 g, 67%). mp 142–144 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.93 (s, 1H), 6.34 (s, 1H), 4.65–4.63 (d, J = 9.3 Hz, 2H), 3.74 (s, 2H), 3.24 (s, 2H), 2.73–2.69 (m, 2H), 2.33–2.31 (m, 2H), 2.24–2.08 (m, 2H), 1.91–1.74 (m, 6H), 1.37–1.34 (m, 6H), 0.98 (s, 6H), 0.75 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.9, 155.4, 135.3, 129.6, 124.7, 121.9, 115.3, 100.9, 67.6, 53.8, 52.9, 43.8, 43.5, 38.5, 35.3, 29.0, 28.8, 27.2, 26.3, 23.4, 23.1, 23.0, 18.2; m/z (relative intensity) 368 (MH^+, 100%); Anal. Calcd. for ($C_{24}H_{35}NO_2$): C, 78.00; H, 9.55; N, 3.79. Found: C, 78.24; H, 9.34; N, 3.99%.

*2.2.33. 2-(1′-Hydroxy-butyl-2′-amino) methyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**14f**)*

Condensation of 2-formyl-17-methylene estratriene **12** (0.4 g, 1.35 mmol) with 2-amino-1-butanol (0.24 mg, 2.7 mmol) followed by sodium borohydride (0.2 g, 5.26 mmol) reduction using the above-mentioned procedure A and subsequent silica gel column chromatography purification (50:50 ethyl acetate/hexane) afforded compound **14f** as a white solid (0.35 mg, 71%). mp 83–85 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.96 (s, 1H), 6.37 (s, 1H), 4.65–4.63 (d, J = 9.3 Hz, 2H), 3.79 (s, 2H), 3.45–3.41 (m, 2H), 3.35–3.31 (m, 1H), 2.73–2.70 (m, 2H), 2.47–2.41 (m 1H), 2.39–2.34 (m, 1H), 2.31–2.25 (m, 1H), 2.0–2.1 (m, 1H), 1.91–1.75 (m, 3H), 1.47–1.34 (m, 9H), 1.26–1.15 (m, 2H), 0.83–0.8 (t, J = 5.9 Hz, 3H), 0.77 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.0, 155.1, 135.6, 129.7, 125.2, 121.5, 115.1, 101.0, 61.4, 59.1, 52.9, 48.1, 43.8, 43.5, 38.5, 35.3, 29.0, 28.8, 27.2, 26.3, 23.4, 18.3, 10.0; HRMS Calcd. for $C_{24}H_{35}NO_2$ (M): 369.2668. Found: 369.2660.

*2.2.34. 2-(2′,4′-Dimethoxybenzylamino) methyl-17-(1′-methylene) estra-1,3,5(10)-triene-3-ol (**14g**)*

Condensation of 2-formyl-17-methylene estratriene **12** (0.4 g, 1.35 mmol) with 2,4-dimethoxybenzylamine (0.45 mg, 2.7 mmol) followed by sodium borohydride (0.2 g, 5.26 mmol) reduction using the above-mentioned procedure A and subsequent silica gel column chromatography purification (20:80 ethyl acetate/hexane) furnished compound **14g** as white solid (0.33 g, 55%). mp 78–80 °C; IR (KBr, cm^{-1}): 3200 (NH & OH), 1612 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.13–7.11 (d, J = 8.2 Hz, 1H), 6.94 (s, 1H), 6.54–6.53 (d, J = 5.2 Hz, 1H), 6.49–6.47 (m, 1H), 6.37 (s, 1H), 4.65–4.63 (d, J = 9.3 Hz, 2H), 3.77 (s, 2H), 3.75 (s, 6H), 3.74 (s, 1H), 3.57 (s, 1H), 2.71–2.70 (m, 2H), 2.39–2.34 (m, 1H), 2.31–2.25 (m, 1H), 2.0–2.1 (m, 1H), 1.91–1.75 (m, 4H), 1.39–1.31 (m, 6H), 1.2–1.1 (m, 2H), 0.77 (s, 3H); m/z (relative intensity) 446 (MH^+, 100%); Anal. Calcd. for ($C_{29}H_{37}NO_3$): C, 77.82; H, 8.33; N, 3.13. Found: C, 77.65; H, 8.20; N, 3.33%.

*2.2.35. 2-(3′,4′,5′-Trimethoxybenzylamino)methyl-17-(1′-methylene) estra-1,3,5(10)-triene-3-ol (**14h**)*

Condensation of 2-formyl-17-methylene estratriene **12** (0.4 g, 1.35 mmol) with 3,4,5-trimethoxybenzylamine (0.53 g, 2.7 mmol) followed by sodium borohydride (0.2 g, 5.26 mmol) reduction using the above-mentioned procedure A and subsequent silica gel column chromatography purification (20:80 ethyl acetate/hexane) afforded compound **14h** as white solid (0.33 g, 52%). mp 116–118 °C; IR (KBr, cm^{-1}): 3277 (NH), 1593 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.98 (s, 1H), 6.66–6.63 (m, 2H), 6.41(s, 1H), 4.65–4.63 (d, J = 9.3 Hz, 2H), 3.93–3.78 (m, 9H), 3.72–3.70 (m, 3H), 3.68–3.57 (m, 2H), 2.71–2.70 (m, 2H), 2.4–2.35 (m, 1H), 2.31–2.27 (m, 1H), 2.25–2.23 (m, 1H), 1.9–1.75 (m, 3H), 1.40–1.32 (m, 6H), 1.27–1.1 (m, 2H), 0.77 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.9, 154.8, 152.7, 152.6, 136.2, 135.5, 135.4, 129.9, 125.5, 121.3, 115.1, 105.8, 105.2, 100.9, 59.9, 55.8, 55.6, 52.9, 51.9, 49.6, 43.8, 43.5, 38.4, 35.3, 29.0, 28.8, 27.2, 26.3, 23.4, 18.2; m/z (relative intensity) 476 (MH^+, 100%); Anal. Calcd. for ($C_{30}H_{39}NO_4$): C, 75.44; H, 8.23; N, 2.93. Found: C, 75.22; H, 8.05; N, 2.72%.

*2.2.36. 3-(tert-Butyldimethylsilyl)-2-formyl-17-methylene estratriene (**15**)*

To the solution of 2-formyl 17-methylene estratriene **12** (10.0 g, 32.9 mmol) in DMF (150 mL) at 10 °C was added triethylamine (9.4 g, 93.5 mmol) and stirred for 10 min, a solution of *tert*-butyldimethylsilyl chloride (10 g, 66.3 mmol) in DMF (100 mL) was added slowly. The resulting mixture was further stirred for another 3 h at 10 °C, the reaction mixture was poured into sodium bicarbonate solution (500 mL, 5% w/v) and washed with hexane (2 × 500 mL). The combined organics were washed with brine solution (2 × 200 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give light yellow viscous oil. The crude product was purified by silica gel column chromatography using hexane as an eluent to yield **15** as white solid (12.1 g, 88%). mp 131–133 °C; IR (KBr, cm^{-1}): 1677 (C=O), 1609 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.44 (s, 1H), 7.58 (s, 1H), 6.68 (s, 1H), 4.66–4.64 (d, J = 8.7 Hz, 2H), 3.5 (m, 1H), 2.84–2.82 (m, 2H), 2.34–2.3 (m, 1H), 2.29–2.1 (s, 2H), 1.94–1.81 (m, 3H), 1.5–1.13 (m, 6H), 1.01 (s, 9H), 0.75 (s, 3H),0.25 (s, 6H); m/z (relative intensity) 411 (MH^+, 100%); Anal. Calcd. for ($C_{26}H_{38}O_2Si$): C, 76.04; H, 9.33. Found: C, 76.32; H, 9.13%.

*2.2.37. 2-Benzoyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**16a**)*

Addition of freshly prepared phenylmagnesium bromide [magnesium turnings (0.2 g, 8.52 mmol) and bromobenzne (4 g, 25.5 mmol] in dry THF (10 mL) to silylated aldehyde **15** (1.5 g, 3.66 mmol) followed by manganese dioxide (3.18 g, 36.6 mmol) oxidation, tetrabutylammonium fluoride trihydrate (7.5 g, 36.6 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (4:96 ethyl acetate/hexane) gave compound **16a** as light yellow solid (0.79 g, 58%). mp 145–146 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.60 (s, 1H), 7.7–7.51 (m, 5H), 7.3 (s, 1H), 6.7 (s, 1H), 4.63 (s, 2H), 2.85 (s, 2H), 2.18–2.12 (m, 3H), 1.88–1.85 (m, 3H), 1.7–1.5 (m, 1H), 1.38–1.11 (m, 6H), 0.77 (s, 3H); m/z (relative intensity) 371 (MH^+, 100%); Anal. Calcd. for ($C_{26}H_{28}O_2$): C, 83.83; H, 7.58. Found: C, 83.65; H, 7.40%.

*2.2.38. 2-(4′-Methoxybenzoyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**16b**)*

Addition of freshly prepared 4-methoxyphenylmagnesium bromide [magnesium turnings (200 mg, 8.52 mmol) and 1-bromo-4-methoxybenzene (4.74 g, 25.5 mmol] in dry THF (10 mL) to silylated aldehyde **15** (1.5 g, 3.66 mmol) followed by manganese dioxide (3.18 g, 36.6 mmol) oxidation, tetrabutylammonium fluoride trihydrate (7.5 g, 36.6 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (5:95 ethyl acetate/hexane) afforded compound **16b** as light yellow solid (0.84 g, 57%). mp 112–114 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.40 (s, 1H), 7.71–7.68 (d, J = 8.6 Hz, 2H), 7.27 (s, 1H), 7.07–7.05 (d, J = 8.6 Hz, 2H), 6.68 (s, 1H), 4.63 (s, 2H), 3.85 (s, 3H), 2.83 (s, 2H), 2.27–2.17 (m, 3H), 1.89–1.74 (m, 3H), 1.44–1.33 (m, 7H), 0.8 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 196.8, 162.7, 160.7, 156.3, 143.4, 131.6, 130.7, 130.2, 127.8, 120.7, 116.5, 113.6, 101.0, 55.4, 52.8, 43.7, 43.0, 38.0, 35.0, 29.2, 28.9, 26.7, 25.9, 23.4, 18.1; m/z (relative intensity) 403 (MH^+, 100%); Anal. Calcd. for ($C_{27}H_{30}O_3$): C, 80.56; H, 7.51. Found: C, 80.40; H, 7.74%.

*2.2.39. 2-(4′-Ethoxybenzoyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**16c**)*

Addition of freshly prepared 4-ethoxyphenylmagnesium bromide [magnesium turnings (0.2 g, 8.52 mmol) and 1-bromo-4-ethoxybenzene (5.1 g, 25.5 mmol] in dry THF (10 mL) to silylated aldehyde **15** (1.5 g, 3.66 mmol) followed by manganese dioxide (3.18 g, 36.6 mmol) oxidation, tetrabutylammonium fluoride trihydrate (7.5 g, 36.6 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (5:95 ethyl acetate/hexane) yielded compound **16c** as light yellow solid (0.93 g, 61%). mp 140–142 °C; IR (KBr, cm^{-1}): 1633 (C=O), 1596 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.38 (s, 1H), 7.69–7.67 (d, J = 8.6 Hz, 2H), 7.26 (s, 1H), 7.05–7.02 (d, J = 8.7 Hz, 2H), 6.68 (s, 1H), 4.63 (s, 2H), 4.15–4.1 (q, J = 6.9 Hz, 2H), 2.83 (s, 2H), 2.50 (m 1H), 2.25–2.17 (m, 3H), 1.89–1.76 (m, 3H), 1.44–1.33 (m, 9H), 0.8 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 197.4, 161.9, 160.6, 157.4, 144.1, 131.5, 130.7, 129.9, 128.3, 119.4, 116.6, 113.8, 100.9, 63.4, 52.8, 43.7, 43.1, 38.0, 35.0, 29.3, 28.9, 26.7, 25.9, 23.4, 18.1, 14.4; HRMS Calcd. for $C_{28}H_{32}O_3$ (M): 416.2351. Found: 416.2357.

*2.2.40. 2-(3′,4′-Dimethoxybenzoyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**16d**)*

Addition of freshly prepared 3,4-dimethoxyphenylmagnesium bromide [magnesium turnings (0.2 g, 8.52 mmol) and 4-bromo-1,2-dimethoxybenzene (5.5 g, 25.5 mmol] in dry THF (10 mL) to silylated aldehyde **15** (1.5 g, 3.66 mmol) followed by manganese dioxide (3.18 g, 36.6 mmol) oxidation, tetrabutylammonium fluoride trihydrate (7.5 g, 36.6 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (20:80 ethyl acetate/hexane) afforded compound **16d** as light yellow solid (0.99 g, 63%). mp 137–138 °C; IR (KBr, cm^{-1}): 3158 (OH), 1654 (C=O), 1630 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.45 (s, 1H), 7.34–7.27 (m, 3H), 7.08–7.06 (d, J = 8.3 Hz, 1H), 6.69 (s, 1H), 4.63 (s, 2H), 3.85 (s, 3H), 3.80 (s, 3H), 2.84 (s, 2H), 2.5 (m, 1H), 2.21–2.17 (m, 3H), 1.89–1.86 (m, 3H), 1.41–1.33 (m, 6H), 0.78 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 196.8, 160.7, 156.5, 152.6, 148.4, 143.5, 130.7, 130.1, 127.9, 124.4, 120.4, 116.5, 111.6, 110.5, 101.0, 55.6, 55.4, 52.8, 43.7, 43.0, 38.0, 35.0, 29.2, 28.9, 26.7, 26.0, 23.4, 18.1; m/z (relative intensity) 431 (MH^+, 100%); Anal. Calcd. for ($C_{28}H_{32}O_4$): C, 77.75; H, 7.46. Found: C, 77.51; H, 7.30%.

*2.2.41. 2-(3′,4′,5-Trimethoxybenzoyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**16e**)*

Addition of freshly prepared 3,4,5-trimethoxyphenylmagnesium bromide [magnesium turnings (0.2 g, 8.52 mmol) and 5-bromo-1,2,3-trimethoxybenzene (6.3 g, 25.5 mmol] in dry THF (10 mL) to silylated aldehyde **15** (1.5 g, 3.66 mmol) followed by manganese dioxide (3.18 g, 36.6 mmol) oxidation, tetrabutylammonium fluoride trihydrate (7.5 g, 36.6 mmol) mediated desilylation using the procedure B as mentioned above and subsequent silica gel column chromatography purification (20:80 ethyl acetate/hexane) furnished compound **16e** as light yellow solid (0.81 g, 48%). mp 65–68 °C; IR (KBr, cm^{-1}): 1630 (C=O), 1569 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.72 (s, 1H), 7.4 (s, 1H), 7.02 (s, 2H), 6.72 (s, 1H), 4.63 (s, 2H), 3.8 (s, 6H), 3.77 (s, 3H), 2.87–2.85 (d, J = 7.4 Hz, 2H), 2.5 (m, 1H), 2.23–2.18 (m, 3H), 1.89–1.87 (m, 3H), 1.43–1.33 (m, 6H), 0.77 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 197.3, 160.7, 157.2, 152.5, 144.5, 141.2, 132.7, 130.9, 128.5, 119.4, 116.7, 107.0, 101.0, 60.1, 56.0, 52.8, 43.7, 43.0, 38.0, 35.0, 29.2, 29.0, 26.6, 26.1, 23.4, 18.1; m/z (relative intensity) 461 (MH^-, 100%); Anal. Calcd. for ($C_{29}H_{34}O_5$): C, 75.30; H, 7.41. Found: C, 75.04; H, 7.70%.

*2.2.42. (2E)-3-(3-Hydroxy-17(1′-methylene)-1,3,5(10)-estratrienyl)-1-(3′-methoxyphenyl)prop-2-en-1-one (**17a**)*

Condensation of 2-formyl-17-methylene estratriene **12** (750 mg, 2.53 mmol) and 3′-methoxyacetophenone (500 mg, 3.33 mmol) using the above-mentioned procedure C followed by silica gel column chromatographic purification (10:90 ethyl acetate/hexane) afforded **17a** as light yellow solid (542 mg, 50%). mp 220–219 °C; IR (KBr, cm^{-1}): 3272 (OH), 1636 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.04 (d, J = 15.7 Hz, 1H), 7.8–7.71 (d, J = 15.7 Hz, 1H), 7.73–7.70 (d, J = 9.8 Hz, 2H), 7.55 (s, 1H), 7.5–7.46 (m, 1H), 7.24–7.21 (m, 1H), 6.62 (s, 1H), 4.68–4.65 (d, J = 12.2 Hz, 2H), 3.84 (s, 3H), 3.56–3.51 (m, 1H), 2.77 (s, 2H), 2.65–2.55 (m, 1H), 2.3–2.15 (m, 2 H), 1.95–1.7 (m, 3H), 1.43–1.1 (m, 6H), 0.8 (s, 3H); m/z (relative intensity) 427 (MH^-, 100%); Anal. Calcd. for ($C_{29}H_{32}O_3$): C, 81.27; H, 7.53. Found: C, 81.06; H, 7.37%.

*2.2.43. (2E)-3-(3-Hydroxy-17(1′-methylene)-1,3,5(10)-estratrienyl)-1-(4′-ethoxyphenyl)prop-2-en-1-one (**17b**)*

Condensation of 2-formyl-17-methylene estratriene **12** (0.75 g, 2.53 mmol) and 4′-ethoxyacetophenone (0.55 g, 3.33 mmol) using the above-mentioned procedure C followed by silica gel column chromatographic purification (10:90 ethyl acetate/hexane) and subsequent trituration with diisopropyl ether afforded **17b** as pale yellow solid (0.62 g, 55%). mp 221–222 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.91 (s, 1H), 8.10–8.09 (d, J = 8.6 Hz, 2H), 7.97 (m, 1H), 7.84 (m, 1H), 7.7 (s, 1H), 7.07–7.05 (d, J = 8.6 Hz, 2H), 6.62 (s, 1H), 4.68–4.65 (d, J = 13.0 Hz, 2H), 4.15–4.13 (m, 2H), 2.77 (s, 2H), 2.7–2.55 (m, 2H), 2.35–2.15 (m, 2H), 1.95–1.7 (m, 3H), 1.41–1.1 (m, 9H), 0.8 (s, 3H); m/z (relative intensity) 441 (MH^+, 100%); Anal. Calcd. for ($C_{30}H_{34}O_3$): C, 81.41; H, 7.74. Found: C, 81.23; H, 7.93%.

*2.2.44. (2E)-3-(3-Hydroxy-17(1′-methylene)-1,3,5(10)-estratrienyl)-1-(2′-trifluoromethylphenyl) prop-2-en-1-one (**17c**)*

Condensation of 2-formyl-17-methylene estratriene **12** (0.75 g, 2.53 mmol) and 2′-trifluoromethylacetophenone (0.62 g, 3.33 mmol) using the above-mentioned procedure C followed by silica gel column chromatographic purification (6:94 ethyl acetate/hexane) and subsequent trituration with diisopropyl ether afforded **17c** as light yellow solid (0.79 g, 67%). mp 208–210 °C; IR (KBr, cm^{-1}): 3321 (OH), 1655 (C=O), 1612 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.00 (s, 1H), 7.88–7.86 (d, J = 7.7 Hz, 1H), 7.82–7.75 (m, 2H), 7.73–7.71 (m, 1H), 7.61–7.59 (d, J = 7.7 Hz, 1H), 7.54–7.48 (m, 2H), 7.24–7.20 (m, 1H), 6.59 (s, 1H), 4.66–4.63 (d, J = 10.5 Hz, 2H), 2.75 (s, 2H), 2.47 (s, 1H), 2.27–2.22 (m, 1H), 2.18–2.1 (m, 1H),1.91–1.72 (m, 3H), 1.42–1.12 (m, 6H), 0.77 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 194.5, 160.8, 155.1, 143.7, 142.0, 132.3, 131.5, 130.0, 128.2, 126.6, 126.5, 125.7, 124.6, 118.2, 115.9, 101.0, 52.9, 43.7, 43.2, 38.1, 35.1, 29.2, 29.0, 26.8, 25.9, 23.4, 18.2; HRMS Calcd. for $C_{29}H_{29}F_3O_2$ (M): 466.2120. Found: 466.2129.

*2.2.45. (2E)-3-(3-Hydroxy-17-(1′-methylene)-1,3,5(10)-estratrienyl)-1-(2′,4′,6′-trimethoxyphenyl) prop-2-en-1-one (**17d**)*

Condensation of 2-formyl-17-methylene estratriene **12** (0.75 g, 2.53 mmol) and 2′,4′,6′-trimethoxyacetophenone (0.7 g, 3.33 mmol) using the above-mentioned procedure C followed by silica gel column chromatographic purification (14:86 ethyl acetate/hexane) and subsequent trituration with diisopropyl ether afforded **17d** as a pale yellow solid (0.56 g, 45%). mp 201–203 °C; IR (KBr, cm^{-1}): 3366 (OH), 1605 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.98 (s, 1H), 7.41 (s, 2H), 6.91–6.87 (m, 1H), 6.66 (s, 1H), 6.30 (s, 2H), 4.66–4.65 (d, J = 7.5 Hz, 2H), 3.85 (s, 6H), 3.70 (s, 3H), 2.75 (s, 2H), 2.47–2.42 (m, 1H), 2.25- 2.11 (m, 2H), 1.92–1.76 (m, 3H), 1.58–1.56 (m, 1H), 1.36–1.23 (m, 6H), 0.77 (s, 3H); m/z (relative intensity) 487 (MH^+, 100%); Anal. Calcd. for ($C_{31}H_{36}O_5$): C, 76.20; H, 7.43. Found: C, 76.44; H, 7.20%.

*2.2.46. (2E)-3-(3-Hydroxy-17(1-methylene)-1,3,5(10)-estratrienyl)-1-(3′,4′,5′-trimethoxyphenyl) prop-2-en-1-one (**17e**)*

Condensation of 2-formyl-17-methylene estratriene **12** (0.75 g, 2.53 mmol) and 3′,4′, 5′-trimethoxyacetophenone (0.7 mg, 3.33 mmol) using the above-mentioned procedure C followed by silica gel column chromatographic purification (40:60 ethyl acetate/hexane) and subsequent trituration with diisopropyl ether yielded **17e** as a pale yellow solid (0.76 g, 52%). mp 204–206 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.04–8.00 (d, J = 15.6 Hz, 1H), 7.81–7.77 (d, J = 15.7 Hz, 1H), 7.69 (s, 1H), 7.36 (s, 2H), 6.62 (s, 1H), 4.68–4.64 (d, J = 14.7 Hz, 2H), 3.89 (s, 6H), 3.76 (s, 3H), 3.17–3.19 (d, J = 5.2 Hz, 1H), 2.46 (m, 1H), 2.77 (s, 2H), 2.26–2.15 (m, 2H), 1.95–177 (m, 3H), 1.46–1.21 (m, 6H), 0.77 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 188.4, 160.9, 155.1, 152.8, 141.7, 141.2, 140.2, 133.6, 131.3, 125.6, 119.8, 119.1, 115.8, 106.0, 101.0, 60.1, 56.1, 52.9, 43.8, 43.4, 35.2, 29.2, 29.0, 26.9, 26.0, 23.4, 18.3; m/z (relative intensity) 487 (MH$^+$, 100%); Anal. Calcd. for ($C_{31}H_{36}O_5$): C, 76.20; H, 7.43. Found: C, 76.01; H, 7.59%.

