Synthesis and biological activity of some D-ring modified estrone derivatives

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3-Methoxy-17-aza-D-homo-1,3,5(10)-estratriene-16,17a-dione 3, 16, 17a-dione-17-aza-D-homo-1,3,5(10)-estratriene-3-ylacetic acid 12 and a series of related compounds have been synthesized from 3-methoxy-16-oximino-1,3,5(10)-estratriene-17-one 1 and 3-hydroxy-16-oximino-1,3,5(10)-estratrien-17-one 2, respectively. The compounds 3 (DPJ-280), 14 (DPJ-360), 17 (DPJ-370), 18 (DPJ-354), 19 (DPJ-320), 21 (DPJ-321), 22 (DPJ-374) and (DPJ-284) have been evaluated for their estrogen/antiestrogenic effects and the compounds 3, 7, 8, 9, 10, 12, 15, 16, 18, 20, 21, 23 and 24 have been screened for antineoplastic activity at NCI, Bethesda.

D-ring modified steroid, testolactone, a derivative of testosterone is used for the treatment of breast carcinoma

It decreases the circulating estrone levels in postmenopausal women with metastatic breast cancer and behaves as time dependent aromatase inactivator (\( t_{1/2} = 32 \text{ min} \); \( K_i = 35 \text{ \mu m} \)). A non-steroidal agent, tamoxifen, containing 2-dimethylaminoethyl side chain, considered to be important for the antiestrogenic actions of tamoxifen and related compounds, is an excellent drug used for the treatment of hormonal-dependent breast cancer. Its principal mode of action has been thought to be as an antioestrogen in contrast to aminoglutethimide, which is a non-steroidal aromatase inhibitor.

The successful introduction of steroidal and non-steroidal compounds for the treatment of breast cancer stimulated our interest in the development of new compounds containing desired functionalities. We have previously reported the synthesis of heterosteroids which have the functionalities of aminoglutethimide and 4-hydroxy-4-androstene-3,17-dione. In this study we wanted to assess the estrogenic/antiestrogenic effects of D-ring modification and fusion involving various groups at the 3-, and 17-position in estrone methyl ether and estrone series.

For this purpose, estrone methyl ether was subjected to oximation with potassium tert-butoxide-isomyl nitrite at room temperature to obtain the keto oxime 1 (Scheme I), the structure of which is well documented. The oxime was subjected to Beckmann rearrangement in refluxing acetic anhydride and gluacial acetic acid to obtain the imide derivative 3' cm. Characteristic vibrational bands for imide system appeared at 1729 and 1673 cm\(^{-1}\). \( ^1\)H NMR spectrum showed singlets at 1.20 (s, 18-CH\(_3\)), 3.80 (s, -OCH\(_3\)) and 8.50 (s, NH, exchangeable). 3-Methoxy-17-aza-D-homo-1,3,5(10)-estratriene-16,17a-dione 2 was treated with hydrochlorides of 2-dimethylaminothelohloride, 2-diethylnitomethylchloride, 1-(2-chloroethyl) pyrrolidine and 1-(2-chloroethyl)piperidine, methyl iodide and n-butyl iodide, allyl bromide and hydrazine hydrate to obtain the
compounds 4,5,6,7,8,9,10 and 11, respectively (Scheme II). Tertiary amines 4 and 5 were obtained as oily residues, so they were characterised as hydrochlorides. All these imide derivatives showed vibrational bands around 1720 and 1670 cm\(^{-1}\) in the IR spectrum for imide system and a peak around 4.00 for \(\text{N-Cl}^+\) in the \(^1\)H NMR spectrum.

Similar sequence of reactions was carried out to prepare estrone derivatives. Estrone was converted into 16-oxime 2 and subsequently to imide derivative 12. Treatment of 16,17a-dioxo-17-aza-D-homo-1,3,5(10)-estratrien-3-yl acetate 12 with \(\text{CH}_3\text{I}\) in presence of anhyd. potassium carbonate afforded 13, which on alkaline hydrolysis and treatment with 1-(2-chloroethyl) pyrrolidine gave 15. Further treatment of 12 with allyl bromide gave 16 and subsequent hydrolysis yielded 17. Reaction of 12 with hydrochlorides of 2-dimethylaminoethylchloride, 1-(2-chloroethyl) pyrrolidine and 1-(2-chloroethyl) piperidine and hydrazine hydrate gave the compounds 18,19,20 and 21. Alkaline hydrolysis of imide 12 gave 3-hydroxy derivative 22 which on treatment with \(\text{CH}_3\text{I}\) in MEK yielded 3-butoxy-17-buty1-17-aza-D-homo-1,3,5(10)-estratrien-16,17a-dione 23 (Scheme III). In \(^1\)H NMR spectrum there appeared signals at \(\delta 1.00\) (t, 6H, \(\text{N(CH}_2\text{)}_2\text{CH}_3\)) and \(\delta 4.00\) (m, 4H, \(-\text{OCH}_2\text{H}_2\) and \(\text{NCH}_2\)). Structures of the compounds were established on the basis of spectral and elemental analyses. Tertiary amines 18,19 and 20 were characterised as hydrochloride salts. Quaternisation of 15 with methyl iodide gave 24 (Scheme IV).

