JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 85–93

Received April 24, 2002
Accepted September 27, 2002
DOI 10.1211/002235702568
ISSN 0022-3573

Synthesis and biochemical evaluation of some novel benzoic acid based esters as potential inhibitors of oestrone sulphatase

Caroline Owen, Karen James, Luther Sampson and Sabbir Ahmed

Abstract

Oestrone sulphatase is an important target in the fight against hormone-dependent breast cancer. In an effort to investigate the reported definitive pharmacophore for oestrone sulphatase and continue our search for potent inhibitors of this enzyme, we have undertaken extensive synthesis, biochemical evaluation and physicochemical property determination of a range of benzoic acid based esters. Here, we report the initial results of our study into a series of straight chain alkyl esters of 4-sulphonylbenzoic acid. