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# Recent advances in the development of steroid sulphatase inhibitors ☆

A. Purohit [a], H.A.M. Hejaz [b], L.W.L. Woo [b], A.E. van Strien [a], B.V.L. Potter [b], M.J. Reed [a,*]

[a] *Endocrinology and Metabolic Medicine, Imperial College School of Medicine, St Mary's Hospital, London, W2 1NY, UK*
[b] *Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK*

**Abstract**

Inhibition of steroid sulphatase is now an important target for the development of new drugs for the treatment of women with endocrine-dependent breast tumours. The first potent sulphatase inhibitor identified, oestrone-3-*O*-sulphamate (EMATE) proved, unexpectedly, to be oestrogenic. A number of strategies have therefore been adopted to design and synthesize a non-oestrogenic inhibitor. For this, a number of modifications have been made to the A and D rings of the oestrone nucleus. 2 Methoxyoestrone-3-*O*-sulphamate, while having similar in vitro and in vivo sulphatase inhibitory potency to that of EMATE, was devoid of oestrogenic activity when tested at 2 mg/kg in an ovariectomised rat uterine weight gain assay. 17-Deoxyoestrone-3-*O*-sulphamate was also a potent steroid sulphatase inhibitor and while it was devoid of oestrogenic activity when tested at 0.1 mg/kg, did stimulate uterine growth at 1.0 mg/kg. As an alternative approach to the use of steroid-based inhibitors a number of single ring, bicyclic non-fused ring, and two fused ring sulphamate analogues were designed, synthesized and tested for their ability to inhibit steroid sulphatase activity. In general, although the single ring and bicyclic non-fused ring sulphamate analogues could inhibit sulphatase activity, they were considerably less potent than EMATE. The mono- and bis-sulphamate derivatives of 5,7-dihydroxyisoflavone were relatively potent, inhibiting in vivo steroid sulphatase activity by 62 and 81% respectively at a single oral dose of 10 mg/kg. A study of the structure-activity relationship of a series of coumarin-based sulphamates has led to the development of a number of potent non-steroidal inhibitors, one of which has a similar potency to that of EMATE. The identification of potent steroid- and non-steroid-based sulphatase inhibitors will enable the therapeutic value of this therapy to be examined in the near future. 

## 1. Introduction

A major impetus to the development of steroid sulphatase inhibitors has been the realisation that in order to improve the therapeutic response to endocrine therapy for women with hormone-dependent breast cancer, not only the aromatase enzyme must be inhibited, but also the synthesis of oestrogenic steroids via other routes. The steroid sulphatase enzyme regulates the formation of oestrone (Fig. 1, **1**) from oestrone sulphate (E1-S, Fig. 1, **2**), a steroid conjugate present at high concentrations in tissues and blood of women with breast cancer [1,2]. This sulphatase also controls the formation of dehydroepiandrosterone (DHA) from DHA-sulphate (DHA-S). DHA can subsequently be reduced to 5-androstene-3$\beta$,17$\beta$-diol (Adiol), a steroid with oestrogenic properties that is capable of stimulating the growth of breast cancer cells in vitro [3] and induced mammary tumours in vivo [4]. In addition to the role that steroid sulphatase has in promoting tumour growth, there is now evidence that it is involved in regulating part of the immune response [5,6] and cognitive function [7,8].

Considerable progress has been made in recent years in developing a number of potent steroid sulphatase

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* Corresponding author. Tel.: +171-886-1738; fax: +171-886-1790.

*E-mail address:* m.reed@ic.ac.uk (M.J. Reed)

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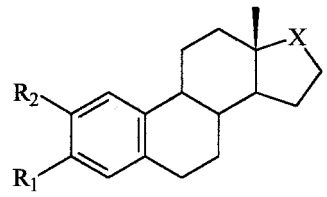
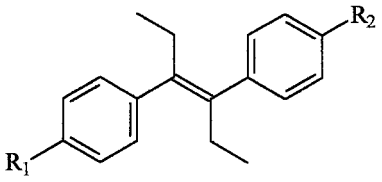
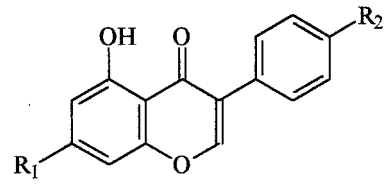


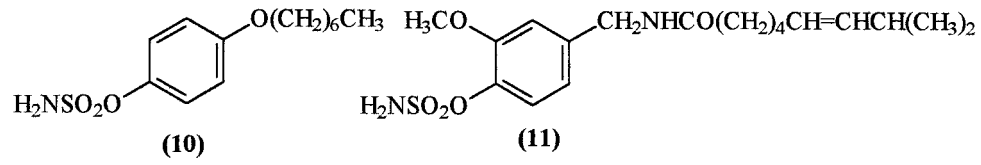

| | R1 | R2 | X |
|---|---|---|---|
| (1) | OH | H | C=O |
| (2) | $OSO_3^-$ | H | C=O |
| (3) | $OSO_2NH_2$ | H | C=O |
| (4) | $OSO_2NH_2$ | $OCH_3$ | C=O |
| (5) | $OSO_2NH_2$ | H | $CH_2$ |

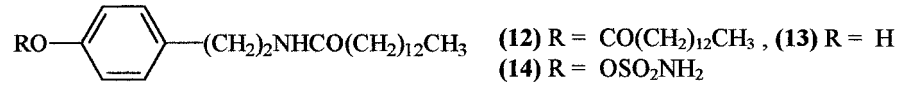



| | R1 | R2 |
|---|---|---|
| (6) | $OSO_2NH_2$ | OH |
| (7) | $OSO_2NH_2$ | $OSO_2NH_2$ |

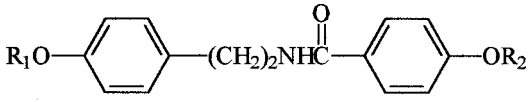



| | R1 | R2 |
|---|---|---|
| (8) | OH | $OSO_2NH_2$ |
| (9) | $OSO_2NH_2$ | $OSO_2NH_2$ |




**(12)** R = $CO(CH_2)_{12}CH_3$ , **(13)** R = H
**(14)** R = $OSO_2NH_2$




| | R1 | R2 | | R1 | R2 |
|---|---|---|---|---|---|
| (15) | $COPhO(CH_2)_5CH_3$ | $(CH_2)_5CH_3$ | (18) | H | $(CH_2)_7CH_3$ |
| (16) | H | $(CH_2)_5CH_3$ | (19) | $OSO_2NH_2$ | $(CH_2)_7CH_3$ |
| (17) | $OSO_2NH_2$ | $(CH_2)_5CH_3$ | | | |

Fig. 1. Structures of oestrone (E1, **1**), oestrone sulphate (E1-S, **2**), oestrone-3-*O*-sulphamate (EMATE, **3**), 2-methoxyoestrone-3-*O*-sulphamate (**4**), 17-deoxyoestrone-3-*O*-sulphamate (NOMATE, **5**), diethylstilboestrol mono-sulphamate (**6**), diethylstilboestrol-bis-sulphamate (**7**), 5,7-dihydroxy-isoflavone-4′-*O*-sulphamate (**8**), 5-hydroxy-isoflavone-4′,-7-bis-sulphamate (**9**), 4-*n*-heptyloxyphenyl-*O*-sulphamate (**10**), *E*-capsaicin sulphamate (*E*-*N*-[(4-*O*-sulphamoyl-3-methoxybenzyl]-8-methyl-6-nonenamide (**11**), (*p*-*O*-tetradecanoyl)-*N*-tetradecanoyl tyramine (**12**), *N*-tetradecanoyl tyramine (**13**), (*p*-*O*-sulphamoyl)-*N*-tetradecanoyl tyramine (**14**), *p*-*O*-(4-*n*-hexyloxybenzoyl)-*N*-(4-*n*-hexyloxybenzoyl) tyramine (**15**), *N*-(4-*n*-hexyloxybenzoyl) tyramine (**16**), (*p*-*O*-sulphamoyl)-*N*-(4-*n*-hexyloxybenzoyl) tyramine (**17**), *N*-(4-*n*-octyloxybenzoyl) tyramine (**18**) and (*p*-*O*-sulphamoyl)-*N*-(4-*n*-octyloxybenzoyl) tyramine (**19**).

