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Novel D-ring modified steroid derivatives as potent, non-estrogenic, steroid sulfatase inhibitors with in vivo activity☆

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Abstract

In pursuit of novel steroid sulfatase (STS) inhibitors devoid of estrogenicity, several D-ring modified steroid derivatives were synthesised. In vitro evaluation of the compounds identified two highly potent inhibitors, **4a** and **4b**, which were 18 times more active than estrone-3-*O*-sulfamate (EMATE), both having IC_{50} values of ca. 1 nM. These 16,17-seco-estra-1,3,5(10)-triene-16,17-imide derivatives were synthesised from estrone, via the intermediate **1**, which was easily alkylated, deprotected and sulfamoylated affording the final compounds in high yields. In order to assess their biological profile, the selected inhibitors were tested for their in vivo inhibitory potency and estrogenicity in ovariectomised rats. After an oral dose of 10 mg/kg per day for 5 days, **4a** and **4b** were found to inhibit rat liver steroid sulfatase by 99%. They were also devoid of estrogenic activity in the uterine weight gain assay, indicating that these two leads have therapeutic potential for the treatment of hormone-dependent breast cancer.

Keywords: Steroid sulfatase; Hormone-dependent breast cancer; D-ring modification; Estrogenicity; In vivo inhibition

1. Introduction

The desulfation of inactive steroids by the enzyme steroid sulfatase (STS) represents the main source for the local productions of active estrogens and, to a lesser extent, of active androgens, in hormone-sensitive tumours. In women with hormone-dependent breast cancer, it is believed that the steroid conjugate estrone-sulfate (E1S) is readily converted in situ into estrone by the enzyme STS [1], accounting for higher estrogen concentration in tumour tissues than circulating plasma levels [2]. Although the conversion of androgens into estrogens by the enzyme aromatase was initially thought to be the main pathway for local synthesis of estrogens [3], there is now substantial evidence that production of estrone via the sulfatase pathway is 10-fold higher than that via the aromatase pathway [1]. Furthermore, androstenediol, which originates from dehydroepiandrosterone (DHEA) after its hydrolysis from DHEA-sulfate, was shown to be capable of binding to the estrogen receptor and stimulating the growth of tumour cells in vitro [4] and induced mammary tumours in vivo [5]. The hydrolysis of E1S and DHEA-sulfate is thought to be carried out by the same steroid sulfatase [6], suggesting that the inhibition of this enzyme offers an attractive strategy for reducing active steroid levels with the view to generating alternative therapeutic regimens for combating hormone-dependent breast cancers.

Estrone-3-*O*-sulfamate (EMATE) was the first highly potent STS inhibitor to be discovered, with an IC_{50} of 65 pM in intact MCF-7 breast cancer cells. It was found to irreversibly inhibit the enzyme activity in a time- and concentration-dependent manner [7] and was active in vivo on oral administration [8]. However, it proved to be more estrogenic than 17-ethinylestradiol on oral application to the rat [9]. In an attempt to design steroidal inhibitors that are devoid of estrogenic activity, molecules combining both the pharmacophore of an STS inhibitor [10] and features that might render reduced estrogenicity have been developed. For example, Li et al. synthesised a series of 3-*O*-sulfamate derivatives of estradiol (E2) bearing 17β-(*N*-alkylcarbamoyl) and 17β-(*N*-alkanoyl) side-chains [11] which turned out to be potent STS inhibitors and did not stimulate the growth of the MCF-7 cell line. Recently, Poirier and co-workers reported 17α-benzyl substituted

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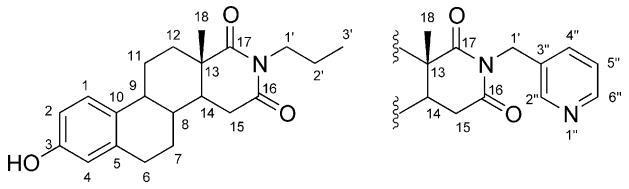


Fig. 1. Example for the numbering of 3-hydroxy-16,17-seco-estra-1,3,5(10)-triene-16,17-imide derivatives.

derivatives of estradiol as a new class of STS inhibitors [12], acting via reversible interactions, as opposed to the active site-directed irreversible inhibition of EMATE. However, these derivatives were shown subsequently to be estrogenic [13] suggesting that the introduction of hydrophobic substituents at the 17α-position of E2 is not entirely effective in abolishing the intrinsic estrogenicity of this type of inhibitor. Non-steroidal inhibitors have also been synthesised and coumarin sulfamate derivatives, developed in our laboratories, have proven to be highly potent non-estrogenic steroid sulfatase inhibitors [14].

