Synthesis of estradiol-cinnamide conjugates

Goreti Ribeiro Morais[a] and Thies Thiemann[b,c,*]

[a]*Instituto Superior Técnico/Instituto Tecnológico e Nuclear, Estrada Nacional 10, Sacavem, Portugal*

[b]*Institute of Materials Chemistry and Engineering, Kyushu University, Kasuga 2686-953, Fukuoka 816-8580, Japan*

[c]*Present address: Faculty of Science, United Arab Emirates University, PO Box 17551, Al Ain, UAE*

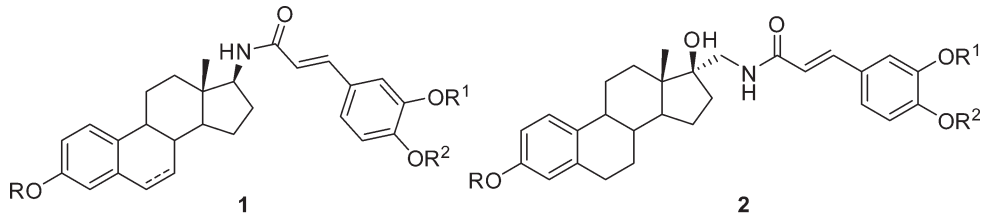
The synthesis of a number of structurally related estradiol-17α-ylmethyl hydroxycinnamides and of one novel estra-1,3,5(10),6-tetraen-3-ol-17β-yl hydroxycinnamide is described, using a microwave assisted amidation of steroidal amines with pentafluorophenol activated, non-protected hydroxycinnamic acids as a key step. A selection of the compounds and of other estra-1,3,5(10)-trien-3-ol 17β-yl hydroxycinnamides was screened against 60 human cancer cell lines, derived from nine neoplastic diseases. From the overall results of the screening, it could be inferred that dihydroxycinnamide derived estradiol conjugates exhibit a better cytotoxic profile when compared with hydroxy-methoxycinnamide derived estradiol conjugates.

Keywords: steroids, estradiols, cinnamides, antiproliferative effect, cytotoxicity

Simple phenolic and polyphenolic structures, many of which are aromatic metabolites of plants, have attracted much attention because of their potential protective role against diseases associated with oxidative damage. They have been studied extensively, and their free radical scavenging and antioxidant activities have been well documented.[1,2] In addition to reports describing the antioxidant properties of phenolic structures, communications have revealed the anticancer activity of some phenolic acids.[3–8] The mechanism associated with the observed antiproliferative and cytotoxic effects of those structures is still not clear, and may depend on the dose and the model studied. The pro-oxidant effect and consequent capacity for generating free radicals has been one of the factors thought to be responsible for the cytotoxicity associated with these molecular structures.[8]

Phytoestrogens, naturally occurring chemicals derived from plants, are known to retard chemical carcinogenesis in experimental models.[3,4] These natural compounds have attracted particular attention due to their structural similarity to steroidal estrogens and their capacity to mimic biological responses of estrogens. On the other hand, steroidal estrogens themselves (*e.g.* estradiol) have been found to exhibit the biological properties of phytoestrogens, in particular, antioxidant activity.[9] This realisation has driven researchers to introduce structural modifications on the estradiol nucleus, with the purpose of increasing the antioxidant properties of these compounds. It has been shown that the introduction of a radicophilic moiety, preferentially of a phenolic group, at the 17α-position of the estradiol framework increases the antioxidant activity of the derivatives.[10,11]

For this reason, we designed a small series of estrogen hybridised with phenolic acids, in an effort to prepare molecules that were biologically active towards neoplastic cells. Three different hydroxycinnamic acid residues were selected to be attached to steroidal derivatives of the estrane series to give the structurally related steroidal-hydroxycinnamide conjugates **1** and **2** (Fig. 1). As candidates, steroids were chosen, in which hydroxycinnamic moieties were connected via an amido function on the β face of the steroids (**1**). Also, steroidal amides were to be prepared, in which the hydroxycinnamate moiety was connected to the α face of the steroids (**2**, Fig. 1). As hydroxycinnamic residues, hydroxyphenyl moieties with different phenolic patterns were chosen, including α,β-dihydroxy- (caffeic acid derived) and α-hydroxy-β-methoxy-cinnamic residues (ferulic acid and isoferulic acid derived). In the steroidal moiety, the structural differences were obtained by replacing the 17β-hydroxyl group of the estradiol with an amino function or by introducing an amino function in the form of a methylamino group at the 17α-position, while preserving the hydroxyl group at the 17β-position. As the influence of a protection of the phenolic function of the estradiol residue in form of a methoxy group and the influence of an unsaturation at the 6,7-position of the steroidal framework on the biological activity of the conjugates were to be studied, the novel steroid **1a** was to be one of the main synthetic targets. Furthermore, with the planned key step being a microwave assisted amidation of non-protected hydroxycinnamic acids, previously developed by the authors,[12] we wished to examine whether this reaction could be used in the neighbourhood of a tertiary hydroxyl group in the amine component, as is present in the steroidal amines leading to target molecules **2**.



1a: R = Me, $R^1 = R^2 = H$, $\Delta^{6,7}$;
1b: $R = R^1 = R^2 = H$
1c: R = Me, $R^1 = R^2 = H$
1d: $R = R^2$ = Me, $R^1 = H$
1e: $R = R^1$ = Me, $R^2 = H$

2a: R - $R^2 = H$
2b: R = Me; $R^1 = R^2 = H$
2c: $R = R^2 = H$; R^1 = Me
2d: $R = R^1$ = Me; $R^2 = H$

Fig. 1 Steroidal-hydroxycinnamide conjugates.

* Correspondent. E-mail: thies@uaeu.ac.ae

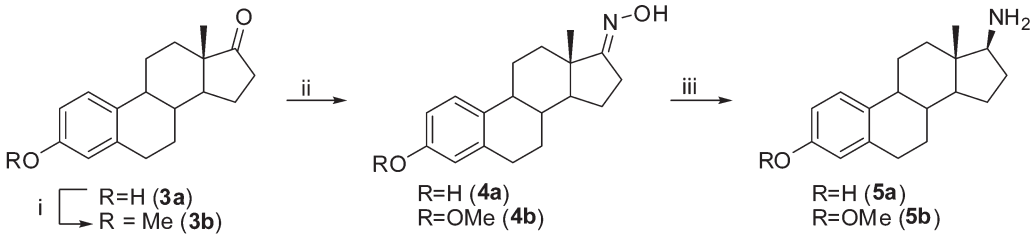
Results and discussion

The syntheses of the compounds **1b**–**e** have been described previously.[12] Treatment of the 3-methoxy-estra-1,3,5(10),6-tetraene-17-one **3c**, prepared according to the literature,[13] with hydroxylamine hydrochloride gave the estra-1,3,5(10),6-tetraene oxime **4c**.[14] The oxime function was reduced using the aluminum amalgam method,[15] in which the 17-amino group was obtained predominantly in the *β*-configuration (Scheme 1). This method has shown to be selective, with no significant hydrogenation of the olefin bond at the 6,7-position. 3-*O*-Methyl-17*β*-aminoestra-1,3,5(10)-triene-3-ol (**5b**) and 17*β*-aminoestra-1,3,5(10)-triene-3-ol (**5a**) were prepared by reducing the respective oximes **4a** and **4b** with sodium in refluxing propanol.

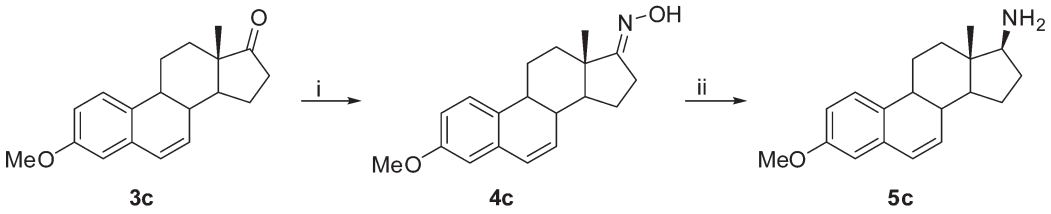
In the other type of *E*-hydroxycinnamides **2**, the amide linkage was to be created on the α face of the steroidal derivatives. Preparation of the steroidal derivative with an amine function on the α side involves the stereoselective transformation of the 17-ketone into a cyanohydrin group and further reduction to the methylamine. Thus, estrone **3a** and 3-methoxyestrone **3b**, respectively, were reacted with trimethylsilyl cyanide in the presence of a Lewis acid (ZnI_2) followed by treatment with acid (HCl).[16] Under these conditions, the addition of the cyano group is *α*-stereoselective. Reduction of the cyano group was carried out with lithium aluminum hydride, in dry THF at room temperature (Scheme 2).[17]

Attempts to isolate the product of the reduction of the cyanohydrin **6** failed. The reason was an excessive solubility of the respective steroidal amine in water, with no possibility to extract it with several organic phases tried. Thus, before carrying out the reduction, the hydroxyl groups in the cyanohydrin **6** were protected as tetrahydropyranyl ethers. The steroidal amine **9** was then isolated without problem. Further, steroidal amine **9** was used without purification, in order to avoid hydrolysis of the acid sensitive protective groups.

With the steroidal amines prepared, the next step consisted of the amidation reaction of the steroids with the phenolic acids, caffeic and ferulic acids. In our group, it was previously established[12] that a solventless mixture of the amine with caffeic acid readily gave the corresponding caffeic amide under microwave (MW) irradiation, when activated with dicyclohexylcarbodiimide (DCC) and pentafluorophenol (PFP). This method was shown to be a general synthetic approach to prepare amides from unprotected hydroxyphenylalkenoic acids.[12] Normally, the pentafluorophenyl alkenoates as the activated acid components are prepared first and the isolated activated alkenoates are then reacted in a second step under MW irradiation. As reported previously by the authors, ferulic and isoferulic acid gave the corresponding, easily isolable pentafluorophenyl esters **12b** and **12c** in high yield, when following a procedure by Zhao et al.,[18] adapted by the authors to the reaction of alkenoic acids. Thus, the phenolic acids were reacted with DCC and PFP in anhydrous dioxane at room temperature (Scheme 3). The formed dicyclohexylurea (DCU) was easily removed by filtration and the product was obtained by column chromatography on silica gel.

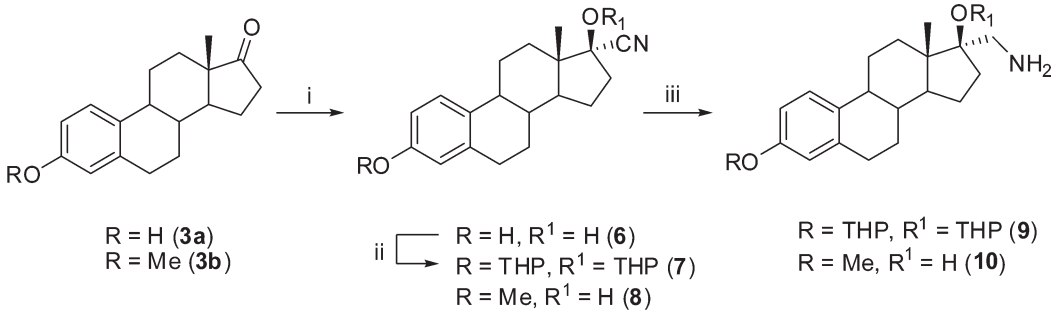


i. KOH, DMSO, MeI; ii. $NH_2OH{\cdot}HCl$, EtOH / water, 50ºC; iii. Na, propanol, reflux.