**Biological effects**

**Estrogenic/antiestrogenic activity**

Potential estrogenic/antiestrogenic activity of the compounds 3 (DPJ-280), 14 (DPJ-369), 17 (DPJ-370), 18 (DPJ-354), 19 (DPJ-320), 21 (DPJ-321), 22 (DPJ-374) and DPJ-284 (6)\(^1\) was assessed by measuring their binding affinity for the estrogen receptor (ER) as well as their potency to induce the synthesis of the progesterone receptor (PgR) in MCF-7 human breast cancer cells. Before assays, MCF-7 cells maintained in monolayer culture at 37°C in minimal essential medium (MEM) supplemented with L-glutamine, antibiotics and 10% heat inactivated fetal calf serum, all from Gibco, Gent, Belgium were cultured for at least 3 days in 10% serum depleted of unconjugated endogenous steroids by dextrancoated charcoal (DCC) treatment.

Figure 1 shows that most of these compounds were characterized by a weakly binding affinity for ER (note that \(\text{E}_1\)-internal control-binds with a 5-fold lower binding affinity than \(\text{E}_2\) in agreement with our previous data\(^{10}\)).

Present data also clearly establish a relationship between the binding affinity of each compound for ER and its potency to induce PgR synthesis (Figure 1 vs Figure 2).

As expected, the presence in the compounds of a phenolic group (i.e. DPJ-374 vs 280) increases the ER binding affinity.

Establishment of this correlation clearly indicates that present structural modifications of ring D of estrone to imide do not suppress estrogenic activity.

**Antineoplastic activity**

The compounds (3-7,8,9,10,12,15,16,18,20,21,23 and 24) were tested at National Cancer Institute, Bethesda, Maryland, USA; in \textit{in vitro} against the cell panel consisting of 60 lines. A 48 hr continuous
Experimental Section

Melting points reported are uncorrected. 1H NMR spectra were recorded on Varian EM-390, 90 MHz and Bruker AC-300 MHz for solutions in CDCl3 using TMS as the internal reference (chemical shifts in δ, ppm). IR and UV spectra were obtained using Perkin-Elmer-882 and Lambda-15 spectrophotometers, respectively. Mass spectra were recorded on a VG-11-250 J 70S. The purity of the compounds was examined by thin-layer chromatography. Elemental analyses were carried out on a Perkin-Elmer-2400. Ultraviolet spectra were recorded in methanol (εmax in nm, figures within parentheses refer to log ε values) and IR spectra were recorded in KBr pellets (vmax in cm⁻¹).

3-Methoxy-17-aza-D-homo-1,3,5(10)-estratriene-16,17a-dione 3. A mixture of 1 (2.0g), gl. acetic acid (64 mL) and acetic anhydride (90 mL) was refluxed for 20 hr. The resultant solution was cooled and solvent removed under reduced pressure. The residue obtained was washed with water, dried and crystallised from methanol to afford 3 (1.0g, 50%). mp 218-20°C; UV: 226.6 (3.63), 277.4 (3.10), 285.6 (3.06); IR: 3210 (-NH), 1729,1673 (imide); 1H NMR: δ 1.20 (3H, s, 18-CH3), 2.03-3.63 [8H, m, CH2(N(CH3)2)], 3.08 (3H, s, -OCH3), 3.72 (2H, t, N-CH2), 6.66 (1H, d, 1-CH), 7.05 (1H, d, 1-CH), 12.46 (br, 1H, N'OH). Anal. Found: C, 65.32; H, 6.90; N, 6.50. Calc. for C19H22N2O2: C, 65.60; H, 7.90; N, 6.60%.

17-(2-Dimethylaminoethyl)-3-methoxy-17-aza-D-homo-1,3,5(10)-estratriene-16,17a-dione 4 hydrochloride. A mixture of 3 (0.2g), 2-dimethylaminoethyl chloride hydrochloride (0.50 g), anhyd. K2CO3 (1.0 g) and KI (0.04 g) in acetonitrile (100 mL) was refluxed for 20 hr with continuous stirring. The slurry obtained was filtered and solvent removed under reduced pressure. The oily residue obtained was purified and characterised by preparing its hydrochloride salt. The residue was taken in dry solvent ether and dry hydrochloric gas was passed through it. The precipitated material was separated and crystallised from dry acetone to give hydrochloride salt of 4 (8.14 g, 54.16%); mp: 235, 278,286; IR: 1723,1674 (imide); 1H NMR: δ 1.20 (3H, s, 18-CH3), 2.66-3.03 [8H, m, CH2(N(CH3)2)], 3.08 (3H, s, -OCH3), 4.20 (2H, t, N-CH2), 6.66 (1H, d, 1-CH), 6.80 (1H, d, 1-CH), 7.20 (1H, d, 1-CH), 12.46 (br, 1H, N'OH). Anal. Found: C, 65.32; H, 7.69; N, 6.50. Calc. for C19H22N2O2Cl: C, 65.60; H, 7.90; N, 6.60%.