To further explore the possibility of hydrophobic interactions in the region neighbouring the D-ring of steroidal compounds as a strategy for enhancing inhibitory activities with a more effective reduction in estrogenicity, several novel D-ring derivatives of EMATE were synthesised. It was reasoned that 3-hydroxy-16,17-seco-estra-1,3,5(10)-triene-16,17-imide [15] would be a suitable template for a structure–activity relationship study (Fig. 1). The piperidinedione moiety is designed to mimic the D-ring of estrone (E1) and is therefore expected to have less intrinsic estrogenicity than E2-derived inhibitors. The imido N-atom of the D-ring also attractively allows the introduction of a variety of alkyl side-chains which might render favourable hydrophobic interactions with the active site, and sulfamoylation at the 3-position is expected to ensure irreversible inactivation of the enzyme. In vitro evaluation of the series of compounds synthesised identified two highly potent STS inhibitor with IC_{50} values of 1 nM, making them ca. 18 times more active than EMATE in the same assay (in vitro data will be reported elsewhere). We report here the chemical synthesis for these structurally modified estrogen derivatives and their in vivo activities.

2. Materials and methods

2.1. General methods for chemical synthesis

All chemicals were either purchased from Aldrich or Lancaster Synthesis (Morecambe, UK). All organic solvents of AR grade were supplied by Fisher Scientific (Loughborough, UK). Anhydrous *N*,*N*-dimethylformamide (DMF) and *N*,*N*-dimethylacetamide (DMA), used for *N*-alkylations and sulfamoylation reactions, were purchased from Aldrich and were stored under a positive pressure of N_2 after use. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger [16] and was stored in the fridge under positive pressure of N_2 as a solution in toluene as described by Woo et al. [17]. An appropriate volume of this solution was freshly concentrated in vacuo immediately before use. E1 was purchased from Sequoia Research Products (Oxford, UK) and E1S from Sigma Chemical Co. (Poole, UK). [6,7-^{3}H]E1S (specific activity, 50 Ci/mmol) and [4-^{14}C]E1 (specific activity, 52 mCi/mmol) were purchased from New England Nuclear (Boston, USA).

Thin layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminium sheets silica gel 60 F_{254}, Art. No. 5554). Product(s) and starting material were detected by either viewing under UV light or treating with an ethanolic solution of phosphomolybdic acid followed by heating. Flash column chromatography was performed on silica gel (Sorbsil C60). IR spectra were determined as KBr discs using a Perkin-Elmer Spectrum RXI FT–IR and peak positions are expressed in cm^{-1}. ^{1}H NMR and *DEPT*-edited ^{13}C NMR spectra were recorded with JMN-GX 400 NMR spectrometer at 400 and 100 MHz, respectively, and chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded at the Mass Spectrometry Service Centre, University of Bath. FAB-MS were carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points were determined using a Reichert-Jung Thermo Galen Kofler block and are uncorrected.

2.2. Synthesis of 3-benzyloxy-16,17-seco-estra-1,3,5(10)-triene-16,17-imide (*1*)

Compound **1** [15] was prepared from 3-benzyloxy-estrone via a haloform reaction, which cleaved the D-ring, and thermal condensation with urea of the resulting marrianolic acid derivative. Full synthetic details of this new route will be reported elsewhere.