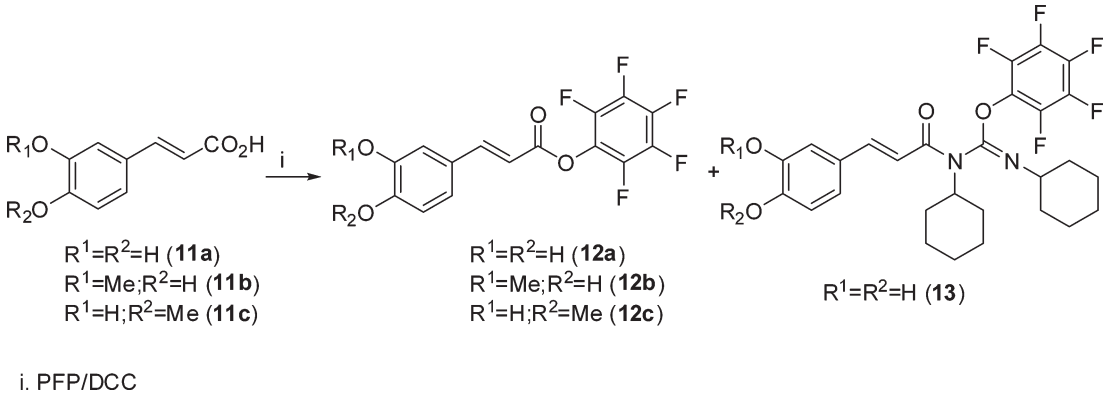
i. $NH_2OH{\cdot}HCl$, EtOH / water, 50ºC; ii. Al / $HgCl_2$, EtOH / water, reflux.

Scheme 1



i. a) TMSCN, ZnI_2, dry DCM, reflux; b) HCl; ii. DHP, *p*-TsOH, DCM, rt; iii. $LiAlH_4$, dry THF, rt

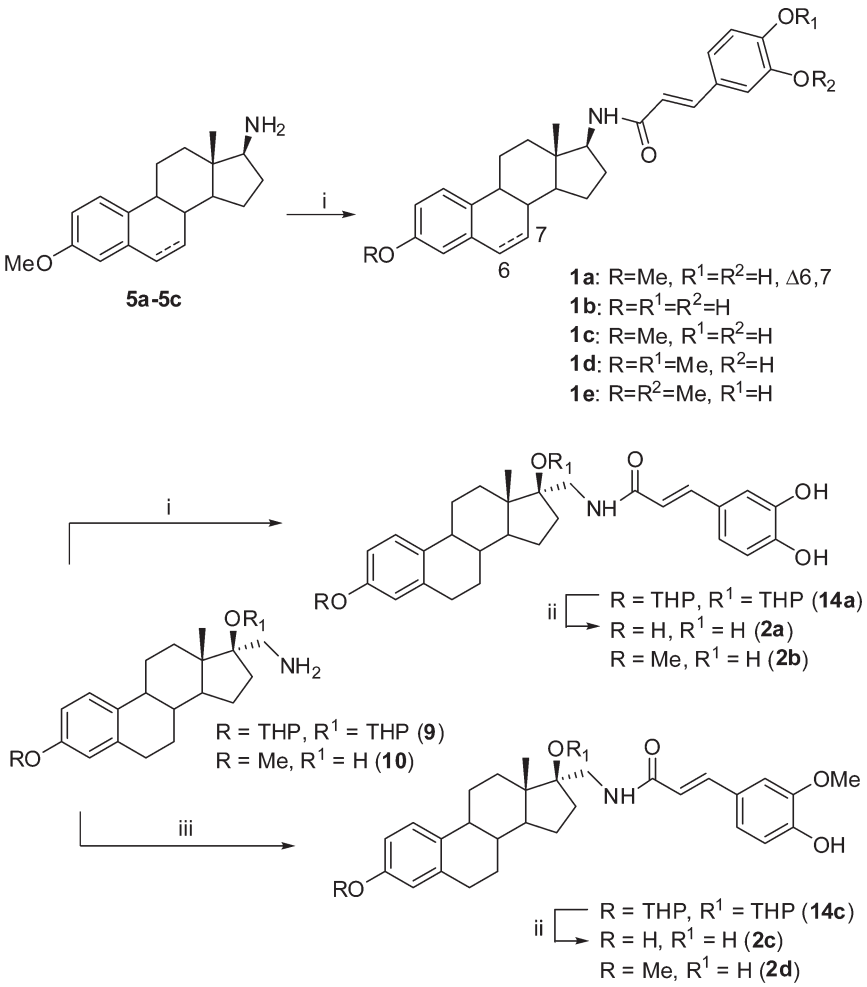
Scheme 2



Scheme 3

In the case of caffeic acid (**11a**), the reaction was found to lead to a by-product along with the formation of the respective, desired activated acid derivative **12a**. However, the by-product was shown not to be the typical ureide, described in the literature as a by-product in the reaction of 2-alkenoic acids with nucleophiles, when using DCC as coupling reagent. Rather, it was the 1,3-dicyclohexyl-1-(3′,4′-dihydroxycinnamoyl)-2-pentafluorophenyl-isourea **13** (Scheme 3). This by-product, which was formed in significant amounts, complicated the work-up of **12a**.

Nevertheless, the reaction of the steroidal amines **5c**, **9**, and **10** with a mixture of **12a** and **13** gave the *E*-3,4-dihydroxyphenylpropenamides **1a**, **14a**, and **2b** in good yield under MW irradiation (Scheme 4). The steroidal amines **9** and **10**, when reacted with the pentafluorophenyl ester of ferulic acid **12b** gave the *E*-3-methoxy-4-hydroxyphenylpropenamides **14c** and **2d** under the same conditions. A small amount of dioxane, necessary to help to mix the reagents adequately, was used. Before column chromatographic separation of the amidation products, the steroidal amides **14a** and **14c** were treated with



i. for **1a-c**, **14a** and **2b**: **12a**, **13**, MW; for **1d**: **12b**, MW; for **1e**: **12c**, MW;
ii. p-TsOH, DCM, rt; iii. **12b**, MW

Scheme 4

Table 1 *In vitro* tumour 50% growth inibition (GI_{50}) of **1c**, **2b**, **2d** and 5-fluorouracil (**FU**)[25]

Panel/cell line	**1c** $\log_{10}GI_{50}$	**2b** $\log_{10}GI_{50}$	**2d** $\log_{10}GI_{50}$	**FU** $\log_{10}GI_{50}$
Leukemia				
CCRF-CEM	–6.14	–6.06	–6.03	–5.00
Non-small cell lung cancer				
NCI-H522	–5.77	–6–30		–5.10
CNS cancer				
SF-539			<–8.00	–7.20
Ovarian cancer				
SK-OV-3	–4.94	–4.83		–4.70
Melanoma				
SK-MEL-2	–5.56	–5.25	–4.41	–4.42
Renal cancer				
UO-31	–5.90	–5.99		–5.80
Prostate cancer				
PC-3	–5.66	–5.72		–5.60
Breast cancer				
BT-549	–5.26	–5.40		–5.00

para-toluenesulfonic acid in order to hydrolyse the tetrahydropyranyloxy groups, giving the non-protected hydroxysteroidal amides **2a** and **2c**.

The starting protocol consists of an *in vitro* pre-screening of the reported compounds **1a–e** and **2a–d** using the three cell lines MCF 7 (breast cancer), NCI-H460 (lung cancer), and SF-268 (Central Nervous System cancer-CMS), in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda.[19–21] The minimum condition for satisfying this test is the reduction of at least 32% of the cell growth in any one of the three lines. Among all the evaluated compounds, compounds **1d** and **1e** failed the pre-screening, while compounds **1a–c** and **2a–d** exhibited positive results and have been further evaluated *in vitro* against a full panel of 60 human cancer cell lines derived from nine neoplastic diseases. The response parameters 50% growth inhibition (GI_{50}), total growth inhibition (TGI), and 50% lethal concentration (LC_{50}) of the compounds obtained for each cell line are given in Table 1 and in the Electronic Supplementary Information (ESI, Tables S1–S3). Compounds **1a–c** and **2a–d** exhibited a non-selective broad spectrum of antitumor activity.

Compounds **1a**, **1c**, **2b**, and **2d** exhibited a better antiproliferative profile than the parents **1b**, **2a**, and **2c**. In the most active compounds, the hydroxyl function at the 3-position of the A-ring of the steroid was protected in the form of methoxy group. Given that a good compromise between lipophilicity *versus* hydrophilicity is required for the molecules to reach the cell, it is believed that the presence of the methoxy group might contribute to the better *in vitro* performance exhibited by the more lipophilic **1a**, **1c**, **2b**, and **2d**. It should be noted that the unsaturation in the B ring in **1a** does not improve the antiproliferative effect of the derivatives. In comparison with 5-fluorouracil (FU), **1c** shows a better antiproliferative effect against a number of cancer cell lines.

Compound **2d** was found to exhibit a very high antiproliferative effect against the CNS cancer cell line SF-539 (Table 1) and the leukemic cancer cell line CCRF-CEM. In both cancer cell lines, the growth inhibition effect of **1d** surpassed the observed effect for the **FU**. However, it was compound **2b** that exhibited a more linear tumour cell growth inhibitory effect among the 60 cancer cell lines, while also exhibiting a better antiproliferative effect than **FU** in several cancer cell lines (Table 1; ESI, Table S1). The good antiproliferative effect of **2b**, in addition to the low cytotoxicity (ESI, Table S3) suggests this compound to be the most favourable candidate as a lead compound for further development of new derivatives along these lines.

Conclusion

A number of novel steroidal-cinnamide conjugates **1a** and **2** were prepared according to a straightforward synthetic strategy in good overall yield. The microwave assisted amidation of non-protected cinnamic acids was found to be possible in the presence of a neighbouring hydroxyl group in the amine component. The *in vitro* anti-tumor screening against a full panel of 60 cancer cell lines revealed the anti-tumor activity of most of the steroidal cinnamide conjugates. The dihydroxyphenyl moiety of the derivatives confers a higher antiproliferation capacity to the derivatives. The presence of the methoxy group at the 3-position of the A ring in the steroid subunit is necessary for a good antiproliferative activity within this series of compounds. Compounds possessing an additional hydroxyl group at 17β (**2b** and **2d**) preserved the desired antiproliferative effect with a moderate decrease in the cytostatic effect and a marked loss of the cytotoxicity.

Experimental

Melting points were measured on a Yanaco hot-stage microscope. IR spectra were measured with a JASCO IR-700 instrument. ^{1}H (270 MHz) and ^{13}C (67.8 MHz) spectra were recorded with a JEOL EX-270 spectrometer. The chemical shifts are relative to TMS. Mass spectra were measured with a JMS-01-SG-2 spectrometer. Column chromatography was carried out on Wakogel 300.

For the microwave irradiation experiments a domestic microwave National NE-S12 (with power settings at 170 W/500 W and 750 W, 2450 MHz) was used. Pyrex glass beakers (20 mL) with a small opening were used. The beakers were sealed with Saran Wrap®, which allows for pressure equilibration. Temperature calibration showed that a temperature plateau of 130 ºC (bulk temperature of the mixture) was reached after 3 min. All chemical reagents were of reagent grade. Estrone was obtained commercially from Wako. 3-Methoxyestrone (**3b**) was prepared according to the literature (KOH, MeI, DMSO).[22] 3-*O*-Methyl-17*β*-aminoestra-1,3,5(10)-triene-3-ol (**5b**) and 17*β*-aminoestra-1,3,5(10)-triene-3-ol (**5a**) were prepared from the corresponding steroidal oximes[23] by reduction with sodium in refluxing propanol.[24] The synthesis of steroids **1b–1e** has been described earlier.[12]

3-O-Methyl-estra-1,3,5(10),6-tetraene-3-ol-17-one-17-oxime (**4c**): 3-*O*-Methyl-estra-1,3,5(10),6-tetraene-3-ol-17-one (**3c**, 1.3 g, 4.7 mmol) was dissolved in EtOH (22 mL) and a solution of $NH_2OH{\cdot}HCl$ (1.3 g, 18.8 mmol) in H_2O (5 mL) was added at room temperature. The reaction mixture was heated at 50 ºC for 11 h 30 min. The solvent was then evaporated, and the crude mixture was submitted to column chromatography on silica gel (EtOAc/$CHCl_3$ 1:3 → 1:2) to give **4c** (850 mg, 61%) as a colourless solid, m.p. 180–184 ºC. (Found: M^+, 297.1730. $C_{19}H_{23}O_2N$ requires M^+, 297.1729). KBr/cm^{-1} ν_{max} 3262, 2924, 1682, 1630, 1603, 1568, 1493, 1452, 1257, 1046, 929; $^1\delta_H$ ($CDCl_3$) 0.96 (3H, s, CH_3), 1.50–2.43 (9H, m), 2.58–2.63 (2H, m), 3.80 (3H, s, $OC\underline{H}_3$), 6.04 (1H, d, ^3J = 9.4 Hz), 6.49 (1H, dd, ^4J = 2.7 Hz ^3J = 9.4 Hz), 6.66 (1H, d, ^4J = 2.7 Hz), 6.75 (1H, dd, ^4J = 2.7 Hz, ^3J 8.3 Hz), 7.17 (1H, d, ^3J = 8.3 Hz), 7.50 (1H, bs, OH); δ_c ($CDCl_3$) 16.9, 22.8, 24.0, 25.1, 33.5, 38.1, 42.1, 44.7, 51.2, 55.3, 111.8, 112.0, 124.2, 128.3, 131.1, 132.0, 135.3, 158.2, 170.7; MS (EI$^+$, 70 eV) *m/z* (%) 297 (100) [M^+].

3-O-Methyl-17β-aminoestra-1,3,5(10),6-tetraene-3-ol (**5c**): Aluminium powder (297 mg, 11 mmol) was added to a solution of estrone oxime **4c** (320 mg, 1.07 mmol) in EtOH (10 mL). A solution of $HgCl_2$ (240 mg, 0.88 mmol) in H_2O (1 mL) and EtOH (2 mL) was added. The reaction mixture was stirred at reflux temperature for 24 h. The reaction mixture was then submitted to centrifugation. The supernatant was separated and was evaporated *in vacuo*. The centrifugate was stirred with conc. HCl (5 mL) and resubmitted to centrifugation. This procedure was repeated twice. An aqueous solution of HCl (50 mL) was added to the acidic supernatant phases was added and poured onto the crude of the first supernatant, followed by extraction with EtOAc (2 x 50 mL). Aqueous 10% NaOH sol. was then added to the aqueous phase until basic pH, and the mixture was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with H_2O (100 mL), dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to provide 3-*O*-methyl-17β-aminoestra-1,3,5(10),6-tetraene-3-ol, **3c** (140 mg, 46%) as an oil. (Found: M^+, 283.1933. $C_{19}H_{25}ON$ requires

M^+, 283.1936). Neat/cm^{-1} ν_{max} 3372, 2924, 1603, 1570, 1491, 1460, 1260; δ_H ($CDCl_3$) 0.68 (3H, s, CH_3), 1.20–2.47 (13H, m), 2.79 (1H, m), 3.79 (3H, s, OCH_3), 5.99 (1H, dd, ^3J = 1.6 Hz ^3J = 9.6 Hz, H7), 6.44 (1H, dd, ^4J = 2.7 Hz ^3J = 9.6 Hz, H6), 6.64 (1H, d, ^4J = 2.7 Hz), 6.73 (1H, dd, ^4J = 2.7 Hz ^3J 8.1 Hz), 7.16 (1H, d, ^3J = 8.1 Hz); MS (EI^+, 70 eV) *m/z* (%) 171 (100), 283 (66) [M^+].

17α-Cyano-estra-1,3,5(10)-triene-3,17β-diol (**6**)–ZnI_2 (95 mg, 0.3 mmol) and estrone **3a** (2 g, 7.4 mmol) were placed in dry CH_2Cl_2 (5 mL), and TMSCN (13.3 mmol) was added. The reaction was carried out under an argon atmosphere, initially under reflux for 1h and then at rt for 3 more hours. Thereafter, conc. HCl (2 mL) was added as well as H_2O (10 mL) and CH_2Cl_2 (10 mL). A white precipitate was formed after 15 min. It was washed with CH_2Cl_2 (10 mL) giving **6** (1.5 g, 68%) as a colourless solid, m.p. 256–260 ºC. (Found: M^+, 297.1728. $C_{19}H_{23}O_2N$ requires M^+, 297.1729). KBr/cm^{-1} ν_{max} 3414, 2924, 2850, 2236, 1649, 1610, 1450, 1220; δ_H (DMSO-d_6) 0.90 (3H, s, CH_3), 1.38–2.54 (14H, m), 2.80–2.84 (2H, m), 4.62 (1H, s, OH), 6.56 (1H, d, ^4J = 2.7 Hz), 6.63 (1H, dd, ^4J = 2.7 Hz ^3J = 8.3 Hz), 7.14 (1H, d, ^3J = 8.3 Hz); MS (EI^+, 70 eV) *m/z* (%) 297 (100) [M^+].

17α-Cyano-3,17β-bis-tetrahydropyranyloxy-estra-1,3,5(10)-triene (**7**): To a solution of the cyanohydrin **6** (1.50 g, 5.05 mmol) in CH_2Cl_2 (15 mL) and THF (4 mL) was added DHP (1.5 ml, 25.9 mmol) and *p*-TsOH (9.6 mg, 0.05 mmol). The reaction mixture was stirred for 10 min at rt. Thereafter, pyridine (0.20 mL) and a solution of H_2O (90 mL) containing $NaHCO_3$ (410 mg) were added, and the mixture was extracted with CH_2Cl_2 (100 mL). The organic phase was washed with H_2O (100 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to dryness. The crude residue was subjected to column chromatography on silica gel (ether/$CHCl_3$/hexane 1:1:3) to give 17α-cyano-3,17β-bis-*O*-tetrahydropyranyl-estra-1,3,5(10)-triene-3,17β-diol, **7** (1.69g, 72%) as an amorphous solid. (Found: MH^+, 466.2956. $C_{29}H_{40}O_4N$ requires MH^+, 466.2957). KBr/cm^{-1} ν_{max} 2938, 2870, 1613, 1499, 1245, 1202, 1037; δ_H (DMSO-d_6) 0.94 (3H, s, CH_3), 1.26–1.90 (22H, m), 2.24–2.48 (3H, m), 2.82–2.87 (2H, m), 3.53–3.63 (2H, m), 3.86–3.96 (2H, m), 5.09–5.11 (1H, m), 5.37–5.40 (1H, m), 6.78 (1H, d, ^4J = 2.7 Hz), 6.84 (1H, dd, ^3J = 8.6 Hz ^4J = 2.7 Hz), 7.19 (1H, d, ^3J = 8.6 Hz); δ_C (DMSO-d_6) 12.7, 18.8, 19.5, 23.3, 25.2, 25.3, 26.1, 27.2, 29.6, 30.4, 31.0, 33.8, 35.2, 38.9, 43.4, 48.3, 48.6, 62.0, 63.1, 86.6, 96.4, 97.7, 114.1, 116.6, 120.7, 126.2, 133.2, 137.7, 155.0; MS (FAB^+, 3-nitrobenzyl alcohol) *m/z* (%) 381 (10.9), 466 (1.7) [MH^+].

3-O-Methyl-17α-cyano-estra-1,3,5(10)-triene-3,17β-diol (**8**) ZnI_2 (89 mg, 0.28 mmol) and 3-methoxy estrone **3b** (2 g, 7 mmol) were placed in dry CH_2Cl_2 (4 mL), and TMSCN (12.6 mmol) was added. The reaction was carried out under an argon atmosphere for 4 hours. The reaction mixture was then treated with conc. HCl (2 mL). H_2O (100 mL) was added to the reaction mixture, and it was extracted with CH_2Cl_2 (2 × 100 mL). The organic phase was washed with H_2O (100 mL), dried over anhydrous $MgSO_4$, and filtered. After concentration of the solvent, the crude product was submitted to column chromatography on silica gel (ether/hexane/$CHCl_3$ 1:1:1) to give **8** (1.8 g, 86%) as a colourless solid. (Found: MH^+, 311.1894. $C_{20}H_{25}O_2N$ requires MH^+, 311.1885). KBr/cm^{-1} ν_{max} 3382, 2918, 2866, 2234, 1499, 1464, 1257, 1147; δ_H ($CDCl_3$) 0.90 (3H, s, CH_3), 1.40–2.54 (14H, m), 2.84–2.87 (2H, m), 3.78 (3H, s, O<u>C</u>H$_3$), 6.63 (1H, d, ^4J = 2.6 Hz), 6.72 (1H, dd, ^4J = 2.6 Hz ^3J = 8.4 Hz), 7.20 (1H, d, ^3J = 8.4 Hz); MS (FAB^+, 3-nitrobenzyl alcohol) *m/z* (%) 311 (28) [MH^+].

3-O-Methyl-17α-aminomethyl-estra-1,3,5(10)-triene-3,17β-diol (**10**): A solution of cyanohydrin **8** (0.72 g, 2.33 mmol) in dry THF (7 mL) at 0 ºC was added to a suspension of $LiAlH_4$ (0.41 g, 10.80 mmol) in dry THF (7 mL). The reaction mixture was stirred for 24 h at rt. The reaction mixture was then poured onto ice (20 g) and H_2O (3 mL) was added, the organic solvent was evaporated and the aluminium salts were filtered. The aluminium salts were washed several times, alternately with H_2O and EtOAc, until no amine could be detected (monitoring by TLCusing a 2% ethanolic sol. of ninhydrin). Conc. HCl was added dropwise to the aqueous phase until pH ~ 1 was reached. EtOAc (75 mL) was then added to the aqueous phase, and the mixture was extracted. Then, the aqueous phase was extracted further with EtOAc (2 × 50 mL). Aqueous 10% NaOH sol. was added to the aqueous phase until basic pH was reached, and the mixture was extracted with EtOAc (4 × 50 mL). The organic phase was washed with H_2O (50 mL), dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to dryness to give 3-*O*-methyl-17α-aminomethyl-estra-1,3,5(10)-triene-3,17β-diol **10** (295 mg, 40%) as a colourless solid, m.p. 155–161 ºC (lit. 163 ºC [17]). (Found: MH^+, 316.2270. $C_{20}H_{30}O_2N$ requires MH^+, 316.2277). KBr/cm^{-1} ν_{max} 3448, 2932, 1614, 1507, 1448; δ_H ($CDCl_3$) 0.93 (3H, s, CH_3), 1.25–2.3 (14H, m), 2.59 (1H, d, ^gJ = 12.5 Hz), 2.82–2.86 (2H, m), 3.01 (1H, d, ^gJ = 12.5 Hz), 3.77 (3H, s, OCH_3), 6.62 (1H, d, ^4J = 2.7 Hz), 6.70 (1H, dd, ^3J = 8.4 Hz ^4J = 2.7 Hz), 7.19 (1H, d, ^3J = 8.4 Hz); δ_C ($CDCl_3$) 14.4, 23.2, 26.3, 27.5, 29.8, 32.6, 34.9, 39.4, 43.8, 45.8, 47.5, 50.0, 55.2, 81.7, 111.5, 113.8, 126.2, 132.6, 138.0, 157.5; MS (FAB^+, 3-nitrobenzyl alcohol) *m/z* (%) 316 (44) [MH^+].

(E)-N-(3′-O-Methyl-estra-1′,3′,5′(10′),6′-tetraen-3′-ol-17′β-yl)-[3-(3,4-dihydroxyphenyl)]propenamide (**1a**): The steroidal amine **5c** (77 mg, 0.27 mmol) was added to a mixture of **12a** and **13** (365 mg, ≅ 0.80 mmol) in the minimum dioxane (1 mL) and the resulting mixture was irradiated at 500 W (2450 MHz) for 4 min. The reaction was carried out under an argon atmosphere. After the reaction was complete, the mixture was subjected directly to column chromatography on silica gel (EtOAc/$CHCl_3$ 1:1) to give **1a** (70 mg, 58%) as a colourless solid; m.p. 157–161 ºC. (Found: MH^+, 446.2326. $C_{28}H_{32}O_4N$ requires MH^+, 446.2331). KBr/cm^{-1} ν_{max} 3434, 2928, 1652, 1602, 1447, 1517, 1258, 1199; δ_H ($CDCl_3$) 0.77 (3H, s, CH_3), 0.85–2.40 (11H, m), 3.79 (3H, s, OCH_3), 4.12–4.15 (1H, m, H17), 5.63 (1H, d, J = 7.8 Hz, NH), 5.98 (1H, d, ^3J = 9.7 Hz), 6.13 (1H, b, OH), 6.27 (1H, d, ^EJ = 15.5 Hz), 6.46 (1H, dd, ^4J = 2.4 Hz, ^3J = 9.7 Hz), 6.65 (1H, d, ^4J = 2.7 Hz), 6.73 (1H, dd, ^3J = 8.1 Hz, ^4J = 2.7 Hz), 6.87 (1H, d, ^3J = 7.4 Hz), 7.02 (1H, d, ^3J = 7.4 Hz), 7.15 (1H, d, ^3J = 8.1 Hz), 7.20 (1H, s), 7.59 (1H, d, ^EJ = 15.5 Hz), 7.70 (1H, b, OH); δ_C ($CDCl_3$) 12.1, 23.5, 24.2, 31.2, 31.5, 36.7, 38.8, 41.8, 44.3, 49.6, 55.4, 59.4, 111.8, 111.9, 112.0, 115.4, 117.4, 120.1, 124.3, 128.1, 131.4, 132.6, 135.4, 142.3, 144.2, 146.5, 158.3, 167.1; MS (FAB^+, 3-nitrobenzyl alcohol) *m/z* (%) 446 (3.4) [MH^+].

(E)-N-(3′-O-Methyl-estra-1′,3′,5′(10′)-triene-3′,17′β-diol-17′α-ylmethyl)-[3-(3,4-dihydroxyphenyl)]propenamide (**2b**): The steroidal amine **10** (110 mg, 0.35 mmol) was added to a mixture of **12a** and **13** (465 mg, ≅ 1.04 mmol) in the minimum dioxane (1 mL) and the resulting mixture was irradiated at 500 W (2450 MHz) for 2 min. After the reaction was complete, the mixture was subjected directly to column chromatography on silica gel (EtOAc/$CHCl_3$ 1:1) to give **2b** (120 mg, 75%) as a colourless solid; m.p. 150–154 ºC. (Found: MH^+, 478.2595. $C_{29}H_{36}O_5N$ requires MH^+, 478.2593). KBr/cm^{-1} ν_{max} 3422, 2928, 1653, 1606, 1533, 1281, 1255; δ_H (DMSO-d_6) 0.82 (3H, s, CH_3), 1.22–1.90 (11H, m), 2.15–2.34 (2H, m), 2.76 (2H, m), 3.13–3.16 (1H, m), 3.56–3.64 (1H, m), 3.68 (3H, s, OCH_3), 4.36–4.61 (1H, b, centered at 4.5, OH), 6.58 (1H, d, ^EJ = 15.4 Hz), 6.59 (1H, d, ^4J = 2.7 Hz), 6.66 (1H, dd, ^4J = 2.7 Hz ^3J = 8.1 Hz), 6.73 (1H, d, ^3J = 8.1 Hz), 6.84 (1H, dd, ^4J = 1.9 Hz ^3J = 8.6 Hz), 6.95 (1H, d, ^4J = 1.9 Hz), 7.16 (1H, d, ^3J = 8.6 Hz), 7.22 (1H, d, ^EJ = 15.4 Hz), 7.76–7.77 (1H, m, NH), 8.83–9.53 (2H, b, centred at 9.23, OH); δ_C (DMSO-d_6) 14.2, 23.0, 25.9, 27.0, 29.3, 30.6, 31.0, 32.7, 43.2, 45.4, 45.8, 49.3, 54.8, 82.2, 111.4, 113.4, 114.0, 115.7, 118.8, 120.3, 126.1, 126.5, 132.1, 137.4, 139.1, 145.5, 147.2, 157.0, 166.3; MS (FAB^+, 3-nitrobenzyl alcohol) *m/z* (%) 478 (22) [MH^+].

(E)-N-(3′-O-Methyl-estra-1′,3′,5′(10′)-triene-3′,17′β-diol-17′α-ylmethyl)-[3-(4-hydroxy-3-methoxyphenyl)propenamide (**2d**): The steroidal amine **10** (60 mg, 0.19 mmol) was added to **12b** (210 mg, 0.60 mmol) in the minimum dioxane (1 mL) and the resulting mixture was irradiated at 500 W (2450 MHz) for 2 min. After the reaction was complete, the mixture was subjected directly to column chromatography on silica gel (EtOAc/$CHCl_3$ 1:1) to give **2d** (60 mg, 64%) as a colourless solid; m.p. 222–224 ºC. (Found: MH^+, 492.2749. $C_{30}H_{38}NO_5$ requires MH^+, 492.2750). KBr/cm^{-1} ν_{max} 3418, 2924, 1655, 1612, 1524, 1253, 1209; δ_H ($CDCl_3$) 0.93 (3H, s, CH_3), 1.25–2.37 (13H, m), 2.83–2.86 (2H, m), 3.39 (1H, dd, ^4J = 2.7 Hz, ^gJ = 13.4 Hz), 3.68–3.73 (1H, dd, ^gJ = 13.4 Hz, ^3J = 7.4Hz), 3.77 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 5.84 (1H, s), 6.07–6.11 (1H, m, NH), 6.31 (1H, d, ^EJ = 15.4 Hz), 6.63 (1H, d, ^4J = 2.7 Hz), 6.70 (1H, dd, ^3J = 8.5 Hz, ^4J = 2.7 Hz), 6.91 (1H, d, ^3J = 8.0 Hz), 7.01 (1H, d, ^4J = 1.7 Hz), 7.07 (1H, dd, ^3J = 8.0 Hz ^4J = 1.7 Hz), 7.18 (1H, d, ^3J = 8.5 Hz), 7.56 (1H, d, ^EJ = 15.4 Hz); δ_C ($CDCl_3$, DEPT 135, DEPT 90) 14.0 (+, CH_3), 23.2 (–), 26.2 (–), 27.3 (–), 29.8 (–), 31.6 (–), 34.7 (–), 39.6 (+, CH), 43.6 (+, CH), 45.7 (C_{quat}), 46.1 (–), 49.6 (+, CH), 55.2 (+, OCH_3), 56.0 (+, OCH_3), 83.7 (C_{quat}), 109.6 (+, CH), 111.5 (+, CH), 113.8 (+, CH), 114.7 (+, CH), 118.2 (+, CH), 122.2 (+, CH), 126.3 (+, CH), 127.4 (C_{quat}), 132.4 (C_{quat}), 137.9 (C_{quat}), 141.2 (+, CH), 146.7 (C_{quat}), 147.4 (C_{quat}), 157.4 (C_{quat}), 167.0 (C_{quat}, CO); MS (FAB^+, 3-nitrobenzyl alcohol) *m/z* (%) 492 (18) [MH^+], 177 (100).