17- (2-Diethylaminoethyl)-3-methoxy-17-aza-D-homo-1,3,5(10)-estratriene-16,17a-dione 5 hydrochloride. A mixture of 3 (0.2g) 2-diethylaminoethyl chloride hydrochloride (0.30 g), anhyd. K2CO3 (1.0 g) and KI (0.04 g) in acetonitrile (300 mL) was refluxed for 20 hr with continuous stirring. The slurry obtained was filtered and solvent removed under reduced pressure. The oily residue obtained was purified and characterised by preparing its hydrochloride salt. The residue was taken in dry solvent ether and dry hydrochloric gas was passed through it. The precipitated material was separated and crystallised from dry acetone to give hydrochloride salt of 5 (0.16 g, 57.89%); mp: 192-94°C; UV: 231.2, 277.9, 286.1; IR: 1722, 1674 (imide) 1H NMR: δ 1.23 (3H, s, 18-CH3), 1.50 (6H, t, N-(CH3CH2)2), 2.70-3.53 [8H, CH2(N(CH3)2) and methylene protons], 3.80 (3H, s, -OCH3), 4.20 (2H, t, N-CH2), 6.80 (1H, s, 4-CH), 6.96 (1H, d, 2-CH), 7.30 (1H, d, 1-CH), 12.20 (br, 1H, N'OH). Anal. Found: C, 65.60; H, 7.90; N, 6.60%;
66.52; H. 7.95; N. 6.29. Calc. for C_{25}H_{37}N_2O_3Cl: C, 66.88; H. 8.31; N. 6.24%.

3-Methoxy-17- (2-pyrrolidinoethyl)- 17-aza-D- homo-1, 3, 5(10)-estratriene-16, 17a-dione 6. A mixture of compound 3 (0.5 g) (2-chloroethyl) pyrrolidine hydrochloride (0.75 g) anhyd. K_2CO_3 (2.5

Figure 1—Relative binding affinity of compounds. MCF-7 cells were incubated with 10^{-9} M \[^3H\]E_2 and increasing amounts of compounds. Bound \[^3H\]E_2 was subsequently measured (100%=4,500 dpm/200μl of cellular extract).

Figure 2—PgR induction. MCF-7 cells were incubated either in the absence (Control = 0%) or the presence of 10^{-10} M of E (optimal induction = 40%) to establish the range of PgR induction. Cells were cultured in parallel with investigated compounds at either 10^{-10}, 10^{-9} and 10^{-8}M. Cytotoxic PgR levels were subsequently measured (PgR levels in fmole/mg protein; 2 sets of experiments; 1st basal = 176; optimal = 521. 2nd basal = 168; optimal = 706).
g) and KI (0.1 g) in acetonitrile (250 mL) was refluxed for 20 hr with continuous stirring. The slurry obtained was filtered and solvent removed under reduced pressure. The residue so obtained was crystallised from dry ether to afford 6 (0.30 g, 45.79%); mp 142-46°C; UV: 229(3.75), 277.4(3.25), 285.4(3.21); IR: 1720, 1671 (imide); 1H NMR: δ 1.16 (3H,s,18-CH3), 2.50-2.80 (m,6H,CH2N<CH2), 3.80 (3H, s, -OCH3), 4.02 (2H,m, N-CH2), 6.80 (2H,m,4-CH and 2-CH2), 7.33 (1H, d, 1-CH); MS: m/z 410. Anal. Found: C, 73.13; H, 8.34; N, 6.80%.

3-Methoxy- 17-(2- pyrroldinoethyl)-17-aza-D-homo-1,3,5(10)-estratriene-16,17a-dione(6) hydrochloride. Through a solution of 7 (0.20 g, 77.96 %) in dry acetone (20 mL) was passed dry HCl gas. The precipitated material was separated and crystallised from dry acetone to afford hydrochloride salt of 6 (0.085 g, 77.96 %); mp 142-46 °C; UV: 229(3.75), 277.4(3.25), 285.4nm (3.15); IR: 1723, 1667 (imide); 1H NMR: δ 1.16 (3H,s,18-CH3), 2.50-2.80 (m,6H,CH2N<CH2), 3.80 (3H, s, -OCH3), 4.02 (2H,m, N-CH2), 6.80 (2H,m,4-CH and 2-CH2), 7.33 (1H, d, 1-CH); MS: m/z 410. Anal. Found: C, 73.13; H, 8.34; N, 6.80%.

3-Methoxy-17-(2-piperidinoethyl)-17-aza-D-homo-l,3,5(10)-estratriene-16,17a-dione 7. A mixture of compound 3 (0.5 g) 1-(2-chloroethyl)peripiperidine hydrochloride (0.75 g), anhyd. K2CO3 (2.5 g) and KI (0.1 g) in acetonitrile (250 mL) was refluxed for 20 hr with continuous stirring. The slurry obtained was filtered and solvent removed under reduced pressure. The residue so obtained was crystallised from dry ether to afford 7 (0.30 g, 44.27%); mp 138-39°C; UV: 228.4 (3.74), 277.4 (3.18), 285.4nm (3.15); IR: 1723, 1667 (imide); 1H NMR: δ 1.13 (3H,s,18-CH3), 2.26-2.63 (6H,t, CH2N<CH2), 3.80 (3H, s, -OCH3), 3.96 (2H,m, N-CH2), 6.70 (1H, s,4-CH), 6.93 (1H, d, 2-CH), 7.30 (1H, d, 1-CH); MS: m/z 424. Anal. Found: C, 73.21; H, 8.71; N, 6.22. Calc. for C25H27N3O3: C, 74.55; H, 7.48; N, 6.59%.

3-Methoxy-17-(2-piperidinoethyl)-17-aza-D-homo-1,3,5(10)-estratriene-16,17a-dione 10. A mixture of 3 (0.5 g), allyl bromide (2 mL) and dry MEK (200 mL) was heated for 10 min. Anhyd. K2CO3 (0.5 g) was added with continuous stirring. The reaction mixture was refluxed for 4 hr with continuous stirring. The slurry obtained was filtered and solvent removed under reduced pressure. The residue so obtained was crystallised from methanol to afford 10 (0.40 g, 71%); mp: 138-40°C; UV: 227 (3.77), 277.4 (3.21), 283.6nm (3.17); IR : 1716, 1667 (imide); 1H NMR: δ 1.20 (3H, s, 18-CH3), 3.76 (3H, s, O-CH3), 4.33 (2H, s, N-CH2), 5.10 (2H, dd, N-CH2CH2CH3), 5.70 (1H, t, N-CH2CH=CH2), 6.63 (1H, s, 4-CH), 6.76 (1H, d, 2-CH), 7.20 (1H, d, 1-CH); MS: m/z 567. Anal. Found: C, 74.55; H, 8.05; N, 4.30. Calc. for C25H27NO3; C, 74.76; H, 8.46; N, 3.79%.

17-Allyl-3-methoxy-17-aza-D-homo-1,3,5(10)-estratriene-16,17a-dione 11. The compound 3 (0.2 g) was added to a solution of compound 3 (0.3 g) in dry acetone (150 mL). Anhyd. K2CO3 (1.5 g) was added to the above hot solution with stirring. The reaction mixture was refluxed for 3 hr with continuous stirring. The slurry obtained was filtered and solvent removed under reduced pressure. The residue so obtained was washed with water and crystallised from methanol to afford 11 (0.2 g), 63.87%; mp 164-69°C; UV: 226 (3.89), 278 (3.33), 285 (3.28); IR: 1721, 1672 (imide); 1H NMR: δ 1.16 (3H,s,18-CH3), 3.16 (3H,s, NCH3), 3.80 (3H, s, OCH3), 6.70 (1H, s, 4-CH), 6.90 (1H, d, 2-CH), 7.26 (1H, d, 1-CH); MS: m/z 327. Anal. Found: C, 73.92; H, 7.48; N, 4.20. Calc. for C25H19NO3: C, 73.36; H, 7.69; N, 4.28%.

17-Butyl-3-methoxy-17-aza-D-homo-1,3,5(10)-estratriene-16,17a-dione 9. n-Butyl iodide (2 mL) was added to a solution of 3 (0.1 g) in dry MEK (50 mL). To the hot solution was added anhyd. K2CO3 (0.5 g) with stirring. The reaction mixture was refluxed for 5 hr with continuous stirring. The slurry obtained was filtered and solvent removed under reduced pressure. The residue so obtained was washed with water and crystallised from n-hexane to afford 9 (0.075 g, 63.69%); mp 83-86°C; UV: 224.27 (3.86),277.4 (3.21); IR: 1721, 1666 (imide); 1H NMR: δ 0.90 (3H,t,N-(CH2)3CH3), 1.16 (3H,s,18-CH3), 3.80 (2H, s, -NCH2), 3.83 (3H, s, OCH3), 6.73 (1H,s,4-CH), 6.90 (1H, d, 2-CH), 7.30 (1H, d, 1-CH); MS: m/z 410. Anal. Found: C, 74.45; H, 7.94; N, 4.06. Calc. for C25H19NO3: C, 74.76; H, 8.46; N, 3.79%.
3-Hydroxy-16-oximino-1,3,5(10)-estratriene-17-one 2. A mixture of estrone (2.0 g) in potassium tert-butoxide [prepared by dissolving potassium metal (0.8 g) in tert-butanol (20 mL)] was stirred for 2 hr at room temperature under nitrogen atmosphere. Isoamyl nitrite (1.8 mL) was then added and reaction mixture was kept at room temperature for 17 hr. Precipitated material was dissolved in water (200 mL) and solution obtained was washed with ether. Aqueous layer was separated and acidified with glacial acetic acid. Separation of product was complete in 2 hr. The separated product was filtered, washed and dried. The solid obtained was crystallised from methanol to afford 2 (1.2 g, 54%); mp 218-20°C; UV: 231, 279; IR: 3366 (N=O), 1733 (C=O); 'H NMR: δ 1.06 (3H, s, 18-CH₃), 6.50 (11H, s, 4-CH), 6.56 (11H, d, 2-CH₃); 7.06 (11H, d, 1-CH); 8.76 (11H, s, exchangeable).  