2.3. Synthesis of *2a* and *2b*

Sodium hydride (60% dispersion in mineral oil, 1.2 eq.) was added to a stirred solution of **1** in anhydrous DMF (15 ml) at 0 °C under an atmosphere of N_2. After evolution of hydrogen had ceased, the parent alkyl halide (2 eq.) was added. The reaction mixture was stirred at room temperature and poured into water (50 ml). The resulting solution was extracted into ethyl acetate (50 ml) and the organic layer washed with brine (4 × 25 ml), dried ($MgSO_4$), filtered and evaporated in vacuo.

2.3.1. 3-Benzyloxy-N-propyl-16,17-seco-estra-1,3,5(10)-triene-16,17-imide (*2a*)

Purification of the crude product by flash chromatography using $CHCl_3$ as eluent gave a white solid (524 mg, 94%). For analysis, a sample was recrystallized from EtOH

to give white crystals: mp 95–98 °C; IR (KBr) ν_{max} 3035, 2960–2870, 1720 (C=O), 1660 (C=O), 1610–1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (3H, t, $J = 7.6$ Hz, C-3′-H_3), 1.16 (3H, s, C-18-H_3), 1.32–2.98 (13H, m), 2.83–2.88 (2H, m, C-6-H_2), 3.64–3.80 (2H, m, *N*-CH_2), 5.03 (2H, s, OCH_2Ar), 6.72 (1H, d, $J = 2.7$ Hz, C-4-H), 6.80 (1H, dd, $J = 8.6$ Hz, $J = 2.7$ Hz, C-2-H), 7.21 (1H, d, $J = 8.6$ Hz, C-1-H) and 7.30–7.44 (5H, m, C_6H_5); MS *m/z* (FAB+) 432.4 [88, $(M + H)^+$]. HRMS *m/z* (FAB+) calcd. for $C_{28}H_{34}NO_3$: 432.2539. Found: 432.2522. Anal. calcd. for $C_{28}H_{33}NO_3$: C, 77.93; H, 7.71; N, 3.25. Found: C, 77.60; H, 7.68; N, 3.26.

2.3.2. 3-Benzyloxy-N-(1″-pyridin-3″-ylmethyl)-16,17-seco-estra-1,3,5(10)-triene-16,17-imide (**2b**)

An additional 2 eq. of sodium hydride were added during the reaction. Purification of the crude product by flash chromatography using $CHCl_3$/acetone (9:1) as eluent gave a white solid (230 mg, 75%). For analysis, a sample was recrystallized from EtOH to give colourless needles: mp 170–172 °C; IR (KBr) ν_{max} 2925–2870, 1720 (C=O), 1670 (C=O), 1610–1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (3H, s, C-18-H_3), 1.28–3.04 (11H, m), 2.84–2.88 (2H, m, C-6-H_2), 4.92 (1H, d, $J = 13.9$ Hz, *N*-CH_AH_B), 4.98 (1H, d, $J = 13.9$ Hz, *N*-CH_AH_B), 5.03 (2H, s, OCH_2Ar), 6.71 (1H, d, $J = 2.7$ Hz, C-4-H), 6.79 (1H, dd, $J = 8.6$ Hz, $J = 2.7$ Hz, C-2-H), 7.17–7.45 (7H, m, C_6H_5, C-1-H and C-5″-H), 7.69 (1H, td, $J = 7.8$ Hz, $J = 1.8$ Hz, C-4″-H), 8.50 (1H, dd, $J = 5.1$ Hz, $J = 1.8$ Hz, C-6″-H) and 8.63 (1H, d, $J = 1.8$ Hz, C-2″-H); MS *m/z* (FAB+) 481.3 [100, $(M + H)^+$]. HRMS *m/z* (FAB+) calcd. for $C_{31}H_{33}N_2O_3$: 481.2491. Found: 481.2504. Anal. calcd. for $C_{31}H_{32}N_2O_3$: C, 77.47; H, 6.71; N, 5.83. Found: C, 77.00; H, 6.75; N, 5.73.

2.4. Synthesis of **3a** and **3b**

Pd–C (10%), 50% (w/w) of substrate used, was added to solutions of the benzyl protected intermediates **2a** and **2b** in MeOH/THF and the resulting suspensions hydrogenated at room temperature using hydrogen-filled balloons. Filtration and evaporation of the filtrates in vacuo gave the crude products.