Representative procedure for the synthesis of the (E)-N-(3′-hydroxy-estra-1′,3′,5′(10′)-triene-3′,17′β-diol-17′α-ylmethyl)-3-phenylpropenamide derivatives

A solution of cyanohydrin **7** (1 mmol) in dry THF (4 mL) at 0 ºC was added to a suspension of $LiAlH_4$ (5 mmol) in dry THF (4 mL). The reaction mixture was stirred at rt for 24 h. Thereafter, H_2O (3 mL) was then added dropwise to the solution and the solvent was evaporated. The aluminium salts were filtered and washed several times alternately with H_2O and ether. The phases were separated and the organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated. The crude residue was dissolved in dioxane (1.5 mL) and the pentafluorophenyl ester derivative, **12b**, (2 mmol) was added. The mixture was submitted to MW irradiation (two cycles of 2 min). *p*-TsOH (0.15 mmol) in MeOH (10 mL) was added to the resulting reaction mixture and the solution was stirred at rt. After 1 h, the mixture was concentrated and the reaction residue was submitted directly to column chromatography on silica gel (EtOAc/$CHCl_3$ 2:8 → 5:5 → EtOAc 100%) to give the respective 3-phenylpropenamide derivatives.

(E)-N-(3′-Hydroxy-estra-1′,3′,5′(10′)-triene-3′,17′β-diol-17′α-ylmethyl)-[3-(3,4-dihydroxyphenyl)]propenamide (**2a**): Overall η = 15%; mp. 256–260 ºC. (Found: MH^+, 464.2430. $C_{28}H_{34}O_5N$ requires MH^+, 464.2437). KBr/cm^{-1} ν_{max} 3418, 2930, 1651, 1603, 1538, 1286, 1253; δ_H (DMSO-d_6) 0.81 (3H, s, CH_3), 1.11–2.23 (13H, m), 2.70 (2H, m), 3.04–3.09 (1H, m), 3.46–3.56 (1H, m), 4.48 (1H, s, OH), 6.42 (1H, s), 6.49 (1H, d, ^3J = 8.1 Hz), 6.58 (1H, d, ^EJ = 15.9 Hz), 6.73 (1H, d, ^3J = 8.1 Hz), 6.84 (1H, d, ^3J = 8.1 Hz), 6.95 (1H, s), 7.03 (1H, d, ^3J = 8.1 Hz), 7.22 (1H, d, ^EJ = 15.9 Hz), 7.75 (1H, m, NH), 8.97 (1H, s, OH), 9.08 (1H, s, OH), 9.34 (1H, s, OH); δ_C (DMSO-d_6) 14.3, 23.0, 26.0, 27.1, 29.2, 31.1, 32.7, 45.4, 45.8, 49.3, 66.3, 82.2, 112.7, 114.0, 114.9, 115.7, 118.8, 120.4, 126.0, 126.5, 130.4, 137.2, 139.1, 145.5, 147.2, 154.9, 166.3; MS (FAB^+, 3-nitrobenzyl alcohol) *m/z* (%) 464 (3) [MH^+].

(E)-N-(3′-Hydroxy-estra-1′,3′,5′(10′)-triene-3′,17′β-diol-17′α-ylmethyl)-[3-(4-hydroxy-3-methoxyphenyl)]propenamide (**2c**): Overall η = 25%; mp: 172–177 ºC (dec.). (Found: MH^+, 478.2598. $C_{29}H_{36}O_5N$ requires MH^+, 478.2593). KBr/cm^{-1} ν_{max} 3428, 2924, 1652, 1588, 1514, 1270; δ_H (DMSO-d_6) 0.81 (3H, s, CH_3), 1.18–2.28 (14H, m), 2.69 (2H, m), 3.15 (1H, dd, ^3J = 2.7 Hz, ^gJ = 13.4 Hz), 3.79 (3H, s, OCH_3), 3.59 (1H, dd, ^3J = 7.8 Hz, ^gJ = 13.4 Hz), 4.49 (1H, s, OH), 6.42 (1H, d, ^4J = 2.3 Hz), 6.49 (1H, dd, ^4J = 2.3 Hz ^3J = 8.1 Hz), 6.71 (1H, d, ^EJ = 15.4 Hz), 6.77 (1H, d, ^3J = 8.1 Hz), 6.98 (1H, d, ^3J = 8.3 Hz), 7.03 (1H, d, ^3J = 8.3 Hz), 7.15 (1H, s), 7.30 (1H, d, ^EJ = 15.4 Hz), 7.71 (1H, dd, ^3J = 2.7 Hz, ^3J = 7.8 Hz, NH), 8.97 (1H, s, OH), 9.39 (1H, s, OH); δ_C (DMSO-d_6) 14.2, 23.0, 26.0, 27.1, 29.2, 31.1, 32.8, 43.2, 45.4, 45.8, 49.3, 55.5, 59.7, 82.2, 110.6, 112.7, 114.9, 115.6, 119.3, 121.7, 126.0, 126.5, 130.4, 137.1, 138.9, 147.8, 148.2, 154.9, 166.2; MS (FAB^+, 3-nitrobenzyl alcohol) *m/z* (%) 478 (16) [MH^+], 177 (35).

In vitro *growth inhibition screening*

The *in vitro* cancer screening was carried out within the Developmental Therapeutics Program of the National Cancer Institute, National Institute of Health, Bethesda, Md, USA. The description of the NCI-60 DTP Human Tumor Cell Line Screening can be obtained at http://dtp.cancer.gov/branches/btb/ivclsp.html.

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Electronic Supplementary Information

Tables S1, S2 and S3 have been deposited in the ESI available through stl.publisher.ingentaconnect.com/content/stl/jcr/suppdata.

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