16.17a-Diexo-17-aza-D-homo-1,3,5(10)-estratrien-3-yl acetate 12. A mixture of compound 2 (0.5 g) of acetic anhydride (25 mL) and gl. acetic acid (15 mL) was refluxed for 20 hr under anhyd. conditions. The solid residue obtained after removing the solvent under reduced pressure was washed with water. The material obtained was crystallised from acetone to afford 12 (0.3 g, 52.63%); mp 196-98°C; UV: 224 (3.64), 266.6 (2.95), 274.2 (2.89); IR: 1759 (-OCOCH₃), 1720,1096 (imide); 'H NMR: δ 1.22 (3H, s, 18-CH₃), 2.29 (3H, s, -OCOCH₃), 2.88 (3H, m, 15-CH₃), 6.82 (11H, s, 18-CH₃), 6.88 (11H, d, 2-CH₃), 7.28 (11H, d, 1-CH₃), 8.50 (11H, s, exchangeable, -NH₂); ¹³C NMR: 16.36 (CH₃), 21.11 (CH₃), 119.0 (CH), 121.44 (CH), 126.38 (CH), 136.61 (C), 137.48 (C), 148.68 (C, 3-C), 169.2 (C=O), 172.16 (C, 16-C, C=O), 178.78 (C, 17a-C, C=O); Anal. Found: C, 69.99; H, 6.52; N, 3.93. Calc. for C₂₆H₃₁NO₄: C, 70.36; H, 6.79; N, 4.10%.  

17-Butyl-16, 17a-dioxo-17-aza-D-homo-1,3,5(10)-estratrien-3-yl acetate 13. A mixture of 12 (0.2 g), dry MEK (50 mL) and anhyd. K₂CO₃ (0.5 g) was refluxed with stirring for 10 min. n-Butyl iodide (2 mL) was added to the stirred solution and mixture was refluxed for 5 hr. The slurry was filtered and solid obtained after removing the solvent was washed with water and crystallised from methanol to afford 13 (0.15 g, 65.2%); mp 174-78°C; UV: 223.6 (3.79), 267 (2.88), 274.2 (2.85); IR: 1754(=OCOCH₃), 1717, 1668 (imide); 'H NMR: δ 0.90 (3H, t, N(CH₃)₂-CH₂), 1.16 (3H, s, 18-CH₃), 2.30 (3H, s, -OCOCH₃), 3.80 (2H, t, N-CH₂), 6.86 (1H, s, 4-CH), 7.00 (11H, d, 2-CH), 7.36 (11H, d, 1-CH); MS: m/z 397. Anal. Found: C, 72.29; H, 7.44; N, 3.79. Calc. for C₂₆H₃₁NO₄: C, 72.51; H, 7.86; N, 3.52%.  

17-Butyl-3-hydroxy-17-aza-D-homo-1,3,5(10)-estratriene-16,17-dione 14. A mixture of 13 (0.5 g), K₂CO₃ (1.0 g) and 10%aq. methanol (50 mL) was stirred at room temperature for 1 hr. The reaction mixture was poured in water and the precipitated material was filtered and dried. The solid obtained was crystallised from methanol to give 14 (0.3 g, 67.35%), mp 210-12°C; UV: 223 (3.72), 280 (3.13); IR: 1716, 1650 (imide); 'H NMR: δ 0.92 (3H, t, N(CH₃)₂-CH₂), 1.16 (3H, s, 18-CH₃), 2.84 (2H, m, 15-C H₂), 3.76 (2H, t, N-CH₂), 5.49 (11H, s, exchangeable, OCH₃), 6.59 (11H, s, 4-CH), 6.65 (11H, d, 2-CH), 7.17 (11H, d, 1-CH); '¹³C NMR: 13.87 (CH₂), 16.58 (CH₃), 113.14 (CH), 115.14 (CH), 126.49 (CH), 131.28 (C), 137.59 (C), 153.94 (C, 3-C), 172.09 (C, 16-C, C=O), 178.91 (C, 17a-C, C=O); Anal. 74.61; H, 8.55; N, 4.25. Calc. for C₂₆H₃₁NO₄: C, 74.33; H, 8.22; N, 3.94%.  

17-Butyl-3-(2-pyridilinoethoxy)-17-aza-D-homo-1,3,5(10)-estratriene-16,17-dione 15. To a solution of 14 (0.3 g) in dry MEK (150 mL) was added 1-(2-chloroethyl) pyrrolidine hydrochloride (0.45 g). Reaction mixture was heated for 10 min. Anhydrous K₂CO₃ and KI (0.0255 g) were then added. The mixture was stirred and refluxed for 5 hr. The slurry was filtered and residue obtained after removal of solvent was crystallised from distilled water to afford 15 (0.31 g, 81.57%); mp 74-76°C; UV: 224.6 (3.89), 277.8 (3.18); IR: 1721, 1667 (imide); 'H NMR: δ 0.92 (3H, t, N(CH₃)₂-CH₂), 1.16 (3H, s, 18-CH₃), 1.80 (6H,m, methylene protons), 2.50-3.03 (10H, m, pyrro-
lidino and methylene protons) 3:74 (2H, m N-CH$_2$), 4.08 (2H, t, CH=CH), 6.66 (1H, s, 4-CH), 6.75 (1H, d, 2-CH), 7.21 (1H, d, 1-CH); $^1$C NMR: 13.83 (CH$_3$), 16.54 (CH$_3$), 67.02 (CH)$_3$, 112.47 (CH) 114.35 (CH), 126.24 (CH), 131.34 (C), 137.27(C), 157.04 (C, 3-C), 171.74 (C, 16-C, C=O); 178.69 (C, 17a-C, C=O); MS, m/z 452. Anal. Found: C, 73.87; H, 8.70; N, 5.83. Calc. for C$_{28}$H$_{43}$N$_2$O$_3$: C, 74.29; H, 8.91; N, 6.19%.

17-Butyl-3-(2-pyrrolidinoxothio)-17-aza-D-homo-1,3,5(10)estratriene-16,17a-dione methiodide

24. Methyl iodide (0.5 mL) was added to a solution of 15 (0.25 g) in absolute ethanol (50 mL) at room temperature and the solution was refluxed for 1 hr. The reaction mixture was cooled, filtered and dried to afford 24 (0.3 g, 90.9%); mp softens at 80°C, melts at 220-222°C. Anal. Found: C, 58.18; H, 7.29; N, 4.39. Calc. for C$_{25}$H$_{41}$N$_3$O$_5$: C, 58.58; H, 7.28; N, 4.71%.

17-Allyl-16-17a-dioxo-17-aza-D-homo-1,3,5(10)-estratrien-3-yl acetate 16. To a solution of 12 (0.5 g) in MEK was added allyl bromide (2 mL) and heated for 10 min. Anhydrous K$_2$CO$_3$ (1.5 g) was then added and reaction mixture was refluxed with stirring for 3.5 hr. The slurry obtained was filtered and residue obtained after removal of solvent was crystallised from methanol to afford 16 (0.47 g, 84.12%) mp 180-82°C; UV: 223.8 (3.67), 267 (2.88), 274.2 (2.84); IR: 1751(-OCOCH$_3$) 1720,1673 (imide); $^1$H NMR: $\delta$ 1.20 (3H, s, 18-CH$_3$), 2.26 (3H, s, -COOC$_2$H$_5$), 4.33 (2H, d, N-CH$_3$), 5.10 (2H, dd, N-CH$_2$=CH$_2$), 5.60 (1H, m, N-CH$_2$=CH$_2$), 6.80 (1H, s, 4-CH), 6.90 (1H, d, 2-CH), 7.23 (1H, d, 1-CH). Anal. Found: C, 72.11; H, 7.38; N, 3.96. Calc. for C$_{23}$H$_{27}$NO$_4$: C, 72.41; H, 7.14; N, 3.67%.

17-Allyl-3-hydroxy-17-aza-D-homo-1, 3, 5 (10)-estratriene-16,17a-dione 17. A mixture of 16 (0.4 g) K$_2$CO$_3$ (1.5 g) and aqueous methanol (50 mL) was stirred at room temperature for 1 hr. The slurry obtained was poured into water. The precipitated material was crystallised from methanol to afford 17 (0.325 g, 91.54%); mp 238-40°C; UV: 226 (3.72), 280 (3.26); IR: 3454(-OH) 1719,1661 (imide); $^1$H NMR: $\delta$ 1.80 (4H, t, N-C=O), 2.00 (2H, d, N-CH$_2$), 3.56 (1H, s, 3-CH), 4.30 (2H, d, N-CH$_2$=CH$_2$), 5.10 (2H, dd, N-CH$_2$CH=CH$_2$), 5.56 (1H, m, N-CH$_2$=CH$_2$), 5.60 (1H, s, 4-CH), 6.00 (1H, d, 2-CH), 7.00 (1H, d, 1-CH), 8.65 (1H, s, exchangeable, OH). Anal. Found: C, 74.16; H, 7.73; N, 4.41. Calc. for C$_{25}$H$_{41}$N$_2$O$_5$: C, 74.30; H, 7.42; N, 4.13%.

17- (2-Dimethylaminoethoxy)-3-hydroxy-17-aza-D-homo-1,3,5 (10) estratriene-16,17a-dione(18) hydrochloride. To a solution of 12 (0.3 g) in dry acetonitrile (200 mL) was added 2- dimethylaminooethylchloride hydrochloride (0.45 g) and heated for 10 min. To the hot solution, was added anhydrous K$_2$CO$_3$ (1.0 g) and KI (0.60 g). The mixture was refluxed for 6 hr with continuous stirring. The slurry obtained was filtered and the residue obtained after removal of solvent was stirred with K$_2$CO$_3$ (0.5 g) in aq. methanol for 3 hr. The resultant mixture was poured into distilled water and extracted with dichloromethane. The oily residue obtained after removal of solvent was purified and characterised by preparing its hydrochloride salt. The residue was taken in dry solvent ether and HCl gas was passed through it. The precipitated material was separated and crystallised from dry acetone to give hydrochloride salt of 18 (5.15 g, 47.9%); mp 280-82°C; UV: 230.9, 280.5; IR: 3401(-OH), 1723,1671 (imide); $^1$H NMR: $\delta$ 1.14 (3H, s, 18-CH$_3$), 2.51 (6H, m, methylene protons), 2.80 (8H, s, -CH$_2$N(CH$_3$)$_2$), 3.95 (2H, m, N-CH$_2$), 6.47 (1H, s, 4-CH), 6.56 (1H, d, 2-CH), 7.07 (1H, d, 1-CH), Anal. Found: C, 64.65; H, 7.80; N, 6.80. Calc. for C$_{27}$H$_{43}$N$_2$O$_3$: C, 64.92; H, 7.67; N, 6.88%.

3-Hydroxy-17-(2-pyrrolidinoxothio)-17-aza-D-homo-1,3,5(10) estratriene-16,17a-dione (19) hydrochloride. To a solution of 12 (0.5 g) in dry acetonitrile (150 mL) was added 1-(2-chloroethyl) pyrrolidine hydrochloride (0.75 g) and heated for 10 min. To the hot solution was added anhyd K$_2$CO$_3$ (0.1 g) and KI (0.06 g). The mixture was refluxed for 6 hr with continuous stirring. The slurry obtained was filtered and the residue obtained after removal of solvent was stirred with K$_2$CO$_3$ (0.5 g) in aq. methanol for 3 hr. The resultant mixture was poured into distilled water and extracted with dichloromethane. The oily residue obtained after removal of solvent was purified and characterised by preparing its hydrochloride salt. The residue was taken in dry solvent ether and dry hydrochloric gas was passed through it. The precipitated material was separated and crystallised from dry acetone to give hydrochloride salt of 19 (0.10 g, 15.8%); mp 176-177°C; UV: 232, 280.4; IR: 3371, 2935, 1723, 1627 (imide); $^1$H NMR: $\delta$ 1.18 (3H, s, 18-CH$_3$), 4.04 (2H, m, N-CH$_2$), 6.48 (1H, s, -CH$_2$), 6.56 (1H, d, 2-CH), 7.01 (1H, d, 1-CH), 8.92 (br, 1H). Anal. Found: C, 66.23; H, 8.08; N, 6.30. Calc. for C$_{27}$H$_{43}$N$_2$O$_3$: C, 66.57; H, 7.68; N, 6.47%.
3-Hydroxy-17-(2-piperidinoethyl)-17-aza-D-homo-1,3,5(10) estratriene-16, 17a-dione (20) hydrochloride. To a solution of 12 (0.2 g) in dry acetonitrile (100 mL) was added 1-(2-chloroethyl) piperidine hydrochloride (0.3 g) and heated for 10 min. To the hot solution was added anhyd. K$_2$CO$_3$ (0.75 g) and KI (0.04 g). The mixture was refluxed for 6 hr with continuous stirring. The slurry obtained was filtered and the residue obtained after removal of solvent was stirred with K$_2$CO$_3$ (0.5 g) in aqueous methanol for 3 hr. The resultant mixture was poured into distilled water and extracted with dichloromethane. The oily residue obtained after removal of solvent was purified and characterized by preparing its hydrochloride salt. The residue was taken in dry solvent ether and dry hydrochloric gas was passed through it. The precipitated material was separated and crystallized from dry ether into xylene. The precipitate was washed with petroleum ether and dried. Solvent was removed overnight and was refluxed with xylene using Dean-Stark apparatus. Solvent was removed and purified and product obtained was crystallized from dry acetone to give 23 (0.15 g, 65.77%); mp 294-96 °C; UV : (imide); IR : 3428 (-OH) 1716,1671 (imide); $^1$H NMR : δ 1.16 (3H, s, 18-CH$_3$), 6.43 (1H, s, 4-CH), 6.46 (1H, d, 2-CH$_2$), 7.03 (1H, d, 1-CH), 8.80 (1H, s, exchangeable). Anal. Found: C, 72.21; H, 7.07; N, 4.67%.

3- Butoxy-17-buty1-17-aza-D-homo-1,3,5(10)-estratriene-16,17a-dione 23. To a stirred solution of 12 (0.4 g) in MEK (150 mL) was added K$_2$CO$_3$ (2.0 g). The reaction mixture was heated and stirred for 10 min. n-Butyl iodide was then added and the mixture refluxed for 6 hr. The slurry was filtered and solid obtained after removal of solvent was characterized from acq. methanol to afford 23 (0.6 g, 68.1%); mp 98-100 °C; UV : 229.4 (3.81); 278.5 (3.23); 286.6 (3.18); IR : 1719,1669 (imide); $^1$H NMR : δ 1.00 [6H, t, N-(CH$_2$)$_3$CH$_3$ and -O-(CH$_2$)$_3$CH$_3$], 1.20 (3H, s, 18-CH$_3$), 3.60-4.10 (4H, m, N-CH$_2$), 4.02 (2H, m, N-CH), 6.47 (1H, s, 4-CH$_3$), 6.56 (1H, d, 2-CH$_2$), 7.10 (1H, d, 1-CH), 9.64 (4H, br, 1H). Anal. Found: C, 75.40; H, 8.81; N, 3.22. Calc. for C$_{26}$H$_{37}$NO$_3$: C, 75.87; H, 9.06; N, 3.40%.

**Pharmacology**

**Material and Methods**

**Binding affinity to estrogen receptor**$^{12}$. Relative binding affinity (RBA) of the compounds 3 (DPl-280), 14 (DPJ-369), 17 (DPJ-370), 18 (DPJ-354), 19 (DPJ-320), 21 (DPJ-321), 22 (DPJ-374) and DPl-284 to estradiol (E$_2$) and estrone (E$_1$) was performed by whole cell assay, a method which provides an index of the ability of the compounds to activate ER$^{12}$. In practice cells were plated in 24-multidishes (1.5x10$^4$ cells / well in mew without phenol red and 10% DCC treated serum. After 4 days of culture, this medium was removed and replaced by a serum free medium containing 10$^3$ M $[^3]$H[E$_2$ (– 80 Ci/mole, Amersham, UK) with or without increasing amounts of investigating compounds. After 1 hr of incubation, this medium was removed and monolayer washed three times with an ice cold buffered saline solution (PBS). Bound $[^3]$H[E$_2$ was then extracted from the monolayer by a final incubation with (250 µL) ethanol at room temperature for 20 min. The radioactivity of these extracts 200 µL was then measured by scintillation counting. Specifically incorporated radioactivity in the presence of unlabeled E$_2$ and a given compound gave the RBA values of the latter for ER ; RBA= [Is$^{13}$H]E$_2$/ [Is$^{15}$O]E$_2$/[Is$^{15}$O]compound ×100. All measurements were performed in triplicate.
Progesterone receptor induction\textsuperscript{13}. Cells were incubated for 3 days in 10\% DCC-treated serum in the presence of a given compound at either 10\(^{-10}\), 10\(^{-8}\) or 10\(^{-6}\) M (T-175 flasks); control cells were maintained in culture with or without E\(_2\) at 10\(^{-10}\) to establish the optimal level in PgR induction (ratio of receptor levels respectively in the absence or presence of E\(_2\)). Treated and untreated cells were subsequently detached from the flasks with 1 mM EDTA in Hank's balanced salt solution (HBSS) without Ca\(^{2+}\) and Mg\(^{2+}\). Cellular suspensions were centrifuged at 800g and the pellets were washed once with (HBBS) and twice with the homogenization buffer (i.e., 10mM phosphate buffer, pH 7.4, containing 1.5 mM EDTA and 1 mM monothioglycerol). Cytosol was obtained by homogenization of the final pellet by means of a teflon-glass homogenizer and centrifugation at 130,000 g for 1 hr (cytosol protein content 1 to 3.5 mg/mL). Cytosol PgR concentrations were measured by Scatchard plot analysis according to the procedure established by the EORTC Receptor group\textsuperscript{14} using [\textsuperscript{3}H]ORG-2058 (50Ci/mmole; Amersham U.K.) as labelled ligand).

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