2.4.1. 3-Hydroxy-N-propyl-16,17-seco-estra-1,3,5(10)-triene-16,17-imide (**3a**)

White solid (256 mg, 81%). An analytical sample was recrystallized from methanol to give colourless crystals: mp 183–186 °C; IR (KBr) ν_{max} 3445 (OH), 3050, 2940–2860, 1725 (C=O), 1655 (C=O), 1585–1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (3H, t, $J = 7.4$ Hz, C-3′-H_3), 1.17 (3H, s, C-18-H_3), 1.30–2.98 (13H, m), 2.82–2.86 (2H, m, C-6-H_2), 3.64–3.80 (2H, m, *N*-CH_2), 4.73 (1H, s, OH), 6.58 (1H, d, $J = 2.7$ Hz, C-4-H), 6.66 (1H, dd, $J = 8.6$ Hz, $J = 2.7$ Hz, C-2-H) and 7.17 (1H, d, $J = 8.6$ Hz, C-1-H); MS *m/z* (FAB+) 342.3 [100, $(M + H)^+$]. HRMS *m/z* (FAB+) calcd. for $C_{21}H_{28}NO_3$: 342.2069. Found: 342.2076. Anal. calcd. for $C_{21}H_{27}NO_3$: C, 73.87; H, 7.97; N, 4.10. Found: C, 73.90; H, 7.98; N, 4.20.

2.4.2. 3-Hydroxy-N-(1″-pyridin-3″-ylmethyl)-16,17-seco-estra-1,3,5(10)-triene-16,17-imide (**3b**)

Creamy solid (141 mg, 91%). An analytical sample was precipitated from hot ethyl acetate to give a white powder: mp 199–223 °C; IR (KBr) ν_{max} 3380 (OH), 2940–2865, 1720 (C=O), 1670 (C=O), 1610–1500 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.11 (3H, s, C-18-H_3), 1.14–2.94 (11H, m), 2.67–2.75 (2H, m, C-6-H_2), 4.82 (1H, d, $J = 14.8$ Hz, *N*-CH_AH_B), 4.87 (1H, d, $J = 14.8$ Hz, *N*-CH_AH_B), 6.44 (1H, d, $J = 2.3$ Hz, C-4-H), 6.52 (1H, dd, $J = 8.5$ Hz, $J = 2.3$ Hz, C-2-H), 7.07 (1H, d, $J = 8.5$ Hz, C-1-H), 7.33 (1H, dd, $J = 7.8$ Hz, $J = 4.7$ Hz, C-5″-H), 7.59 (1H, m, C-4″-H), 8.42–8.47 (2H, m, C-2″-H and C-6″-H) and 9.05 (1H, s, OH); MS *m/z* (FAB+) 391.2 [88, $(M + H)^+$]. HRMS *m/z* (FAB+) calcd. for $C_{24}H_{27}N_2O_3$: 391.2022. Found 391.2019.

2.5. Synthesis of **4a** and **4b**

2.5.1. 3-Sulfamoyloxy-N-propyl-16,17-seco-estra-1,3,5(10)-triene-16,17-imide (**4a**)

The phenol derivative **3a** (100 mg, 293 μmol) was added to a stirred solution of sulfamoyl chloride (2.2 eq.) in anhydrous DMA (2 ml) at 0 °C under an atmosphere of N_2. The reaction mixture was stirred for 6 h and then poured into cold brine (15 ml), and the resulting solution extracted with ethyl acetate (2 × 20 ml). The organic layers were combined, washed with brine (5 × 20 ml), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography using $CHCl_3$/acetone (95:5) as eluent to give a white solid (107 mg, 87%). An analytical sample was recrystallized from acetone/hexane (1:2) to give white crystals: mp 202–204 °C; IR (KBr) ν_{max} 3365 (NH_2), 3255 (NH_2), 3095, 2965–2880, 1710 (C=O), 1660 (C=O), 1600–1500, 1380 (SO_2), 1180 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (3H, t, $J = 7.4$ Hz, C-3′-H_3), 1.17 (3H, s, C-18-H_3), 1.32–3.00 (13H, m), 2.88–2.93 (2H, m, C-6-H_2), 3.64–3.80 (2H, m, *N*-CH_2), 4.90 (2H, s, NH_2), 7.06 (1H, d, $J = 2.5$ Hz, C-4-H), 7.11 (1H, dd, $J = 8.4$ Hz, $J = 2.5$ Hz, C-2-H) and 7.33 (1H, d, $J = 8.4$ Hz, C-1-H); MS *m/z* (FAB+) 421.0 [100, $(M + H)^+$]. HRMS *m/z* (FAB+) calcd. for $C_{21}H_{29}N_2O_5S$: 421.1797. Found 421.1800. Anal. calcd. for $C_{21}H_{28}N_2O_5S$: C, 59.98; H, 6.71; N, 6.66. Found: C, 60.00; H, 6.60; N, 6.49.