inhibitors since Carlstrom first showed that danazol could inhibit the activity of this enzyme in breast tumour tissue [9]. Subsequently a bis-sulphate derivative of a 2-(hydroxyphenyl)indole was found to inhibit steroid sulphatase [10] and oestrone-3-*O*-methylthiophosphonate (E1-MTP) [11] was the first compound specifically designed to inhibit steroid sulphatase activity. While these inhibitors possessed greater potency

compared with danazol, their ability to inhibit sulphatase activity was still relatively modest. E1-MTP, for example, had a $K_i$ of 37.5 μM for the inhibition of sulphatase activity in microsomes prepared from breast tumour tissue [11]. The design, synthesis and testing of a series of steroid sulphate surrogates led to the identification of the most potent inhibitor developed to-date, oestrone-3-*O*-sulphamate (EMATE, Fig. 1, **3**) [12,13]. Subsequent structure-activity studies have confirmed that the sulphamate group attached to an aryl ring is the active pharmacophore required for potent inhibition of steroid sulphatase activity [14].

EMATE inhibited steroid sulphatase activity in a time- and concentration-dependent manner [13] and was active in vivo on oral administration [15]. Unexpectedly, this inhibitor proved to be a potent oestrogen, being five times more active than the synthetic oestrogen, ethinyloestradiol on oral administration to rats [16]. Several strategies were therefore adopted in order to develop a steroid sulphatase inhibitor which, while active in vivo, was devoid of any oestrogenicity that would limit its use for breast cancer therapy. In this paper we review the modifications that have been made to the A- and D-rings of the steroid nucleus and the development of 1, 2 and 3-ring sulphamate analogues which, while potent sulphatase inhibitors, lack any oestrogenic properties. We also report the synthesis of some new single ring and bicyclic non-fused ring inhibitors.

## 2. Materials and methods

### 2.1. Synthesis of single ring and bicyclic non-fused ring sulphamates

All chemicals were either purchased from Aldrich Chemical Co. (Gillingham, Dorset, UK) or Lancaster Synthesis (Morecambe, Lancashire, UK). All organic solvents, of A.R. grade, were supplied by Fison plc (Loughborough, UK) and stored over 4 Å molecular sieves. Anhydrous dimethylformamide (DMF), used for all the sulphamoylation reactions, was purchased from Aldrich and was stored under a positive pressure of $N_2$ after use.

Sulphamoyl chloride was prepared by an adaptation of the method of Appel and Berger [17] and was stored as a solution in toluene as described by Woo et al. [14]. The procedure for the sulphamoylation reaction is described in detail for (Fig. 1, **10**) and sulphamates reported thereafter were synthesised in a similar manner from their corresponding parent phenolic compound.

E1-S and E1 were purchased from Sigma Chemical Co. (Poole, UK). [6,7-$^3$H]-E1 S (50 Ci/mmol) and [4-$^{14}$C]-E1 (52 mCi/mmol) were purchased from NenDuPont (Boston, MA). [6,7-$^3$H]-E1 (97 Ci/mmol) was obtained from the Amersham International Radiochemical Centre (Amersham, UK).

Thin Layer Chromatography (TLC) was performed on pre-coated plates (Merck TLC aluminium sheets silica gel 60 $F_{254}$, Art. No. 5554). Product(s) and starting material (SM) were detected by either viewing under UV light or treating with a methanolic solution of phosphomolybdic acid followed by heating. Flash column chromatography was performed on silica gel (Sorbsil C60). IR spectra were determined by a Perkin–Elmer 782 infrared spectrophotometer, and peak positions are expressed in $cm^{-1}$. $^1$H NMR spectra were recorded with a Jeol JMN-GX270 and JMN-GX400 NMR spectrometers, and chemical shifts are reported in parts per million (ppm, $\delta$) relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded at the Mass Spectrometry Service Centre, University of Bath. FAB-mass spectra were carried out using *m*-nitrobenzyl alcohol (NBA) as the matrix and 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. 4-n-Heptyloxyphenyl-O-sulphamate (Fig. 1, **10**)

A stirred solution of 4-*n*-heptyloxyphenol (1.0 g, 4.80 mmol) in anhydrous DMF (20 ml) was treated with sodium hydride (60% dispersion, 230 mg, 5.76 mmol) at 0°C under an atmosphere of $N_2$. After evolution of hydrogen had ceased, sulphamoyl chloride in toluene [excess, ca. 5 equiv.] was added. The reaction mixture was stirred overnight at room temperature, poured into brine (150 ml) and the resulting mixture extracted with ethyl acetate. The combined organic extracts were washed exhaustively with brine, dried ($MgSO_4$), filtered and evaporated in vacuo. Purification of the crude product obtained by flash chromatography with chloroform/acetone (8:1) gave a white residue (757 mg) which was further purified by recrystallization from acetone/hexane (1:5) to give **10** as white crystals (557 mg, 40%): mp > 42°C (dec.); TLC (chloroform/acetone 8:1 and 4:1) $R_f$s 0.56 and 0.69 respectively; $\nu_{max}$ (KBr) 3440, 3320 (—$NH_2$), 1370 (—$SO_2$N—) $cm^{-1}$; $\delta_H$ (270 MHz, Acetone-$d_6$) 0.89 (3H, *t*, C$\underline{H}_3$), 1.34 (8H, *m*), 1.79 (2H, quintet, OCH$_2$C$\underline{H}_2$CH$_2$), 4.0 (2H, *t*, $J$ = 6.4 Hz, —OC$\underline{H}_2$—), 7.0 (4H, *m*, 2H exchanged with $D_2O$, C-3—$\underline{H}$, C-5—$\underline{H}$ and —OSO$_2$N$\underline{H}_2$), 7.23 (2H, *m*, C-2—$\underline{H}$ and C-6—$\underline{H}$); MS (FAB+) *m*/*z* (rel. intensity) 287.1 [100, $(M)^+$], 208.2 [30, $(M-SO_2NH)^+$]; MS: (FAB[mom0) *m*/*z* (rel. intensity) 286.0 [100, $(M-H)^-$]; Acc. MS (FAB+) *m*/*z* 288.1246 $C_{13}H_{22}NO_4S$ requires 288.1269. Found C, 54.2; H, 7.35; N, 4.7; $C_{13}H_{21}NO_4S$ requires C, 54.33; H, 7.37; N, 4.87%.