*2.2.47. Representative procedure for preparation 2-ethenyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol **18a** (Procedure E)*

To a suspension of methyltriphenylphosphonium iodide (3.41 g, 8.44 mmol) in anhydrous THF (25 mL) at 0 °C under nitrogen was added 1 M solution of Li[N(SiMe$_3$)$_2$] (11 mL, 11 mmol) in THF and stirred for 10 min at 0 °C to get yellow clear solution. To this, compound **12** (0.5 g 1.69 mmol) in anhydrous THF (5 mL) was added and the turbid mixture was stirred for 4 h at room temperature. The resulting mixture was poured into the mixture of ethyl acetate/water (150 mL, 3:1), and acidified with 5 N HCl to pH 5.0. The organic layer was washed with brine (2 × 25 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give yellow viscous oil. The crude product was purified by silica gel column chromatography using (4:96 ethyl acetate/hexane) as an eluent to yield compound **18a** as colorless liquid (0.4 g, 81%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.27 (s, 1H), 7.29 (s, 1H), 6.9–6.83 (m, 1H), 6.51 (s, 1H), 5.73–5.68 (m, 1H), 5.11–5.08 (d, J = 11.3 Hz,1H), 4.66–4.63 (d, J = 9.9 Hz, 2H), 2.74–2.7 (m, 2H), 2.25–2.12 (m, 4H), 1.98–1.73 (m, 3H), 1.38–1.23 (m, 6H), 0.78 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.0, 152.5, 137.0, 132.2, 130.5, 122.9, 121.4, 115.4, 112.2, 101.0, 59.7, 52.9, 43.8, 38.4, 35.2, 29.0, 27.1, 26.1, 23.4, 20,7, 18.3, 14.0; m/z (relative intensity) 293 (MH$^+$, 100%); Anal. Calcd. for ($C_{21}H_{26}O$): C, 85.67; H, 8.90. Found: C, 85.95; H, 8.71%.

*2.2.48. 2-(E)-Propenyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**18b**)*

Wittig reaction of aldehyde **12** (0.5 g, 1.69 mmol) with ethyltriphenylphosphonium iodide (3.53 g, 8.45 mmol) using the above-mentioned procedure E followed by column chromatographic purification (3:97 ethyl acetate/hexane) afforded pure *trans*-isomer **18b** as a colorless liquid (0.45 g, 86%). IR (KBr, cm^{-1}): 3343 (OH), 1615 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.11 (s, 1H), 7.19 (s, 1H), 6.56–6.52 (d, J = 15.9 Hz, 1H), 6.47 (s, 1H), 6.17–6.12 (m, 1H), 4.65–4.63 (d, J = 9.4 Hz, 2H), 2.72–2.67 (m, 2H), 2.26–2.12 (m, 4H), 198–1.72 (m, 6H), 1.42–1.11 (m, 6H), 0.77 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.9, 151.9, 135.5, 130.3, 126.6, 123.0, 122.7, 121.6, 115.2, 100.8, 52.9, 43.8, 43.4, 38.4, 35.2, 29.0, 28.8, 27.2, 26.2, 23.4, 18.7, 18.2; m/z (relative intensity) 307 (MH$^+$, 100%); Anal. Calcd. for ($C_{22}H_{28}O$): C, 85.66; H, 9.15. Found: C, 85.87; H, 9.23%.

*2.2.49. Ethyl-2-(E)-2″-acryl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**18c**)*

A solution of aldehyde **12** (0.75 g, 2.53 mmol) and ethyl(triphenylphosphoranylidene)acetate (1.32 g, 3.8 mmol) in dry THF (50 mL) was heated at reflux for 10 h. Removal of solvent followed by silica gel column chromatography purification (5:95 ethyl acetate/hexane) yielded **18c** as a colorless solid (0.77 g, 83%). mp 227–229 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.94 (s, 1H), 7.85–7.81 (d, J = 16.1 Hz, 1H), 7.47 (s, 1H), 6.62–6.58 (m, 1H), 6.47 (s, 1H), 4.69–4.66 (d, J = 12.0 Hz, 2H), 4.19–4.15 (m, 2H), 2.77–2.74 (m, 2H), 2.35–1.76 (m, 7H), 1.48–1.17 (m, 9H), 0.78 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.9, 160.9, 154.6, 140.7, 140.6, 131.2, 125.6, 118.3, 115.9, 115.7, 101.0, 59.6, 52.9, 43.8, 43.3, 38.2, 35.2, 29.0, 26.9, 25.9, 23.4, 18.2, 14.2; m/z (relative intensity) 365 (MH$^+$, 100%); Anal. Calcd. for ($C_{24}H_{30}O_3$): C, 78.65; H, 8.25. Found: C, 78.68; H, 8.02%.

*2.2.50. 2-(E)-3′-Hydroxy-1′-propenyl-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**18d**)*

A 1 M solution of DIBAL in toluene (10 mL, 10 mmol) was slowly added to a solution of ester **18c** (0.75 g, 2.05 mmol) in dry THF (10 mL) at 10 °C under nitrogen. After the addition was completed, the reaction mixture was stirred at the same temperature for 1 h. It was then poured into the mixture of ethyl acetate/water (100 mL, 3:1). The organic layer was washed with brine (2 × 25 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give yellow viscous oil. The crude product was purified by silica gel column chromatography (30:70 ethyl acetate/hexane) to yield compound **18d** as yellow solid (0.52 g, 78%). mp 171–173 °C; IR (KBr, cm^{-1}): 3386 (OH), 1616 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.27 (s, 1H), 7.29 (s, 1H), 6.76–6.72 (d, J = 16.0 Hz, 1H), 6.55 (s, 1H), 6.34–6.28 (m, 1H), 4.80–4.77 (m, 1H), 4.71–4.69 (d, J = 10.3 Hz, 2H), 4.13–4.07 (m, 2H), 2.79–2.76 (m, 2H), 2.35–1.76 (m, 7H), 1.48–1.17 (m, 6H), 0.83 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.9, 152.3, 136.1, 130.5, 128.7, 124.5, 123.1, 121.0, 115.3, 101.0, 62.2, 52.9, 43.8, 43.4, 38.4, 35.2, 29.0, 28.8, 27.2, 26.2, 23.4, 18.2; HRMS Calcd. for $C_{22}H_{28}O_2$ (M): 324.2089. Found: 324.2086.

*2.2.51. 2-(E)-3′4′,5′-Trimethoxyphenyl-1′-vinly1-17-(1′-methylene) estra-1,3,5(10)-triene-3-ol (**18e**)*

Wittig reaction of aldehyde **12** (0.5 g, 1.69 mmol) with 3,4,5-trimethoxybenzyl triphenylphosphonium bromide (4.41 g, 8.45 mmol) was performed using the above-mentioned procedure E except that the reaction time was extended up to 12 h. The usual workup followed by column chromatographic purification (15:85 ethyl acetate/hexane) afforded pure *trans*-isomer **18e** as a white solid (0.61 g, 79%). mp 215–217 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (s, 1H), 7.44 (s, 1H), 7.30 (d, 1H), 7.26 (s, 1H), 6.84 (s, 2H), 6.55 (s, 1H), 4.67–4.64 (d, J = 12.0 Hz, 2H), 3.82 (s, 6H), 3.66 (s, 3H), 2.74–2.7 (m, 2H), 2.26–2.15 (m, 3H), 198–1.78 (m, 4H), 1.48–1.2 (m, 6H), 0.78 (s, 3H); m/z (relative intensity) 459 (MH$^+$, 100%); Anal. Calcd. for ($C_{30}H_{36}O_4$): C, 78.23; H, 7.88. Found: C, 78.06; H, 7.63%.

*2.2.52. Preparation of 3-hydroxy-17α-methyl estratriene (**19**)*

The solution of 17-methylene estratriene **11** (5.0 g, 18.6 mmol) in methanol (200 mL) was hydrogenated under 5 kg hydrogen pressure for 3 h at RT. The mixture was filtered through celite powder and concentrated via rotary evaporation to yield the title compound **19** as white solid (5 g, 99%). mp 133–134 °C ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.99 (s, 1H), 7.06–7.04 (d, J = 8.5 Hz, 1H), 6.65–6.49 (m, 1H), 6.44 (s, 1H), 2.80 (s, 2H), 2.28–2.25 (m, 2H), 2.1 (m, 1H), 1.81–1.76 (m, 4H), 1.45–1.24 (m, 7H), 0.85 (s, 3H), 0.6 (s, 3H); m/z (relative intensity) 271 (MH$^+$, 100%); Anal. Calcd. for ($C_{19}H_{26}O$): C, 84.39; H, 9.69. Found: C, 84.20; H, 9.93%.