2.5.2. 3-Sulfamoyloxy-N-(1″-pyridin-3″-ylmethyl)-16,17-seco-estra-1,3,5(10)-triene-16,17-imide (**4b**)

A stirred solution of **3b** (45 mg, 115 μmol) in anhydrous DMF (3 ml) was treated with sodium hydride (60% dispersion in mineral oil, 1.2 eq.) at 0 °C under an atmosphere of N_2. After evolution of hydrogen had ceased, sulfamoyl chloride (6 eq.) was added. The reaction mixture was stirred at room temperature for 3 h and then poured into water

(25 ml) and the resulting solution extracted with ethyl acetate (2 × 20 ml). The organic layers were combined, washed with water (2 × 20 ml) then brine (2 × 20 ml), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography using $CHCl_3$/acetone (8:2) as eluent to give a creamy powder (42 mg, 78%). This was recrystallized from acetone/hexane to give colourless crystals (28 mg, 52%): mp 215–218 °C; IR (KBr) ν_{max} 3335 (br, NH_2), 3100–2850, 1720 (C=O), 1675 (C=O), 1590–1495, 1380 (SO_2), 1190 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.10 (3H, s, C-18-H_3), 1.15–2.97 (11H, m), 2.79–2.84 (2H, m, C-6-H_2), 4.81 (1H, d, J = 14.8 Hz, N-CH_AH$_B$), 4.86 (1H, d, J = 14.8 Hz, N-CH$_A$$H_B$), 6.96 (1H, d, J = 2.7 Hz, C-4-H), 7.01 (1H, dd, J = 8.6 Hz, J=2.7 Hz, C-2-H), 7.31 (1H, dd, J = 7.8 Hz, J = 4.7 Hz, C-5″-H), 7.36 (1H, d, J = 8.6 Hz, C-1-H), 7.57 (1H, m, C-4″-H), 7.89 (2H, s, NH_2) and 8.41–8.44 (2H, m, C-2″-H and C-6″-H); MS *m/z* (FAB+) 470.3 [48, $(M + H)^+$]. HRMS *m/z* (FAB+) calcd. for $C_{24}H_{28}N_3O_5S$: 470.1750. Found 470.1767. Anal. calcd. for $C_{24}H_{27}N_3O_5S + (H_2O)_{1/2}$: C, 60.03; H, 5.90; N, 8.78. Found: C, 60.00; H, 5.86; N, 8.57.

2.6. In vivo studies

Ovariectomised female Wistar rats (200 g) were obtained from Charles River (Kent, UK) and kept under conditions meeting institutional requirement with free access to food and water. Groups of rats, with three rats in each group, were treated with EMATE (50 μg/kg, s.c.) or **4a** or **4b** (10 mg/kg, p.o.) once daily for a 5-day period. Control groups of rats were given vehicle (propylene glycol, 200 μl, p.o.).

2.6.1. Uterotrophic estrogenicity study

Animals were killed 24 h after administration of the last dose of compound and uteri were excised of fat and weighed. Total body weights of the rats were also recorded, and the results were expressed as (uterine weight × 100)/total body weight.