### 2.3. (*E*)-N-[(4-O-Sulphamoyl-3-methoxybenzyl]-8-methyl-6-nonenamide (Fig. 1, **11**)

Upon sulphamoylation, *E*-capsaicin (100 mg, 327.4 μmol) gave the crude product which was fractionated by flash chromatography with chloroform/acetone (2:1) and the white residue that obtained (85 mg) was further purified by recrystallization from acetone/hexane (1:2) to give **11** as white crystals (63 mg, 50%): mp 114–116°C; TLC (chloroform/acetone 2:1 and 4:1) $R_f$s 0.4 and 0.15 respectively; $\nu_{max}$ (KBr) 3490, 3300 (—$NH_2$), 1650 (C═O), 1380 (—$SO_2N$—) $cm^{-1}$; $\delta_H$ (270 MHz, $CDCl_3$) 0.94 (6H, *d*, $J=6.6$ Hz, 2 × C$\underline{H}_3$), 1.4 (2H, quintet, $J \approx 7.2$ Hz), 1.62 (2H, quintet, $J \approx 7.7$ Hz), 2.0 (2H, *q*, $J \approx 6.9$ Hz, —C$H_2$CH═CH—), 2.2 (3H, *m*, —C$\underline{H}_2$CONH— and —C$\underline{H}$($CH_3$)$_2$), 3.87 (3H, *s*, —OC$\underline{H}_3$), 4.39 (2H, *d*, $J=5.9$ Hz, ArC$\underline{H}_2$NHCO), 5.14 (2H, *br s*, exchanged with $D_2O$, —$OSO_2N\underline{H}_2$), 5.34 (2H, *m*, —C$\underline{H}$═C$\underline{H}$—), 5.87 (1H, *br t*, $J \approx 5.8$ Hz, —N$\underline{H}$CO—), 6.84 (1H, *dd*, $J_{C\text{-}2—H,C\text{-}6—H}=1.9$ Hz and $J_{C\text{-}5—H,C\text{-}6—H}=8.2$ Hz C-6—$\underline{H}$), 6.86 (1H, *d*, $J_{C\text{-}6—H,\ C\text{-}2—H}=1.8$ Hz, C-2-$\underline{H}$) and 7.26 (1H, *d*, $J_{C\text{-}6—H,C\text{-}5—H}=8.1$ Hz, C-5-$\underline{H}$); MS (FAB+) *m*/*z* (rel. intensity) 385.2 [100, $(M+H)^+$], 304.2 [20, $(M-SO_2NH_2)^+$], 287.1 [10, $(M-OH)^-$]; MS (FAB−) *m*/*z* (rel. intensity) 383.1 [100, $(M-H)^-$], 303.2 [10, $(M-H—SO_2NH_2)^-$]. Found C, 56.2; H, 7.38; N, 7.29; $C_{18}H_{28}N_2O_5S$ requires C, 56.23; H, 7.34; N, 7.29%.

### 2.4. (*p*-O-Tetradecanoyl)-N-tetradecanoyl tyramine (Fig. 1, **12**)

Myristoyl chloride (2.7 g, 10.94 mmol) was added dropwise to a cold solution of tyramine (1.5 g, 10.94 mmol) and triethylamine (3 ml, 21.52 mmol) in THF (50 ml). After being stirred at room temperature for 48 h, the reaction mixture was poured into 10% HCl solution (100 ml), and the resulting mixture extracted with ethyl acetate (3 × 100 ml). The combined organic extracts were washed with brine until neutral, dried ($MgSO_4$) and evaporated. The crude product that obtained was fractionated by flash chromatography with chloroform/acetone (8:1) and the residue that isolated (4.96 g) was further purified by recrystallization from hot toluene to give **12** as white crystals (3.85 g). Upon recrystallization from toluene of the residue recovered from the evaporation of mother liquor, a second crop of **12** was obtained (630 mg, 73% overall yield): mp 110–112°C; TLC (chloroform/acetone 8:1) $R_f$ 0.83; $\nu_{max}$ (KBr) 3340 (NH), 1750 (C═O) $cm^{-1}$; $\delta_H$ (400 MHz, DMSO-$d_6$, 100°C) 0.85 (6H, *t*, $J=6.9$ Hz, 2 × C$\underline{H}_3$), 1.25 (40 H, m), 1.43 (2H, quintet, $J \approx 7.0$ Hz, —NHCOC$H_2$C$\underline{H}_2$—), 1.64 (2H, quintet, $J \approx 7$ Hz, ArOCOC$H_2$C$\underline{H}_2$—), 2.03 (2H, *t*, $J=7.3$ Hz, —NHCOC$\underline{H}_2$—), 2.51 (2H, *t*, $J=7.2$ Hz, —ArOCOC$\underline{H}_2$—), 2.72 (2H, *t*, $J=7.3$ Hz, ArC$\underline{H}_2$C$H_2$NHCO—), 3.28 (2H, *q*, $J=7.3$ Hz, —C$\underline{H}_2$NHCO—), 6.98 (2H, *d*, $J=8.5$ Hz, C-2—$\underline{H}$ and C-6-$\underline{H}$), 7.2 (2H, *d*, $J=8.55$ Hz, C-3—$\underline{H}$ and C-5—$\underline{H}$) and 7.41 (1H, *br s*, $J \approx 5.5$ Hz, —N$\underline{H}$CO—); MS (FAB+) *m*/*z* (rel. intensity) 558.6 [100, $(M+H)^+$]; MS (FAB−) *m*/*z* (rel. intensity) 710.9 [100, $(M+H+NBA)^-$], 556.5 [70, $(M-H)^-$]. Found C, 77.60; H, 11.46; N, 2.51; $C_{36}H_{63}NO_3$ requires C, 77.50; H, 11.38; N, 2.51%.

### 2.5. N-Tetradecanoyl tyramine (Fig. 1, **13**)

A mixture of **12** (2.0 g, 3.585 mmol), DMF (50 ml), 10% aqueous $NaHCO_3$ (15 ml) and methanol (70 ml) was heated under reflux for 1 h and then stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic extracts were washed with brine until neutral, dried ($MgSO_4$) and evaporated to yield the crude product which was further purified by recrystallization from acetone/hexane (1:2) to give **13** as white crystals (1.15 g, 91%): mp 99–101°C; TLC (chloroform/acetone 8:1) $R_f$ 0.50. $\nu_{max}$ (KBr) 3320 (OH), 3300 (NH), 1640 (C═O) $cm^{-1}$; $\delta_H$ (400 MHz, DMSO-$d_6$) 0.85 (3H, *t*, $J=6.7$ Hz, C$\underline{H}_3$), 1.25 (20 H, *m*), 1.45 (2H, quintet, $J \approx 6.9$ Hz, NHCOC$H_2$C$\underline{H}_2$—), 2.01 (2H, *t*, $J=7.5$ Hz, —NHCOC$\underline{H}_2$), 2.56 (2H, *t*, $J=7.63$ Hz, ArC$\underline{H}_2$—), 3.17 (2H, *q*, $J \approx 6.6$ Hz, —C$\underline{H}_2$NHCO—), 6.6 (2H, *d*, $J=8.5$ Hz, C-3—$\underline{H}$ and C-5—$\underline{H}$), 6.97 (2H, *d*, $J=8.3$ Hz, C-2—$\underline{H}$ and C-6—$\underline{H}$), 7.79 (1H, *br t*, $J=5.4$ Hz, —N$\underline{H}$CO—) and 9.16 (1H, *br s*, exchange with $D_2O$, O$\underline{H}$); MS (FAB+) *m*/*z* (rel. intensity) 348.3 [100, $(M+H)^+$]; MS (FAB−) *m*/*z* (rel. intensity). 500.2 [15, $(M+NBA)^-$], 346.2 [100, $(M-H)^-$]; Acc. MS (FAB+) *m*/*z* 348.29029 $C_{22}H_{38}NO_2$ requires 348.290294. Found C, 74.5; H, 10.7; N, 4.13; $C_{22}H_{37}NO_2$ requires C, 76.03; H, 10.73; N, 4.03%.

### 2.6. (*p*-O-Sulphamoyl)-N-tetradecanoyl tyramine (Fig. 1, **14**)

Upon sulphamoylation, **13** (500 mg, 1.441 mmol) gave the crude product which was fractionated by flash chromatography with chloroform/acetone (8:1) and the white residue that isolated (486 mg) was further purified by recrystallization from acetone/hexane (1:2) to give **14** as white crystals (376 mg, 61%): mp 110–112°C; TLC (chloroform/acetone 8:1) $R_f$ 0.23; $\nu_{max}$ (KBr) 3420, 3320 (—$NH_2$), 1650 (C═O), 1390 (—$SO_2$—) $cm^{-1}$; $\delta_H$ (400 MHz, DMSO-$d_6$) 0.85 (3H, *t*, $J=6.7$ Hz, C$\underline{H}_3$), 1.24 (20H, *m*), 1.45 (2H, quintet, $J \approx 7.0$ Hz, —NHCOC$H_2$C$\underline{H}_2$—), 2.0 (2H, *t*, $J=7.3$ Hz, —NHCOC$\underline{H}_2$—), 2.71 (2H, *t*, $J=7.3$ Hz, ArC$\underline{H}_2$—),

3.25 (2H, *q*, $J \approx 7.0$ Hz, —C<u>H</u>$_2$NHCO—), 7.2 (2H, *d*, $J = 8.24$ Hz, Ar—H), 7.27 (2H, *d*, $J = 8.55$ Hz, Ar—H), 7.88 (1H, *br t*, $J = 5.5$ Hz, —N<u>H</u>CO—) and 7.95 (2H, *br s*, exchanged with $D_2O$, $OSO_2N\underline{H}_2$); MS (FAB+) *m/z* (rel. intensity) 427.3 [100, $(M+H)^+$], 348.3 [10, $(M+H+SO_2NH)^+$]; MS (FAB−) *m/z* (rel. intensity) 425.3 [100, $(M-H)^-$]; Acc. MS (FAB+) *m/z* 427.2637 $C_{22}H_{39}N_2O_4S$ requires 427.2631. Found C, 62.1; H, 9.02; N, 6.66; $C_{22}H_{38}N_2O_4S$ requires C, 61.94; H, 8.98; N, 6.57%.