*2.2.53. Preparation of 2-formyl-3-hydroxy-17α-methyl estratriene (**20**)*

To the suspension of magnesium turnings (2.1 g, 86.4 mmol) in THF (20 mL) was added solution of bromoethane (13.1 g, 120 mmol) in THF (10 mL) slowly at RT during 20 min and stirred for another 30 min at RT. To the resulting clear solution was added the solution of methyl compound **19** (4 g, 14.5 mmol) in THF (40 mL) at RT and further stirred for 30 min. To the resulting suspension was added hexamethylphosphoramide (7.4 g, 41.3 mmol) followed by paraformaldedhyde (7 g, 233 mmol) and heated to 60 °C for 20 h. The resulting yellow reaction mixture was poured in to water (100 mL) and washed with ethyl acetate (2 × 100 mL) at pH 4.0. The combined organics were washed with brine (2 × 25 mL) and concentrated via rotary evaporation to give yellowish oil. The crude product was suspended in methanol (80 mL) at 10 °C and added sodium hydroxide solution (20% w/v, 8 mL) and stirred for 1 h. The resulting yellow clear solution was poured into the water (160 mL) and washed with ethyl acetate (2 × 150 mL) at pH 4.0. The combined organics were washed with brine (2 × 50 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give light yellow viscous oil. The crude product was purified by silica gel column chromatography using hexane as an eluent to yield 2-formyl methyl compound **20** as off white solid (3.65 g, 83%). mp 194–196 °C ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.42 (s, 1H), 10.13 (s, 1H), 7.56 (s, 1H), 6.67 (s, 1H), 2.8 (s, 2H), 2.28–2.25 (m, 1H), 2.1 (m, 1H), 1.81–1.76 (m, 4H), 1.45–1.24 (m, 8H), 0.85 (s, 3H), 0.60 (s, 3H); *m*/*z* (relative intensity) 297 (MH^+, 100%); Anal. Calcd. for ($C_{20}H_{26}O_2$): C, 80.50; H, 8.78. Found: C, 80.75; H, 8.59%.

*2.2.54. 2-(N,N-Dimethylhydrazinimino)methyl-17-(1′-methyl)estra-1,3,5(10)-triene-3-ol (**21a**)*

Condensation of 2-formyl compound **20** (0.2 g, 0.67 mmol) with *N*,*N*-dimethylhydrazine (52 mg, 0.87 mmol) using the above-mentioned procedure D followed by trituration of the crude product with diisopropyl ether afforded compound **21a** as a white solid (0.2 g, 87%). mp 208–209 °C; IR (KBr, cm^{-1}): 3360 (OH), 1590 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.0 (s, 1H), 7.6 (s, 1H), 7.24 (s, 1H), 6.5 (s, 1H), 2.85 (s, 6H), 2.74 (s, 2H), 2.30–2.27 (m, 1H), 2.12 (m, 1H), 1.80–1.68 (m, 6H), 1.47–1.23 (m, 6H), 0.8 (s, 3H), 0.55 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.1, 137.9, 137.4, 131.0, 125.5, 116.9, 115.3, 54.2, 44.5, 43.3, 42.4, 41.7, 38.5, 36.9, 29.7, 29.1, 27.3, 26.0, 23.9, 13.5, 11.6; *m*/*z* (relative intensity) 341 (MH^+, 100%); Anal. Calcd. for ($C_{22}H_{32}N_2O$): C, 77.60; H, 9.47; N, 8.23. Found: C, 77.79; H, 9.20; N, 8.02%.

*2.2.55. 2-(2′-(N,N-Dimethylaminoethyl)imino)methyl-17-(1′-methyl)estra-1,3,5(10)-triene-3-ol (**21b**)*

Condensation of 2-formyl compound **20** (0.2 g, 0.67 mmol) with *N*,*N*-dimethylethylenediamine (76 mg, 0.87 mmol) using the above-mentioned procedure D followed by trituration of the crude product with diisopropyl ether afforded compound **21b** as a white solid (0.2 g, 81%). mp 130–132 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.1 (s, 1H), 8.31 (s, 1H), 7.26 (s, 1H), 7.14 (s, 1H), 6.68 (s, 1H), 3.7 (m, 2H), 2.86–2.85 (m, 2H), 2.63–2.59 (m, 2H), 2.29 (s, 6H), 2.17–2.15 (m, 1H), 1.89–1.69 (m, 6H), 1.5–1.1 (m, 6H), 0.88 (s, 3H), 0.58 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.7, 158.1, 141.2, 130.6, 127.9, 116.4, 115.7, 59.5, 56.7, 54.2, 45.2, 44.5, 43.2, 41.8, 38.4, 36.8, 29.7, 29.3, 27.2, 26.0, 23.9, 13.7, 11.7; HRMS Calcd. for $C_{24}H_{36}N_2O$ (M): 368.2828. Found: 368.2821.

*2.2.56. 2-(Cyclopropylimino) methyl-17-(1′-methyl)estra-1,3,5(10)-triene-3-ol (**21c**)*

Condensation of 2-formyl compound **20** (0.2 g, 0.67 mmol) with cyclopropylamine (50 mg, 0.87 mmol) using the above-mentioned procedure D followed by trituration of the crude product with diisopropyl ether afforded compound **21c** as a white solid (0.19 g, 84%). mp 152.5–153 °C; IR (KBr, cm^{-1}): 3432 (OH), 1623 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.4 (s, 1H), 8.43 (s, 1H), 7.25 (s, 1H), 7.11 (s, 1H), 6.64 (s, 1H), 2.94–2.91 (m, 1H), 2.85–2.82 (m, 2H), 2.29–2.17 (m, 3H), 1.89–1.17 (m, 4H), 1.55–1.37 (m, 10H), 0.88 (s, 3H), 0.6 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.6, 157.2, 140.6, 130.8, 127.4, 116.7, 115.5, 54.2, 44.5, 43.3, 41.8, 38.4, 36.8, 29.7, 29.3, 27.2, 26.0, 23.9, 13,7, 11.7, 8.8; *m*/*z* (relative intensity) 338 (MH^+, 100%); Anal. Calcd. for ($C_{23}H_{31}NO$): C, 81.85; H, 9.26; N, 4.15. Found: C, 81.70; H, 9.03; N, 4.39%.

*2.2.57. 2-(Hydroxyethylimino)methyl-17-(1′-methyl)estra-1,3,5(10)-triene-3-ol (**21d**)*

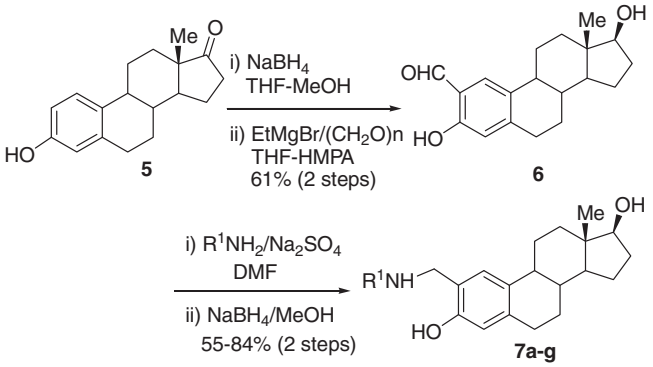
Condensation of 2-formyl compound **20** (0.2 g, 0.67 mmol) with ethanolamine (53 mg, 0.87 mmol) using the above-mentioned procedure D followed by trituration of the crude product with diisopropyl ether afforded compound **21d** as a white solid (0.18 g, 79%). mp 195–197 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.86 (s, 1H), 8.36 (s, 1H), 7.26 (s, 1H), 7.16 (s, 1H), 6.68 (s, 1H), 3.92–3.89 (t, *J* = 5.1 Hz, 2H), 3.74–3.72 (t, *J* = 5.1 Hz, 2H), 2.87 (s, 2H), 2.28–2.18 (m, 2H), 1.90–1.17 (m, 10H), 0.82 (s, 3H), 0.6 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.5, 158.2, 141.3, 130.6, 128.1, 116.7, 115.7, 61.2, 60.7, 54.2, 44.5, 43.2, 41.8, 38.5, 36.8, 29.7, 29.2, 27.2, 26.0, 24.0, 13.8, 11.8; *m*/*z* (relative intensity) 342 (MH^+, 100%); Anal. Calcd. for ($C_{22}H_{31}NO_2$): C, 77.38; H, 9.15; N, 4.10. Found: C, 77.18; H, 9.39; N, 4.32%.

3. Results and discussion

3.1. Chemical synthesis

Commercially available estrone **5** upon $NaBH_4$ reduction followed by selective 2-formylation of resulting 17β-estradiol using non-lithiation protocol [15] led to aldehyde **6**, which on condensation with various amines/amino acid ethyl esters followed by reduction afforded amino derivatives **7a–g**, Scheme 1.

As per the objective, the synthesis of estradiol derivatives containing aroyl units at the 2-position was initiated. Accordingly, interaction of the aldehyde **8** with freshly prepared aryl Grignards



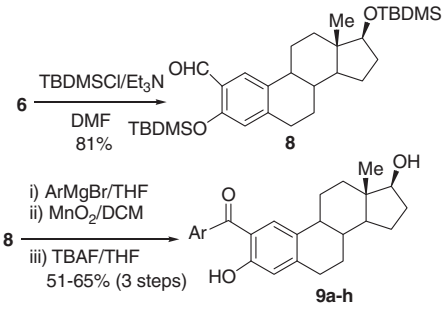
Compound	R^1	Yield (%)
7a	$(CH_2)_2NMe_2$	60
7b	$(CH_2)_3NMe_2$	63
7c	Cyclopropyl	84
7d	CH_2CO_2Et	55
7e	$CH(Et)CO_2Et$	66
7f	CH_2CH_2OH	77
7g	$CH(Et)CH_2OH$	73

Scheme 1. Synthesis of 2-alkylaminomethyl 17β-estradiols **7a–g**.

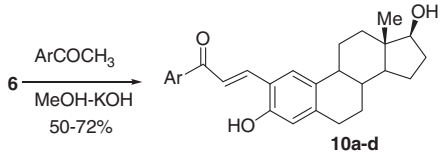
followed by MnO_2 oxidation and subsequent desilylation gave 2-aroyl estradiols **9a**–**h**, Scheme 2. Next, the synthesis of vinyl tethered 2-aroyl estradiols was planned. As expected, condensation of 17β-estradiol-2-carboxaldehyde **6** with substituted acetophenones [16] using methanolic KOH as a base, furnished *trans*-chalcones **10a**–**d**.

Since, recent reports identified that the replacement of 17β-OH group with a methylene unit exhibited maximum cytotoxicity [3d,10], a synthesis of 2-substituted estratriene derivatives containing an exocyclic methylene at C-17 was planned. Accordingly, the Wittig reaction of estrone **5** with methyltriphenylphosphonium iodide using NaH as a base in dry DMSO, afforded **11**, which on regio-selective formylation as mentioned in the case of 17β-estradiol [15] gave 17-methylene aldehyde **12**. The condensation of aldehyde **12** with amines led to the isolation of 17-methylene imine analogs **13a**–**d**. Alternatively, condensation of **12** with amines followed by $NaBH_4$ reduction afforded 2-alkylaminomethyl estradiol analogs **14a**–**h**, Scheme 3.

The preparation of additional analogs of 17-methylene estratrienes is depicted in Scheme 4. TBDMS protection of phenolic OH group of **12** furnished **15**. Addition of arylmagnesium bromides to aldehyde **15**, followed by oxidation and subsequent de-silylation, produced the 17-methylene 2-aroyls estratrienes **16a**–**e**. Condensation of **12** with substituted acetophenones [16] using methanolic KOH furnished vinyl tethered 17-methylene 2-aroyl estratrienes **17a**–**e**. The Wittig reaction of aldehyde **12** with alkyl phosphonium salts using LiHMDS as base yielded 2-substituted 17-methylene estratrienes **18a**–**e**, Scheme 4.

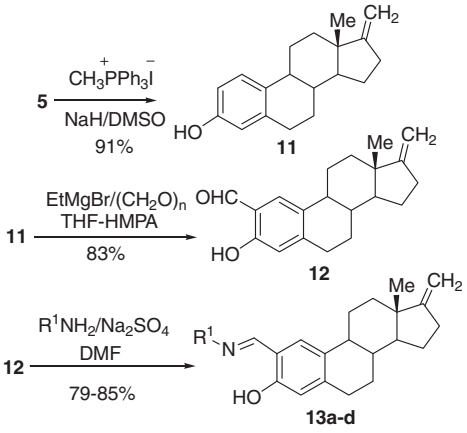


Compound	Ar	Yield (%)
9a	phenyl	63
9b	*p*-tolyl	60
9c	4-fluorophenyl	64
9d	*p*-anisyl	65
9e	4-ethoxyphenyl	63
9f	3,4-dimethoxyphenyl	53
9g	3,5-dimethoxyphenyl	55
9h	3,4,5-trimethoxyphenyl	51

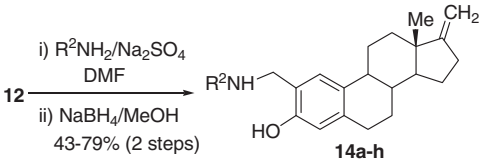


Compound	Ar	Yield (%)
10a	*m*-anisyl	50
10b	2-trifluoromethyl	52
10c	2,4,6-trimethoxyphenyl	72
10d	3,4,5-trimethoxyphenyl	69

Scheme 2. Synthesis of 2-aroyl and 2-aroylvinyl 17β-estradiols.



Compound	R^1	Yield (%)
13a	$(CH_3)_2N$	82
13b	$(CH_2)_2NMe_2$	79
13c	cyclopropyl	83
13d	CH_2CH_2OH	85



Compound	R^2	Yield (%)
14a	$(CH_2)_2NMe_2$	60
14b	$(CH_2)_3NMe_2$	43
14c	cyclopropyl	76
14d	CH_2CH_2OH	79
14e	$CH(Me)_2CH_2OH$	67
14f	$CH(Et)CH_2OH$	71
14g	2,4-dimethoxybenzyl	55
14h	3,4,5-trimethoxybenzyl	52

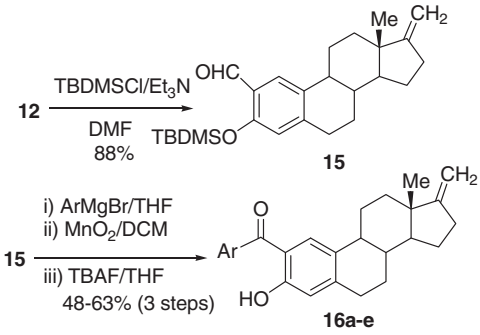
Scheme 3. Synthesis of 2-imino and 2-alkylaminomethyl 17-methylene estratrienes.

Finally, 17α-methyl-2-iminoestratrienes **21a**–**d** were prepared using the routine procedure as mentioned in Scheme 5. As expected, heterogenous reduction of 17-methylene unit of **11** produced compound **19**. A selective 2-formylation of **19** using non-lithiation protocol [15] led to aldehyde **20**, which on condensation with various amines provided 17α-methyl 2-iminoestratriene derivatives **21a**–**d**, Scheme 5.

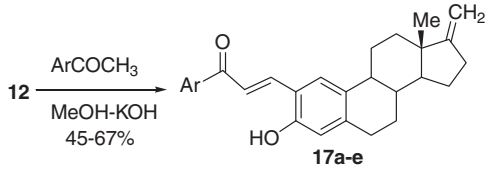
3.2. Antiproliferative activity

The synthesized 2-substituted estratriene analogs were evaluated for their antiproliferative activities against four different human cell lines of diverse tumour origin. The *in vitro* GI_{50} (growth inhibition) values of estratrienes against individual cell lines are presented in Table 1. The MGM values were calculated by averaging the GI_{50} values of the four cell lines tested. It should be noted that in those cases wherein growth inhibition values for one or more cell lines are >100 μM, the respective MGM values are not calculated.

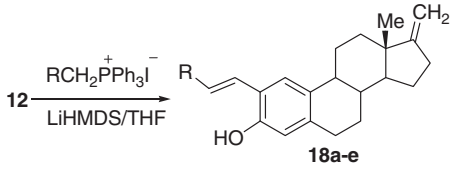
The Table 1 also includes antiproliferation values of the most active reference compounds, 2-methoxyestradiol **1** [3a], 2-ethoxyestradiol **2** [3a], 2-ethoxy-17-methylene estratriene-3-ol **3** [3d], and 2-methoxy-17-methylene estratriene-3-ol **4** [10]. The



Compound	Ar	Yield (%)
16a	phenyl	58
16b	*p*-anisyl	57
16c	4-ethoxyphenyl	61
16d	3,4-dimethoxyphenyl	63
16e	3,4,5-trimethoxyphenyl	48

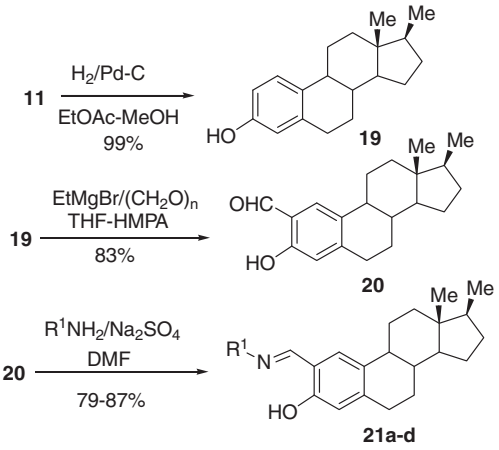


Compound	Ar	Yield (%)
17a	3-methoxyphenyl	50
17b	4-ethoxyphenyl	55
17c	3-trifluoromethylphenyl	67
17d	2,4,6-trimethoxyphenyl	45
17e	3,4,5-trimethoxyphenyl	52



Compound	R	Yield (%)
18a	H	81
18b	Me	86
18c	CO_2Et	83
18d	CH_2OH	78
18e	3,4,5-trimethoxyphenyl	79

Scheme 4. Synthesis of 2-aroyl, 2-aroylvinyl and 2-vinyl 17-methylene estratrienes.



Compound	R^1	Yield (%)
21a	Me_2N	87
21b	$(CH_2)_2NMe_2$	81
21c	cyclopropyl	84
21d	CH_2CH_2OH	79

Scheme 5. Synthesis of 2-imino 17β-methyl estratrienes **21a–d**.

compounds containing 2-alkylamino side chain **7a–g** were found to have moderate inhibitory effects (MGM 23–50 μM) on cell proliferation. The compound **7d**/**7e** containing an electron withdrawing ester unit in the 2-alkylamino side chain was found to be completely inactive (>100 μM). Although, it has been confirmed that the electron releasing substituents at 2-position of estratrienes are favorable for growth inhibition [3], the moderate activity displayed by the estratrienes **7a–c**, **7f** and **7g** are surprising. It should be noted that the corresponding unsaturated imine analogs of **7a–c** exhibited comparatively better antiproliferative activity with MGM value of ∼16 μM [3c,3d]. The steric-bulkiness of the 2-substituents of **7a–g** did not have any significant influence on their activity. The compounds **9a–h** possessing an electron withdrawing benzoyl unit at the 2-position exhibited GI_{50} values in the range of 3.8–40 μM. Indeed, electronic withdrawing effect of cyano group imposed on A-ring is responsible for completely inactive nature of 2-cyanoestradiol [3c]. However, moderate activity portrayed by compounds **9a–h** containing an electron withdrawing carbonyl function seems to indicate that the electronic factor alone may not be a sole criteria for the observed activity of 2-substituted estratrienes. The SAR of **9a–h** against the four cell lines indicated that the presence of either varying number of methoxy groups or its absence have only negligible influence on their antiproliferation potential. Among the four chalcone derivatives **10a–d** tested, only **10c** and **10d** containing three methoxy groups displayed antiproliferative activities comparable to that of reference compounds **1–4**. The compound **10d** wherein three methoxy groups are far away from the carbonyl function showed maximum cytotoxicity with an MGM value of 1.8 μM. The remaining two chalcone derivatives **10a**/**10b** containing methoxy/trifluoromethyl unit showed moderate activity (MGM value 6–9 μM). Comparison of SAR of **9h** and **10d** clearly indicated the separation of estradiol and 3,4,5-trimethoxybenzoyl unit using a vinyl tether has significantly enhanced the antiproliferative potential.

Among the four imine derivatives **13a–d** tested for their antiproliferative potential, three of them showed comparable activity. All the eight 2-alkylamino side chain compounds **14a–h** containing 17-methylene displayed growth inhibition values (MGM ∼2 μM) comparable to that of the reference compounds **1–4**. Contrary to the 17β-OH containing 2-alkylamino compounds **7a–g**, in the case of 17-methylene system **13a–d** reduction of imine-double bond led to a significant increase in the antiproliferative potential. In particular, saturation of imine unit of **13a** led to dramatic increase in the cytotoxicity value (from MGM 53.7 to 2.3 μM). Most of the 17-methylene compounds **16a–c** and **16e** possessing an electron withdrawing benzoyl unit at the 2-position were found to be inactive. Surprisingly, the transformation of the 17β-OH of **9f** into the exocyclic methylene compound **16d** has significantly enhanced its activity in HCT-116 and U-251 human cancer cell lines. To reach any further conclusions, however, the observed selectivity pattern needs to be further verified with additional estratriene analogs. The 17-methylene chalcone derivatives **17a–d** did not show any significant cytotoxicity in all the four cancer lines tested. However, a 17-methylene chalcone derivative **17e** containing 3,4,5-trime-

Table 1
Antiproliferative assays of 2-substituted estradiol analogs.

Compound	Colon HCT-116	Lung NCIH-460	Glioma U-251	Breast MDA MB-435	MGM[b]
Growth inhibition (GI_{50} in μM)[a]					
1	0.47	0.7	0.36	0.08	0.4
2	0.26	0.18	0.016	<0.01	0.058
3	0.6	0.56	0.54	0.08	0.44
4	0.65	0.6	0.62	0.15	0.54
7a	25	30	25	10	22.5
7b	52.5	38	60	ND	50.2
7c	38	14	20	20	23
7d	>100	41	>100	ND	
7e	52	23	>100	ND	
7f	46.5	14.5	21	25	26.7
7g	40	10	39	21	27.5
9a	13	20	15	20	17
9b	14	17	17	21	17.2
9c	14	15	13	29	17.7
9d	16	19	19	ND	18
9e	12	17	17	26	18
9f	3.8	19	13	18	13.4
9g	13	28	40	ND	27
9h	12	16	15	20	15.7
10a	10	5	7	3	6.2
10b	9	7	18	15	9.1
10c	1.8	4.5	4.8	8	4
10d	1.9	2.2	2.1	2	1.8
13a	35	100	50	30	53.7
13b	2.5	2.5	2.9	2.1	2.5
13c	3.2	6.8	38	8.9	5.7
13d	3	3.6	3	4.8	3.6
14a	2.5	2.8	2.2	1.8	2.3
14b	2	0.9	1.9	1.6	1.6
14c	1.8	2.4	1.8	1.5	1.9
14d	2	1.8	1.8	2	1.9
14e	1.8	1.7	2.3	1.6	2
14f	2	2.2	2.8	2.8	2.4
14g	1.7	2.3	1.9	2	2
14h	2	2.2	2.5	2.2	2.2
16a	70	>100	>100	>100	
16b	50	>100	>100	>100	
16c	45	>100	>100	>100	
16d	0.45	50	0.6	30	20.2
16e	>100	70	30	60	
17a	34	14	>100	18	
17b	62	3.2	>100	24	
17c	15	18	19	18	17.5
17d	8	6	15	14	10.8
17e	1.6	2.6	1.4	1.6	1.8
18a	18	17	21	1.5	14.4
18b	19	17	16	1.2	13.3
18c	>100	>100	>100	>100	
18d	25	22	24	24	23.8
18e	60	>100	>100	>100	
21a	28	40	20	20	27
21b	6	6.5	10	13	8.8
21c	3.5	6.5	8.5	13	7.8
21d	3	3.2	2.6	2.5	2.8

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition.
[b] MGM values were calculated by averaging the GI_{50} values of the four cell lines tested.

thoxyphenyl unit displayed the maximum cytotoxicity with MGM value of 1.8 μM. As observed in the case of 17β-OH system **9h** and **10d**, comparison of SAR of 17-methylene derivatives **16e** and **17e** clearly indicated that the separation of estradiol and 3,4,5-trimethoxybenzoyl unit using a vinyl tether has enhanced the antiproliferative potential. The cytotoxicity profile of 2-alkenyl-17-methylene estratrienes **18a**–**e** confirmed that conversion of 17β-OH into exocyclic methylene indeed diminished their activity. It should be noted that the corresponding 17-β-OH analogs of **18a**, **18b**, **18d** and **18e** exhibited MGM value of 5.7 μM, 0.14 μM, 1.1 μM and 3.4 μM, respectively [3a]. Hence, it is reasonable to conclude that presence of simultaneous double bonds at the C-2 and C-17 positions of estratrienes **18a**–**e** are not favorable for growth inhibition. The antiproliferative potential of imino compounds **21a**–**d** containing 17α-methyl unit was found to be moderate and the activity was comparatively better compared to the corresponding imino derivatives containing 17-β-OH and 17-methylene units.

4. Conclusions

In conclusion, different types of 2-substituted estratrienes containing 17β-OH, 17-methylene, 17α-methyl units were synthesized and tested for their antiproliferative activity by using four different cell lines from colon, lung, glioma and breast cancers. Among the various 2-substituted estratrienes, ten compounds **10d**, **14a**–**h** and **17e** were found to have *in vitro* antiproliferative activity comparable to that of parent compounds **1**–**4**. Comparison of the SAR pattern of these 2-susbtituted estratriene derivatives confirmed that relatively, 17-methylene estratrienes are more active. Work is in progress to synthesize additional analogs of 17-methylene and 17α-methyl estratriene analogs to further understand their antiproliferative potential.

Acknowledgements

The authors thank Mr. J. Venkatesan and Mr. K. Parthasarathy, Analytical Division, Orchid Pharma, for providing HPLC, NMR and mass spectral data. The authors thank the Department of Science and Technology (DST-FIST) for a 300-MHz NMR spectrometer.

References

[1] Lottering ML, Haag M, Seegers JC. Effects of 17β-estradiol metabolites on cell cycle events in MCF-7 cells. Cancer Res 1992;52:5926–32;
Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, Schweigerer L. The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. Nature 1994;368:237–9.
[2] (a) D'Amato RJ, Lin CM, Flynn E, Folkman J, Hamel E. 2-Methoxyestradiol, an endogenous mammalian metabolite, inhibits tubulin polymerization by interacting at the colchicine site. Proc Natl Acad Sci USA 1994;91:3964–8;
(b) Hamel E, Lin CM, Flynn E, D'Amato RJ. Interactions of 2-methoxyestradiol, an endogenous mammalian metabolite, with unpolymerized tubulin and with tubulin polymers. Biochemistry 1996;35:1304–10.
[3] (a) Cushman M, He HM, Katzenellenbogen JA, Lin CM, Hamel E. Synthesis, antitubulin and antimitotic activity, and cytotoxicity of analogs of 2-methoxyestradiol, an endogenous mammalian metabolite of estradiol that inhibits tubulin polymerization by binding to the colchicine binding site. J Med Chem 1995;38:2041–9;
(b) Cushman M, He HM, Katzenellenbogen JA, Varma RK, Hamel E, Lin CM, et al. Synthesis of analogs of 2-methoxyestradiol with enhanced inhibitory effects on tubulin polymerization and cancer cell growth. J Med Chem 1997;40:2323–34;
(c) Cushman M, Mohanakrishnan AK, Hollingshead M, Hamel E. The effect of exchanging various substituents at the 2-position of 2-methoxyestradiol on cytotoxicity in human cancer cell cultures and inhibition of tubulin polymerization. J Med Chem 2002;45:4748–54;
(d) Edsall AB, Mohanakrishnan AK, Yang D, Fanwick PE, Hamel E, Hanson AD, et al. Effects of altering the electronics of 2-methoxyestradiol on cell proliferation on cytotoxicity in human cancer cell cultures, and on tubulin polymerization. J Med Chem 2004;47:5126–39.
[4] (a) Verdier-Pinard P, Wang ZQ, Mohanakrishnan AK, Cushman M, Hamel E. A steroid derivative with paclitaxel-like effects on tubulin polymerization. Mol Pharmacol 2000;57:568–75;
(b) Wang Z, Yang D, Mohanakrishnan AK, Fanwick PE, Nampoothiri P, Hamel E, et al. Synthesis of B-ring homologated estradiol analogues that modulate tubulin polymerization and microtubule stability. J Med Chem 2000;43:2419–29.
[5] (a) Miller TA, Bulmann AL, Thompson CD, Garst ME, Macdonald TL. The synthesis and evaluation of functionalized estratropones: potent inhibitors of tubulin polymerization. Bioorg Med Chem Lett 1997;7:1851–6;
(b) Miller TA, Bulmann AL, Thompson CD, Garst ME, Macdonald TL. Synthesis and structure-activity profiles of A-homoestranes, the estratropones. J Med Chem 1997;40:3836–41.
[6] (a) Rao PN, Cessac JW, Tinley TL, Mooberry SL. Synthesis and antimitotic activity of novel 2-methoxyestradiol analogs. Steroids 2002;67:1079–89;

(b) Rao PN, Cessac JW, Boyd JW, Hanson AD, Shah J. Synthesis and antimitotic activity of novel 2-methoxyestradiol analogs – Part II. Steroids 2008;73:158–70;
(c) Rao PN, Cessac JW, Boyd JW, Hanson AD, Shah J. Synthesis and antimitotic activity of novel 2-methoxyestradiol analogs – Part III. Steroids 2008;73:171–83.

[7] Newman SP, Ireson CR, Tutill HJ, Day JM, Parsons MFC, Leese MP, et al. The role of 17β-hydroxysteroid dehydrogenases in modulating the activity of 2-methoxyestradiol in breast cancer cells. Cancer Res 2006;66:324–30.

[8] (a) Leese MP, Hejaz HAM, Mahon MF, Newman SP, Purohit A, Reed MJ, et al. A-ring-substituted estrogen-3-*O*-sulfamates: potent multitargeted anticancer agents. J Med Chem 2005;48:5243–56;
(b) Leese MP, Leblond B, Smith A, Newman SP, Di Fiore A, de Simone G, et al. 2-Substituted estradiol bis-sulfamates, multitargeted antitumor agents: synthesis, in *vitro* SAR, protein crystallography, and in *vivo* activity. J Med Chem 2006;49:7683–96;
(c) Leese MP, Jourdan FL, Gaukroger K, Mahon MF, Newman SP, Poster PA, et al. Structure–activity relationships of C-17 cyano-substituted estratrienes as anticancer agents. J Med Chem 2008;51:1295–308.

[9] Ireson CR, Chander SK, Purohit A, Perera S, Newman SP, Parish D, et al. Pharmacokinetics and efficacy of 2-methoxyoestradiol and 2-methoxyoestradiol-bis-sulphamate *in vivo* in rodents. Br J Cancer 2004;90:932–7.

[10] Shah JH, Agoston GE, Suwandi L, Hunsucker K, Pribluda V, Zhan XH, et al. Synthesis of 2- and 17-substituted estrone analogs and their antiproliferative structure–activity relationships compared to 2-methoxyestradiol. Bioorg Med Chem 2009;17:7344–52.

[11] (a) Liou JP, Chang JY, Chang CW, Chang CY, Mahindroo N, Kuo FM, et al. Synthesis and structure-activity relationships of 3-aminobenzophenones as antimitotic agents. J Med Chem 2004;47:2897–905;
(b) Liou JP, Chang YL, Kuo FM, Chang CW, Tseng HY, Wang CC, et al. Concise synthesis and structure-activity relationships of combretastatin A-4 analogues, 1-aroylindoles and 3-aroylindoles, as novel classes of potent antitubulin agents. J Med Chem 2004;47:4247–57.

[12] Boumendjel A, Bocard J, Carrupt PA, Nicolle E, Blanc M, Geze A, et al. Antimitotic and antiproliferative activities of chalcones: forward structure–activity relationship. J Med Chem 2008;51:2307–10.

[13] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990;82:1107–12.

[14] Papazisis KT, Geromichalos GD, Dimitriadis KA, Kortsaris AH. Optimization of the sulforhodamine B colorimetric assay. J Immunol Methods 1997;208:151–8.

[15] Peters RH, Chao WR, Sato B, Shigeno K, Zaveri NT, Tanabe M. Steroidal oxathiazine inhibitors of estrone sulfatase. Steroids 2003;68:97–110.

[16] Ducki S, Forest R, Hadfield JA, Kendall A, Lawrence NJ, McGown AT, et al. Potent antimitotic and cell growth inhibitory properties of substituted chalcones. Bioorg Med Chem Lett 1998;8:1051–6.