2.6.2. In vivo inhibition of steroid sulfatase activity

Samples of liver tissue obtained from animals 24 h after administration of the last dose of the drug, were immediately frozen on solid carbondioxide and stored at −20 °C until assay [8]. Briefly, tissue was homogenised in PBS (pH 7.4, 50 mM containing 250 mM sucrose). Nuclei and cell debris were removed by centrifugation. Duplicate aliquots of tissue supernatants were incubated with [6,7-^{3}H]E1S (4×10^5 dpm, adjusted to a final concentration of 20 μM with unlabelled E1S) for 1 h at 37 °C. [4-^{14}C]E1 (1×10^4 dpm) was used to monitor procedural losses. Product formed was partitioned into toluene. The mass of E1S hydrolysed was calculated from the ^{3}H counts detected and recovery of [4-^{14}C]E1. The protein concentration in supernatants was measured by the method of Bradford [19].

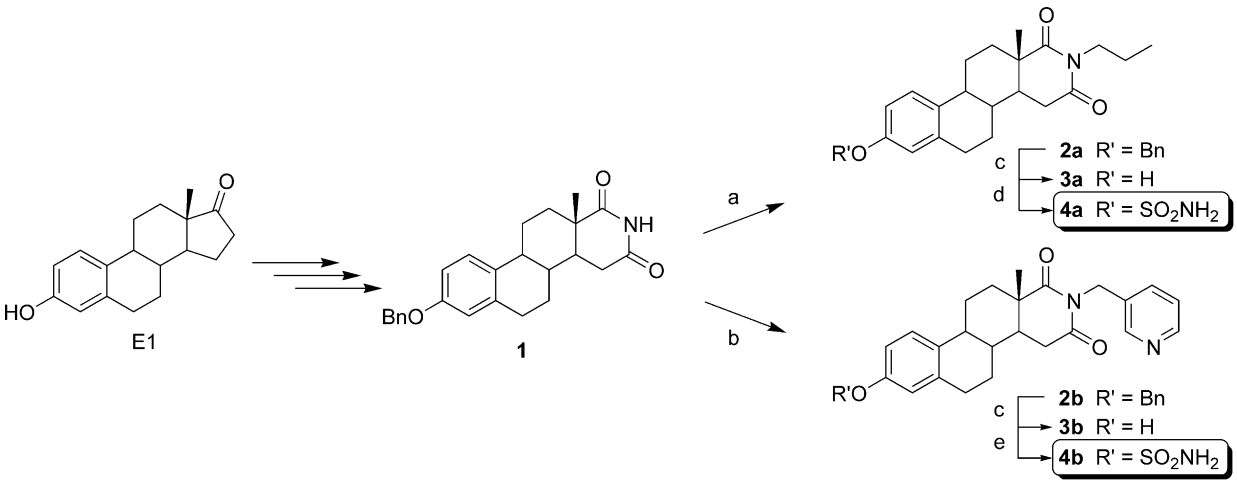
2.7. Statistics

Student's *t*-test was used to assess the significance of the effect of drugs on STS activity.

3. Results and discussion

3.1. Chemical synthesis of the inhibitors

The synthesis of 3-hydroxy-16,17-seco-estra-1,3,5(10)-triene-16,17-imide derivatives has previously been investigated by Gupta and Jindal and the related compounds were initially developed in order to assess the estrogenic/anti-estrogenic effects of D-ring modifications [15]. In our search for D-ring modified steroid derivatives that could inhibit STS without being estrogenic, these estrone derivatives appeared to be ideal candidates for further studies. The piperidine-



Scheme 1. Synthesis of new D-ring derivatives of EMATE from E1. Reagents: (a) NaH/DMF, $CH_3(CH_2)_2I$; (b) NaH/DMF, 3-(bromomethyl)pyridine hydrobromide; (c) Pd/C, H_2, MeOH/THF; (d) $ClSO_2NH_2$/DMA; (e) NaH/DMF, $ClSO_2NH_2$.

dione D-ring has the advantage of allowing a very easy introduction of substituents to the imido N-atom. We therefore decided to investigate the synthesis of a series of these compounds bearing different side-chains on the D-ring and a sulfamate at the 3-position.