### 2.7. p-O-(4-n-Hexyloxybenzoyl)-N-(4-n-hexyloxybenzoyl) tyramine (Fig. 1, **15**)

4-Hexyloxybenzoyl chloride (2.63 g, 10.93 mmol) was added dropwise to a cold solution of tyramine (1.0 g, 7.29 mmol) and triethylamine (1.52 ml, 10.91 mmol) in THF (35 ml). After being stirred at room temperature for 48 h, the reaction mixture was poured into 10% HCl solution (100 ml) and the resulting mixture extracted with ethyl acetate (3 × 100 ml). The combined organic extracts were washed with brine until neutral, dried ($MgSO_4$) and evaporated. The crude product that obtained was fractionated by flash chromatography with chloroform/acetone (8:1) and the white residue that isolated (3.65 g) was further purified by recrystallization from methanol to give **15** as white crystals (3.2 g, 80%): mp 145–156°C; TLC (chloroform/acetone 8:1) $R_f$ 0.7; $\nu_{max}$ (KBr) 3320. (NH), 1740 (C═O) $cm^{-1}$; $\delta_H$ (400 MHz, DMSO-$d_6$) 0.86 (6H, *m*, 2 × C<u>H</u>$_3$), 1.31 (8H, *m*), 1.42 (4H, quintet, $J \approx 6.8$ Hz, 2 × OCH$_2$CH$_2$C<u>H</u>$_2$), 1.72 (4H, quintet, $J \approx 7.3$ Hz, 2 × ArOCH$_2$C<u>H</u>$_2$—, 2.86 (2H, *t*, $J = 7.3$ Hz, —ArC<u>H</u>$_2$—), 3.48 (2H, *q*, $J \approx 5.9$ Hz, —C<u>H</u>$_2$NHCO—), 4.0 (2H, *t*, $J = 6.4$ Hz, —ArOC<u>H</u>$_2$—), 4.08 (2H, *t*, $J = 6.3$ Hz, ArOC<u>H</u>$_2$), 6.97 (2H, *d*, $J = 8.3$ Hz, Ar—H), 7.1 (2H, *d*, $J = 8.3$ Hz, Ar—H), 7.16 (2H, *d*, $J = 7.82$ Hz, Ar—H), 7.3 (2H, *d*, $J = 8.3$ Hz, Ar—H), 7.8 (2H, *d*, $J = 8.3$ Hz, Ar—H), 8.05 (2H, *d*, $J = 8.3$ Hz, Ar—H) and 8.43 (1H, *br t*, $J = 5.5$ Hz, —N<u>H</u>CO—); MS (FAB+) *m/z* (rel. intensity) 546.3 [100, $(M+H)^+$], 342.2 [20, $(M+H\text{-}OCOPhO(CH_2)_5CH_3)^+$]; MS (FAB−) *m/z* (rel. intensity) 544.3 [40, $(M-H)^-$], 340.2 [100, $(M\text{-}H\text{-}OCOPhO(CH_2)_5CH_3)^-$]; Acc. MS (FAB+) *m/z* 546.3233 $C_{34}H_{44}NO_5$ requires 546.3220. Found, C, 74.2; H, 8.0; N, 2.55; $C_{34}H_{43}NO_5$ requires C, 74.84; H, 7.94; N, 2.57%.

### 2.8. N-(4-n-Hexyloxybenzoyl) tyramine (Fig. 1, **16**)

A mixture of **15** (2.0 g, 3.665 mmol), DMF (50 ml), 10% aqueous $NaHCO_3$ (15 ml) and methanol (50 ml) was heated under reflux for 1 h and then stirred at room temperature overnight. The mixture was diluted with water and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$) and evaporated. The crude product that obtained was recrystallized from methanol to give **16** as white crystals (1.05 g, 87%): mp 160–162°C; TLC (chloroform/acetone 8:1) $R_f$ 0.37; $\nu_{max}$ (KBr) 3340 (NH), 3100 (OH), 1640 (C═O) $cm^{-1}$; $\delta_H$ (400 MHz, DMSO-$d_6$) 0.88 (3H, *t*, $J = 6.7$ Hz, C<u>H</u>$_3$), 1.31 (4H, *m*), 1.41 (2H, *m*), 1.71 (2H, quintet, $J \approx 7.0$ Hz, —ArOCH$_2$C<u>H</u>$_2$—), 2.70 (2H, *t*, $J = 7.6$ Hz, —ArC<u>H</u>$_2$—), 3.37 (2H, *q*, $J \approx 7.0$ Hz, —C<u>H</u>$_2$NHCO—), 4.0 (2H, *t*, $J = 6.4$ Hz, —ArOC<u>H</u>$_2$—), 6.67 (2H, *d*, $J = 7.3$ Hz, Ar—H), 6.96 (2H, *d*, $J = 7.6$ Hz, Ar—H), 7.01 (2H, *d*, $J = 7.6$ Hz, Ar—H), 7.78 (2H, *d*, $J = 7.3$ Hz, Ar—H), 8.36 (1H, *t*, $J = 5.5$ Hz, —N<u>H</u>CO—) and 9.17 (1H, *br s*, exchanged with $D_2O$ OH); MS (FAB+) *m/z* (rel. intensity) 342.2 [100, $(M+H)^+$]; MS (FAB−) *m/z* (rel. intensity) 340.2 [100, $(M-H)^-$]; Acc. (FAB+) MS *m/z* 342.2067 $C_{21}H_{28}NO_3$ requires 342.2069. Found C, 73.73; H, 7.94; N, 3.99; $C_{21}H_{27}NO_3$ requires C, 73.87; H, 7.97; N, 4.10%.

### 2.9. ( p-O-Sulphamoyl)-N-(4-n-hexyloxybenzoyl) tyramine (Fig. 1, **17**)

Upon sulphamoylation, **16** (1.0 g, 2.928 mmol) gave the crude product which was fractionated by flash chromatography with chloroform/acetone (8:1) and the white residue that isolated (860 mg) was further purified by recrystallization from acetone/hexane (1:2) to give **17** as white crystals (650 mg, 53%): mp 159–160°C; TLC (chloroform/acetone 8:1) $R_f$ 0.2; $\nu_{max}$ (KBr) 3320, 3220 (—$NH_2$), 1620 (C═O), 1390 (—$SO_2$—) $cm^{-1}$; $\delta_H$ (400 MHz, DMSO-$d_6$) 0.88 (3H, *t*, $J \approx 7.0$ Hz, C<u>H</u>$_3$), 1.31 (4H, *m*), 1.41 (2H, *m*), 1.71 (2H, quintet, $J \approx 7.0$ Hz, OCH$_2$C<u>H</u>$_2$—), 2.85 (2H, *t*, $J = 7.3$ Hz, ArC<u>H</u>$_2$—), 3.48 (2H, *q*, $J = 6.3$ Hz, —C<u>H</u>$_2$NHCO—), 4.01 (2H, *t*, $J = 6.3$ Hz, —ArOC<u>H</u>$_2$—), 6.97 (2H, *d*, $J = 8.9$ Hz, Ar—H), 7.2 (2H, *d*, $J = 8.6$ Hz, Ar—H), 7.32 (2H, *d*, $J = 8.6$ Hz, Ar—H), 7.8 (2H, *d*, $J = 8.5$ Hz, Ar—H), 7.95 (2H, *br s*, exchanged with $D_2O$, $OSO_2N\underline{H}_2$) and 8.43 (1H, *br t*, $J = 5.5$ Hz, —N<u>H</u>CO—); MS (FAB+) *m/z* (rel. intensity) 421.2 [100, $(M+H)^+$]; MS (FAB−) *m/z* (rel. intensity) 573.4 [30, $(M+NBA)^-$], 419.3 [100, $(M-H)^-$], 340.3 [10, $(M\text{-}H\text{-}SO_2NH)^-$]. Found C, 60.1; H, 6.8; N, 6.63; $C_{21}H_{28}N_2O_5S$ requires C, 59.98; H, 6.71; N, 6.66%.