An efficient synthetic pathway, different to that proposed by Gupta and Jindal, was developed (Scheme 1) in order to access the intermediate **1** and full details of this will be reported elsewhere. Alkylation on the N-atom of the D-ring was easily performed using sodium hydride in DMF and the corresponding alkyl halide. The resulting alkylated compounds **2a** and **2b** were obtained in yields of 94 and 75%, respectively. The benzyl ether of the products was then cleaved by catalytic hydrogenation using Pd/C affording the

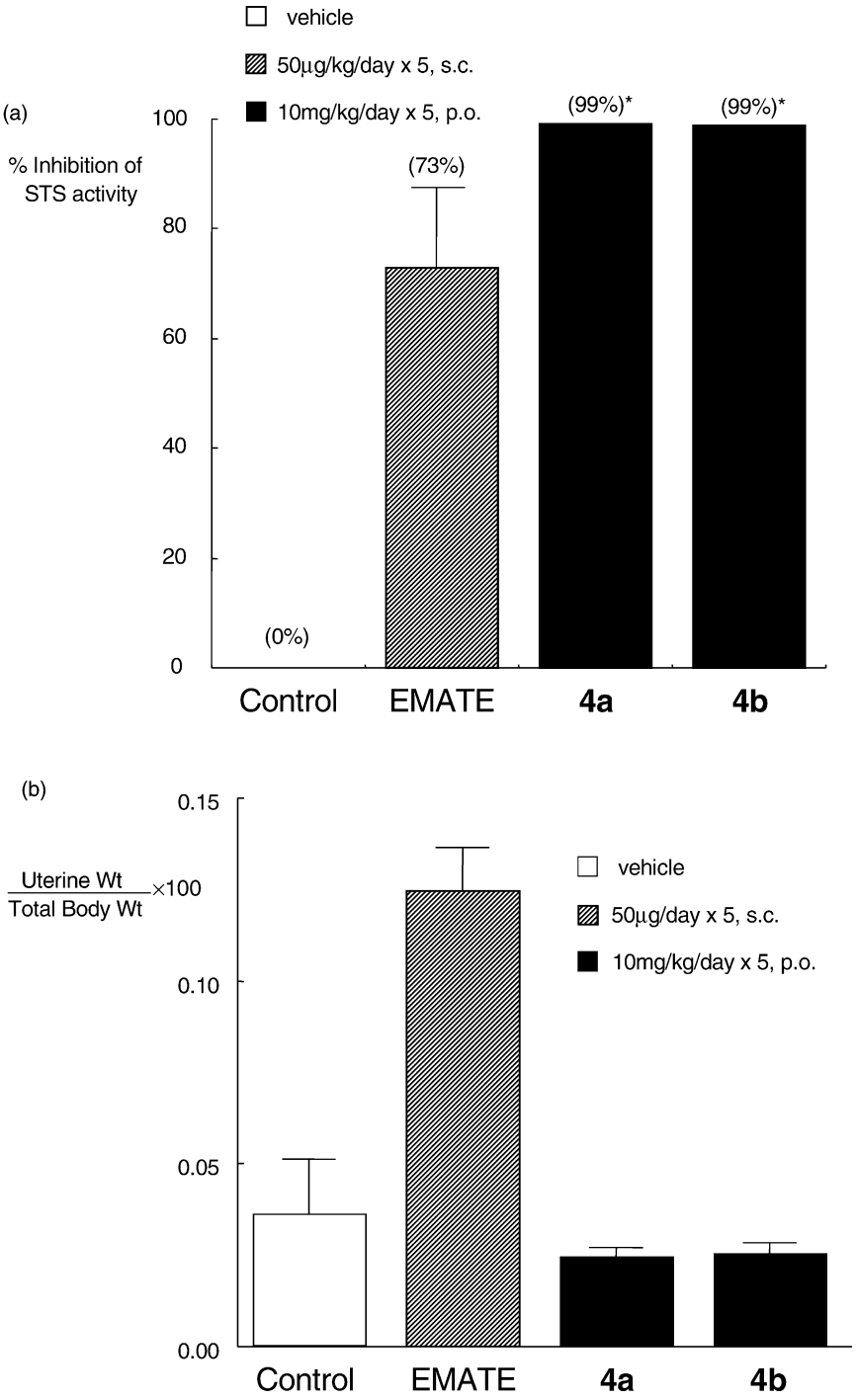


Fig. 2. (a) Inhibition of rat liver STS activity by **4a** and **4b**, means ± S.D. (for **4a** and **4b** the S.D were <10%). Basal sulfatase activity in liver homogenates of untreated animals was 8.75 nmol/h/mg protein. Figure in parentheses represent the percent inhibition compared with the control. The degree of inhibition by **4a** and **4b** was significantly greater than by EMATE at the dose used in this study (* $P < 0.05$) (b) Effects of **4a** and **4b** on uterine growth in the ovariectomised rat. All results are expressed as mean ± S.D. of triplicate measurements.

corresponding phenols **3a** and **3b** in high yields. Sulfamoylation of the hydroxysteroid derivative **3a** was then performed following a recent procedure described by Okada et al. [18] in which sulfamoylation of phenolic compounds is conducted in the aprotic solvent *N*,*N*-dimethylacetamide in the absence of base. This method, which requires only a slight excess of sulfamoyl chloride, is expected to give a better yield of sulfamate than the usual procedure with DMF, where a large excess of the reagent is required. The sulfamate **4a** was obtained in high yield after a short reaction time using 2.2 eq. of sulfamoyl chloride in DMA. Attempts to use this method on **3b** however succeeded only with poor yield and the classic method (NaH/DMF) using 6 eq. of sulfamoyl chloride was chosen to obtain the desired final compound **4b**. In general, we have noticed difficulty in sulfamoylating compounds possessing pyridine motifs and this may be due to the interference of a sulfamoyl-pyridinium species in the process.

3.2. In vivo inhibition of steroid sulfatase

Preliminary in vitro studies carried out by our group identified **4a** and **4b** as potent STS inhibitors, both having IC_{50} values of 1 nM when tested on human placental microsomes [20]. In a limited study on 3-hydroxy-16,17-seco-estra-1,3,5(10)-triene-16,17-imide derivatives, Gupta and Jindal reported that the D-ring modification did not suppress in vitro estrogenic activity [15]. However, the ability of the compounds to bind to the estrogen receptor was shown to be dependent on the type of moiety introduced on the D-ring. Compounds bearing a propyl or a 1-pyridin-3-ylmethyl side-chain on the D-ring were not synthesised in their study. In order to better assess the ability of such compounds as development candidates for the treatment of hormone-dependent breast cancer, an in vivo investigation of their biological profile was carried out. Treatment of ovariectomised rats with an oral dose of 10 mg/kg per day of either inhibitor for 5 days resulted in almost complete inhibition (99%) of liver STS activity (Fig. 2a), as suggested by the IC_{50} values obtained from the in vitro study. Both compounds therefore resemble EMATE by being orally active and highly potent as an STS inhibitor in vivo. However, unlike EMATE, **4a** and **4b** had no effect on uterine growth meaning that they are devoid of estrogenic activity (Fig. 2b). As expected, EMATE was found to be highly estrogenic when tested at a 200-fold lower dose in the same assay. It however remains a powerful inhibitor, as exemplified by the 73% inhibition of liver STS activity obtained after treatment at 50 μg/kg per day subcutaneously. The apparent lack of estrogenicity of compounds **4a** and **4b** further supports our design strategy that the piperidinedione moiety is a more successful template for introducing hydrophobic substituents to the D-ring of an estrogen than other strategies which are based on derivatives of E2.

In conclusion, these in vivo results demonstrate that the *N*-propyl and *N*-(1-pyridin-3-ylmethyl) piperidinedione derivatives of EMATE, **4a** and **4b**, respectively, possess high inhibitory activity against STS with no estrogenic activity. These compounds therefore represent an advance in the development of orally active non-estrogenic steroidal STS inhibitors and are potential candidates for further development for the treatment of hormone-dependent breast cancer.

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