### 2.10. N-(4-n-Octyloxybenzoyl) tyramine (Fig. 1, **18**)

To a mixture of 4-*n*-octyloxybenzoic acid (2.63 g, 10.51 mmol) and triethylamine (1.52 ml, 10.91 mmol) in THF (35 ml), at 0°C was added isobutylchloroformate (2.0 g, 14.64 mmol), followed by tyramine (1.0 g, 7.290 mmol). After being stirred at 0°C for 48 h, the reaction mixture was poured into 10% HCl solution

(100 ml), and the resulting mixture was extracted with ethyl acetate (3 × 100 ml). The combined organic extracts were washed with brine until neutral, dried ($MgSO_4$) and evaporated. The crude product that obtained was fractionated by flash chromatography with chloroform/acetone (8:1) and the white residue that isolated (2.2 g) was further purified by recrystallization from methanol to give **18** as white crystals (1.93 g, 72%): mp 141–143°C; TLC (chloroform/acetone 8:1) $R_f$ 0.36; $\nu_{max}$ (KBr) 3280 (NH), 1620 (C═O) $cm^{-1}$; $\delta_H$ (400 MHz, DMSO-$d_6$) 0.9 (3H, *t*, $J = 6.6$ Hz, $C\underline{H}_3CH_2$—), 1.31 (8H, *m*), 1.44 (2H, *m*), 1.74 (2H, quintet, $J \approx 7.3$ Hz, $OCH_2C\underline{H}_2$—), 2.74 (2H, *t*, $J = 7.5$ Hz, $ArC\underline{H}_2$—), 3.42 (2H, *q*, $J \approx 7.0$ Hz, —$C\underline{H}_2NHCO$—), 4.04 (2H, *t*, $J = 6.4$ Hz, —$ArOC\underline{H}_2$—), 6.72 (2H, *d*, $J = 8.2$ Hz, Ar—H), 7.0 (2H, *d*, $J = 8.5$ Hz, Ar—H), 7.05 (2H, *d*, $J = 8.2$ Hz, Ar—H), 7.82 (2H, *d*, $J = 8.6$ Hz, Ar—H), 8.4 (1H, *t*, $J = 5.5$ Hz, —N$\underline{H}$CO—) and 9.21 (1H, *br s*, OH); MS (FAB+) *m/z* (rel. intensity) 370.2 [100, $(M+H)^+$]; MS (FAB−) *m/z* (rel. intensity) 368.3 [100, $(M-H)^-$]; Acc. MS (FAB+) *m/z* 370.2400 $C_{23}H_{32}NO_3$ requires 370.2382. Found C, 74.7; H, 8.54; N, 3.78; $C_{23}H_{31}NO_3$ requires C, 74.76; H, 8.46; N, 3.79%.

### 2.11. (p-O-Sulphamoyl)-N-(4-n-octyloxybenzoyl) tyramine (Fig. 1, **19**)

Upon sulphamoylation, **18** (1.0 g, 2.706 mmol) gave the crude product which was fractionated by flash chromatography with chloroform/acetone (8:1) and the white residue that isolated (967 mg) was further purified by recrystallization from acetone/hexane (1:2) to give **19** as white crystals (817 mg, 67%): mp > 120°C (dec.); TLC (chloroform/acetone 8:1) $R_f$ 0.2; $\nu_{max}$ (KBr) 3420, 3340 (—$NH_2$), 1680 (C═O), 1380 (—$SO_2$—) $cm^{-1}$; $\delta_H$ (400 MHz, DMSO-$d_6$) 0.86 (3H, *m*), 1.25 (8H, *m*), 1.40 (2H, quintet, $J \approx 7.0$ Hz), 1.71 (2H, quintet, $J \approx 7.3$ Hz, $OCH_2C\underline{H}_2$—), 2.7 (2H, *t*, $J = 7.3$ Hz, $ArC\underline{H}_2$—), 3.45 (2H, *q*, $J \approx 6.3$ Hz, —$C\underline{H}_2NHCO$—), 4.01 (2H, *t*, $J = 6.3$ Hz, —$ArOC\underline{H}_2$—), 6.96 (2H, *d*, $J = 9.2$ Hz, Ar—H), 7.11 (2H, *d*, $J = 8.3$ Hz, Ar—H), 7.3 (2H, *d*, $J = 8.3$ Hz, Ar—H), 7.80 (2H, *d*, $J = 8.79$ Hz, Ar—H), 7.95 (2H, *br s*, exchanged with $D_2O$, $OSO_2N\underline{H}_2$) and 8.4 (1H, *t*, $J = 5.9$ Hz, —N$\underline{H}$CO—); MS (FAB+) *m/z* (rel. intensity) 449.2 [90, $(M+H)^+$]; MS (FAB−) *m/z* (rel. intensity) 447.2 [100, $(M-H)^-$]; Acc. (FAB+) MS: *m/z* 449.2099 $C_{23}H_{33}N_2O_5S$ requires 449.2110. Found C, 58.1; H, 6.94; N, 7.07; $C_{23}H_{32}N_2O_5S$ requires C, 61.58; H, 7.19; N, 6.24%.

### 2.12. In vitro screening for sulphatase inhibitory activity

Potential steroid sulphatase inhibitors were initially tested in MCF-7 breast cancer cells using a physiological (2 nM) substrate concentration. Briefly, MCF-7 cells were maintained in minimal essential medium with 5% foetal calf serum and other essential nutrients [11]. Steroid sulphatase activity was measured in intact cell monolayers by incubation with [$^3$H] E1-S ($4 \times 10^5$ dpm, 2 nM) [11]. Compounds showing sulphatase inhibitory properties were further examined using placental microsomes (100,000 g fractions) using [$^3$H] E1-S ($4 \times 10^5$ dpm) adjusted to 20 μM with unlabelled substrate (Sigma). After incubation of the substrate, ± inhibitor, with placental microsomes (125 μg protein/ml) for 30 min, the product formed was isolated from the mixture by extraction with toluene [11].

### 2.13. In vivo studies

Compounds which inhibited oestrone sulphatase activity in vitro were selected for in vivo testing. For this female Wistar rats (150–250 g) were obtained from Harlan Olac (Bicester, Oxon, UK) and kept under conditions meeting institutional requirements. Groups of rats were treated with inhibitors (0.1–10 mg/kg/day) as indicated. At the end of the treatment period animals were killed using an approved procedure and livers obtained for assay of oestrone sulphatase activity [15].

To examine for oestrogenicity in vivo rats were ovariectomised and 14 days later treated with vehicle (propylene glycol) or sulphatase inhibitor at concentrations indicated for 5 days. Animals were killed 24 h after administration of the last dose of the drug, and uteri were excised of fat and weighed. Total body weights of animals were also recorded, and the results expressed as uterine weight × 100/total body weight. Oestrone sulphatase activity was measured in uteri obtained from the animals used for this study.

The ability of selected oestrone sulphatase inhibitors to block the E1-S stimulated growth of uteri in ovariectomised animals was also assessed. For this, animals received either vehicle (propylene glycol) or inhibitor, at the dose indicated, for 2 days. Animals either continued to receive vehicle only, E1-S or drug plus E1-S. Twenty-four hours after administration of the last dose, animals were killed and uterine and total body weights recorded.

### 2.14. Statistics

The significance of differences for oestrone sulphatase activities in cells, placental microsomes or tissues and uterine weights were assessed using Student's *t* test.

## 3. Results and discussion

### 3.1. Steroidal A- and D-ring Modified Analogues

#### 3.1.1. A-ring Modifications

Based on previous observations that modifications to the A- and D-ring of the oestrone steroid nucleus could greatly reduce their oestrogenicity [18,19], a number of A- and D-ring modified sulphamate analogues were synthesized and tested. A-ring modified compounds included the 2-methoxy (Fig. 1, **4**), 2/4-nitro, 2/4-*n*-propyl and 2/4-allyl EMATE analogues [20]. An examination of the ability of these EMATE analogues to inhibit oestrone sulphatase (E1-STS) activity in placental microsomes revealed that the 4-nitro derivative was the most efficient inhibitor ($IC_{50}$ = 800 pM) and was five times more potent than EMATE. The 2-methoxy and 2-nitro analogues were also efficient inhibitors with $IC_{50}$s of 30nM and 70nM respectively. In contrast, the 2- and 4-allyl substituted compounds were only relatively weak inhibitors ($IC_{50}$s, 2.5 μM and 9.0 μM respectively), but were more active than the 2-*n*- and 4-*n*-propyl derivatives ($IC_{50}$s, 29 μM and >100 μM). The 2,4-di-*n*-propyl and 2,4-diallyl analogues were devoid of activity.

In vivo testing of selected A-ring modified analogues revealed that the 2-methoxy and 4-nitro EMATE derivatives inhibited liver oestrone sulphatase activity to a similar extent to that of EMATE (95%). In contrast, the 4-*n*-propyl and 4-allyl EMATE analogues only inhibited liver sulphatase activity by 70 and 40% respectively. When tested for oestrogenicity in ovariectomised rat uteri, the 2-methoxy, 4-*n*-propyl and 4-allyl EMATE analogues were devoid of oestrogenic properties. 4–Nitro EMATE, while being less oestrogenic than EMATE, did increase the growth of uteri in ovariectomised rats by 158% compared to control animals [20]. The similar in vivo potency of 2-methoxy EMATE to that of EMATE, but lack of any oestrogenicity, has identified this analogue as a potential new lead compound for development as a potent steroid sulphatase inhibitor.

To some extent the relative potencies of the EMATE derivatives tested as sulphatase inhibitors reflected the effects that A-ring modifications have on their oestrogenicity [18]. Alkylation of the C-2 carbon atom with the allyl or propyl groups reduced the in vitro potency of the resulting analogues as E1-STS inhibitors. However, in contrast to the effect that substitution with these groups has on oestrogenicity, with 2-allyl oestrone being less oestrogenic than the 2-*n*-propyl derivative, 2-allyl EMATE was about eleven-times more potent as a sulphatase inhibitor in vitro than the propyl analogue. In keeping with the results of Patton and Dmochowski [18] who observed that substitution at both C-2 and C-4 position by allyl groups, greatly reduced oestrogenicity. 2,4-diallyl and 2,4-*n*,*n*-dipropyl EMATE were both inactive in vitro even when tested at 100 μM. As discussed by Patton and Dmochowski, alkyl groups at both C-2 and C-4 position would shield the function at C-3. In our case this would be the sulphamoyl group, making its entry into the active site of the enzyme more difficult.

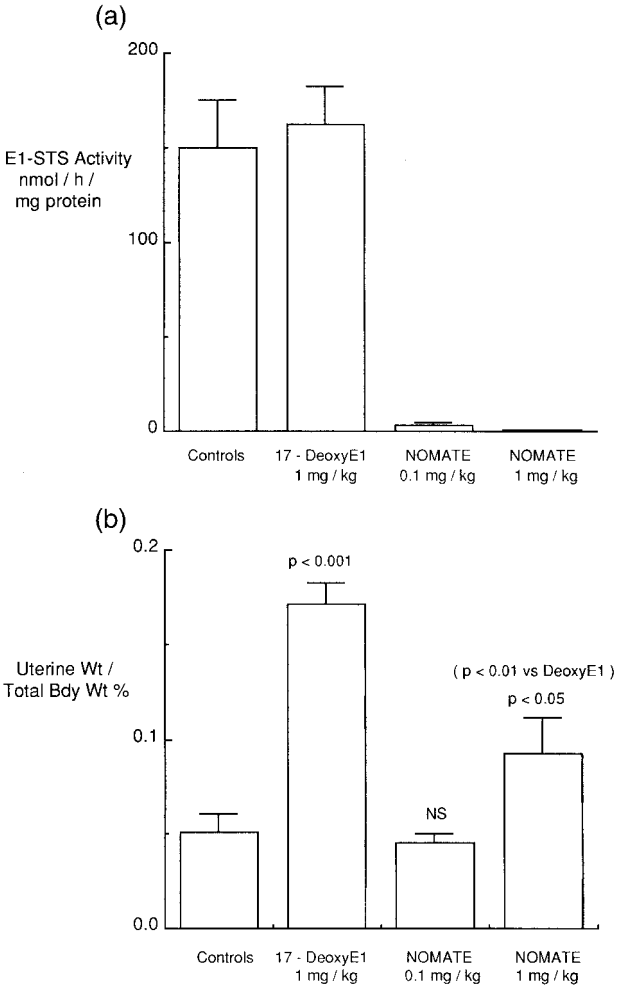



Fig. 2. (a) Effect of 17-deoxyoestrone (17-Deoxy E1) and 17–deoxyoestrone-3-*O*-sulphamate (NOMATE) on rat liver oestrone sulphatase activity. Animals were treated at the doses indicated for 5 days; (b) effect of 17-deoxyoestrone or NOMATE on uterine growth in the ovariectomised rat.

#### 3.1.2. D-ring modifications

Dorfman and Kincl [19] previously examined the effect of removal of the oxygen function at C-17 of the D-ring of the oestrone nucleus on oestrogenicity. Using a mouse uterotrophic assay the relative potency of oestra-1,3,5(10)-trien-3-ol was only 11% as active as oestrone by the subcutaneous route. We are currently examining a number of D-ring modified EMATE analogues for their ability to inhibit E1-STS activity in vitro and in vivo. As an example 17-deoxyoestrone-3-

Table 1
$IC_{50}$ values for single ring and bicyclic non-fused ring sulphatase inhibitors (ND = Not determined)

| | $IC_{50}$ Values (μM) | |
|---|---|---|
| | Placental microsomes | MCF-7 cells |
| (**10**) 4-*n*-heptyloxyphenyl-*O*-sulphamate | 40 | 1 |
| (**11**) (E)-*N*-[(4-*O*-sulphamoyl-3-methoxyphenyl)methyl]-8-methyl-6-nonenamide | 25 | 0.1 |
| (**14**) (*p*-*O*-sulphamoyl)-*N*-tetradecanoyl tyramine | 2 | ND |
| (**17**) (*p*-*O*-sulphamoyl)-*N*-(4-hexyloxybenzoyl)tyramine | 10 | ND |
| (**19**) (*p*-*O*-sulphamoyl)-*N*-(4-octyloxybenzoyl)tyramine | 20 | ND |

*O*-sulphamate (Fig. 1, **5**, NOMATE) inhibited activity in MCF-7 cells by 97% at 0.01μM, similar to the inhibition achieved with EMATE [12].

In vivo, when given to ovariectomised rats at 0.1 mg/kg or 1.0 mg/kg per day p.o. for 5 days NOMATE almost completely inhibited (98%) liver oestrone sulphatase activity (Fig. 2a). Although the parent compound, 17-deoxyoestrone (1 mg/kg), had no significant effect on oestrone sulphatase activity, it did increase uterine growth in the ovariectomised rat by 235% compared with control animals (Fig. 2b). NOMATE at 0.1 mg/kg, a dose that almost completely inhibited E1-STS activity, had no effect on uterine growth. This shows that at 0.1 mg/kg NOMATE is devoid of oestrogenic activity. At a ten-fold higher dose, however, NOMATE did stimulate uterine growth (80% vs controls) but to a significantly lower degree ($P < 0.05$) than that caused by 17-deoxyoestrone. Modifications to the D-ring of the oestrone steroid nucleus, therefore, offers an additional route for the development of potent sulphatase inhibitors lacking oestrogenicity.

### 3.2. Single ring and bicyclic non-fused ring sulphatase inhibitors

Our initial studies to develop a non-steroidal sulphatase inhibitor involved the sulphamoylation of diethylstilboestrol (DES) which has two non-fused aryl rings. The mono-sulphamate (Fig. 1, **6**) and bis-sulphamate (Fig. 1, **7**) derivatives of DES were considerably more potent inhibitors than the 2-fused ring tetrahydronaphthol (THN) sulphamates [21,22]. DES-bis-sulphamate had an $IC_{50}$ of 10 nM as assessed in intact MCF-7 cells.

The finding that it was not necessary to have a fused ring system for sulphatase inhibition led to the synthesis of a series of sulphamate analogues with a single ring or bicyclic non-fused ring structure (Fig. 1, **10, 11, 14, 17, 19**). In the placental microsome oestrone sulphatase assay, these compounds proved to be relatively weak inhibitors with $IC_{50}$s ranging from 10–40 μM (Table 1). A single 10 mg/kg dose of *E*-capsaicin sulphamate (Fig. 1, **11**) did result in a modest degree of inhibition of E1-STS activity in rat uterine (57%) and

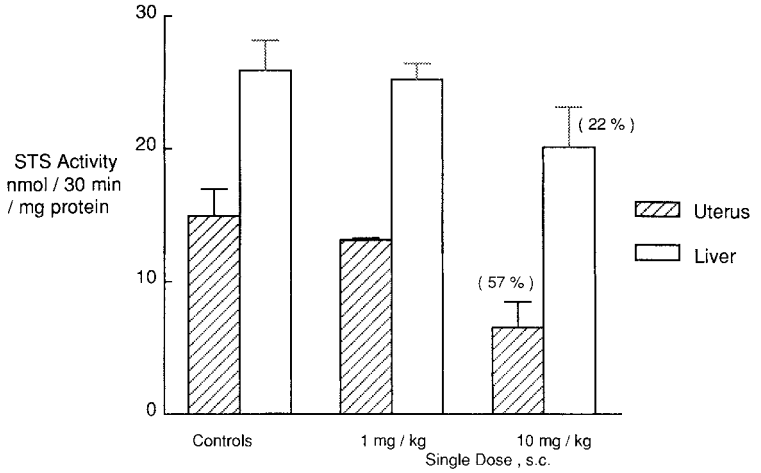



Fig. 3. Inhibition of rat uterine and liver oestrone sulphatase activity by a single 1 mg/kg or 10 mg/kg dose of a single ring sulphatase inhibitor *E*-capsaicin sulphamate (Fig. 1, **11**).

liver (22%) tissues (Fig. 3). The (*p*-*O*-sulphamoyl)-*N*-(4-*n*-hexyloxybenzoyl) tyramine (Fig. 1, **17**) and (*p*-*O*-sulphamoyl)-*N*-(4-*n*-octyloxybenzoyl) tyramine (Fig. 1, **19**) were also tested in vivo. After a single oral 10 mg/kg dose, no inhibition of rat liver E1-STS activity was detected.

Using the active pharmacophore that our studies had identified, Li and his colleagues have designed, synthesized and tested a series of (*p*-*O*-sulphamoyl)-*N*-alkanoyl tyramines as potential inhibitors [23]. It was reasoned by this group that the phenyl portion of such an inhibitor would mimic the A-ring of EMATE with the alkanoyl group providing hydrophobic bulk to mimic the B, C and D rings.

In a placental microsomal assay, an increase in potency was detected as the length of the alkanoyl chain increased from heptanoyl [$-CO(CH_2)_5CH_3$] which had an $IC_{50}$ of 14,300 nM to tetradecanoyl [$CO(CH_2)_{12}CH_3$] with an $IC_{50}$ of 56 nM. A hexadecanoyl derivative was also shown to inhibit E1-STS activity in MDA-MB-231 cells by 82% at 1 μM and the E1 S-stimulated growth of MCF-7 cells in a dose-dependent manner [24].

Li and his colleagues have obtained convincing evidence that inhibition of steroid sulphatase activity in rats has a role in countering scopolamine-induced amnesia. Both EMATE [7] and one non-steroidal (*p*-*O*-sulphamoyl)-*N*-alkanoyl derivative of tyramine [8] were found to be effective in reversing scopolamine-induced amnesia. This finding offers the prospect of developing new therapeutic strategies for the treatment of Alzheimer's disease in humans.

We have further extended the work of Li et al. [23] in this paper by synthesising several single ring and bicyclic non-fused ring sulphamates. We initially synthesised 4-*n*-heptyloxyphenyl-*O*-sulphamate (**10**) as a minimal structure possessing the pharmacophore for sulphatase inhibition and a hydrophobic side chain. The sulphamate of *E*-capsaicin (**11**) was also prepared in a similar fashion, as were the two bicyclic non-fused long chain sulphamates, (*p*-*O*-sulphamoyl)-*N*-(4-*n*-hexyloxybenzoyl) tyramine (**17**) and the *n*-octyloxybenzoyl congener (**19**), which were analogous to that of the compounds of Li et al. We also synthesised (*p*-*O*-sulphamoyl)-*N*-tetradecanoyl tyramine (**14**), a compound previously synthesised by Li et al., but made some different observations.

The preparation of compounds **13** and **16** were attempted by reacting tyramine with the corresponding acid chloride at room temperature in the presence of triethylamine as described by Li et al. [23] for the synthesis of *N*-tridecanoyl tyramine. However, in contrary to the finding of Li et al. which showed the phenolic amide (*N*-tridecanoyl tyramine) as the major product, our reactions gave the amido esters (**12** and **15**) at 73 and 80% respectively instead. Compounds **13** and **16** were then synthesised by hydrolysing the ester group of **12** and **15** respectively in refluxing aqueous methanol containing sodium bicarbonate. For the synthesis of compound **18**, a modified approach was adopted in which tyramine was allowed to react with the corresponding acid anhydride at $0^0$C. As expected, **18** was isolated as the major product with a yield of 72%, which confirms our reasoning that the nucleophilicity of the phenolic moiety of tyramine is reduced and hence the selectivity for the formation of the phenolic amide improved by conducting the reaction at a lower temperature.

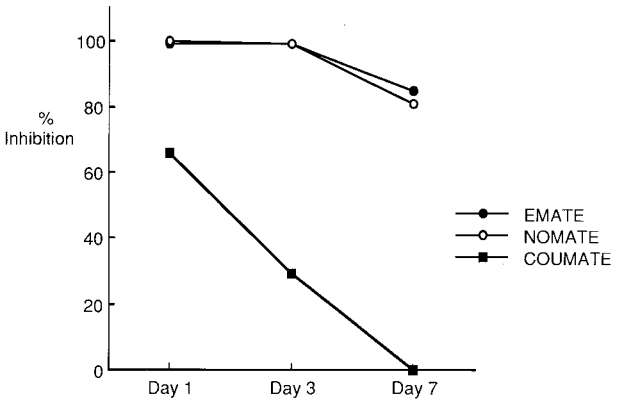



Fig. 4. Recovery of rat liver oestrone sulphatase activity after a single 10 mg/kg dose of oestrone-3-*O*-sulphamate (EMATE), 17-deoxyoestrone-3-*O*-sulphamate (NOMATE) or 4-methylcoumarin-7-*O*-sulphamate (COUMATE).

Under the experimental conditions employed in our placental microsome E1-STS assay the $IC_{50}$ for (*p*-*O*-sulphamoyl)-*N*-tetradecanoyl tyramine (Fig. 1, **14**) was 2 μM. Administration of a single 10 mg/kg oral dose of this compound did not result in any inhibition of rat liver E1-STS activity.

### 3.3. 2- and 3-Ring sulphatase inhibitors

In order to explore the structure-activity relationship of a number of 2-ring structures, and also to investigate the possibility of developing a non-oestrogenic inhibitor via this approach, a series of sulphamate derivatives of THN were synthesized and tested [12]. Sulphamate derivatives of THN were relatively weak inhibitors of oestrone sulphatase activity with THN-sulphamate having an $IC_{50}$ of 1 μM when tested in MCF-7 cells.

The finding that phenolic compounds such as THN could be utilised as oestrone sulphatase inhibitors led to the development of a series of coumarin sulphamate derivatives [22]. It was reasoned that the two-ring fused structure of coumarin analogues should mimic the A- and B-rings of the oestrone steroid nucleus. The analogues synthesized and tested in this series included coumarin-7-*O*-sulphamate, 4-methylcou-

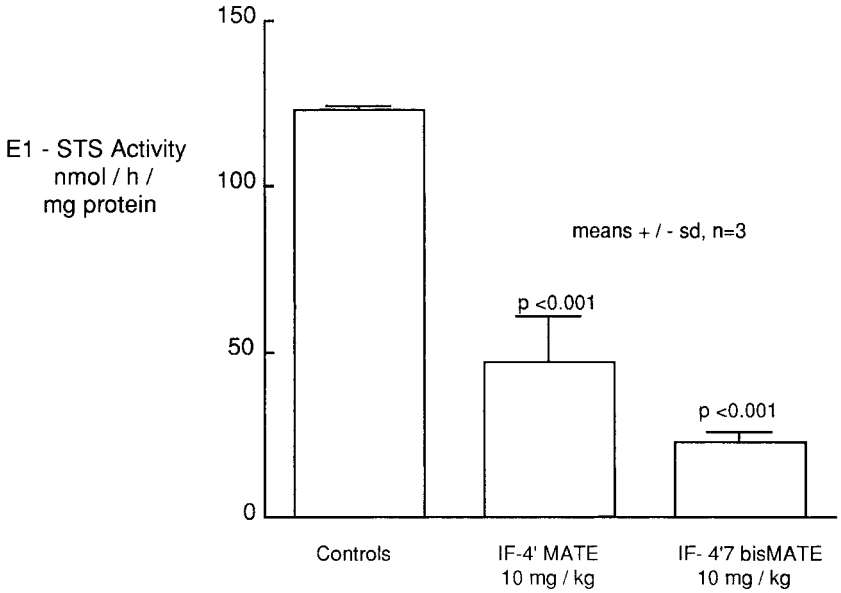



Fig. 5. Inhibition of rat liver oestrone sulphatase activity 24 h after a single dose of 5,7-dihydroxy-isoflavone-4′-*O*-sulphamate (IF-4′MATE, Fig. 1, **8**) or 5-hydroxy-isoflavone-4′,7-bis-sulphamate (IF-4′,7-bisMATE, Fig. 1, **9**).

marin-7-*O*-suphamate, 3,4,8-trimethylcoumarin-7-*O*-sulphamate and 4-(trifluoromethyl)coumarin-7-*O*-sulphamate. In intact MCF-7 cells the $IC_{50}$ for inhibition of oestrone sulphatase by 4-methylcoumarin-7-*O*-sulphamate (COUMATE) was 380 nM. As previously found for EMATE [13], COUMATE also inhibited oestrone sulphatase activity in a time- and concentration-dependent manner [22].

COUMATE was tested in vivo in rats and daily dosing at 10 mg/kg/day for 7 days, resulted in 85% inhibition of liver oestrone sulphatase activity [25]. Recovery of liver oestrone sulphatase activity after single or multiple doses of COUMATE was relatively rapid with almost complete restoration of activity by seven days after cessation of drug administration (Fig. 4). This is in contrast to the lengthy duration of E1-STS inhibition after administration of EMATE or NOMATE (Fig. 4). For these two steroidal sulphamates activity was still inhibited by more than 80% one week after a single dose of drug. In ovariectomised rats COUMATE administration did not stimulate uterine growth but was able to inhibit the E1-S stimulation of uterine growth [25].

Recently, a number of further modifications have been made to the basic coumarin sulphamate structure including the synthesis of several novel tricyclic coumarin-based sulphamates. Preliminary results from investigations with these compounds show that it will be feasible to develop a non-steroidal, non-oestrogenic sulphatase inhibitor that is equipotent to EMATE.

A number of compounds of the flavonoid class have also been sulphamoylated and tested for their ability to inhibit E1-STS activity (the synthesis of flavonoid-based inhibitors will be reported elsewhere). Flavonoids have previously been reported to inhibit aromatase and oestradiol 17$\beta$-hydroxysteroid dehydrogenase activities [26,27]. The natural flavonoids, quercetin, kaempferol and narigenin, can all inhibit oestrone sulphatase activity in liver microsomes with the most potent, quercetin, having an $IC_{50}$ of $< 10$ μM [26]. It has been postulated that dietary isoflavones may exert their chemopreventive role, at least in part, by inhibition of E1-STS activity [26]. Daidzein-4′-*O*-sulphate and daidzein-7,4′-*O*-disulphate are both competitive inhibitors of oestrone sulphatase activity with $K_i$ values of 5.9 and 1.0 μM respectively [27].

The structures of two of the many isoflavone sulphamates we have synthesized and tested are shown in Fig. 1 (**8**, **9**). In MCF-7 cells at 1 μM, the bis- and mono-sulphamates inhibited oestrone sulphatase activity by 90 and 83% respectively. In vivo in the rat both isoflavone sulphamates were active (Fig. 5) but were considerably less potent than EMATE. After a single 10 mg/kg dose liver oestrone sulphatase activity was inhibited by 62% by the mono-sulphamate and 81% by the bis-sulphamate analogue.

### *3.4. Anti-oestrogens and progestogens as sulphatase inhibitors*

In parallel with studies to those involving the design and synthesis of sulphatase inhibitors, other research groups have explored the ability of drugs already in clinical use to inhibit oestrone sulphatase activity. Pasqualini and his colleagues originally reported that antioestrogens could decrease the concentration of oes-

tradiol in breast cancer cells after incubation with E1-S [2]. This finding suggested that such compounds may also act as sulphatase inhibitors. Using rat mammary tumour tissue Santner and Santen obtained evidence in support of antioestrogens inhibiting oestrone sulphatase activity [28]. Tamoxifen, 4-hydroxytamoxifen and the 'pure' antioestrogen ICI 164 384 all inhibited sulphatase activity although the $K_i$ values of 11–1130 μM were relatively high [28].

The progestogens, Progmesterone (R5020) and Nomegestrol acetate also possess sulphatase inhibitory properties [29,30]. At a relatively high concentration ($5 \times 10^{-5}$M), Nomegestrol acetate inhibited sulphatase activity in MCF-7 and T47-D cells by 70–80% [29]. Progmesterone also inhibits the expression of oestrone sulphatase mRNA in both MCF-7 and T47-D breast cancer cells [30].

## 4. Conclusion

It is now generally acknowledged that in order to improve the response rate to endocrine therapy using enzyme inhibitors in women with breast cancer, it will be necessary to achieve complete oestrogen deprivation. While potent, specific aromatase inhibitors are now available, the clinical response has been somewhat disappointing [31,32]. During the last few years, since the identification of the active pharmacophore required for potent sulphatase inhibition [14], considerable progress has been made in the development of steroidal and non-steroidal inhibitors and several potent candidates have now been identified and are currently undergoing pre-clinical testing. The availability of these steroid sulphatase inhibitors should enable the therapeutic value of this therapy to be examined in a clinical trial in the near future.

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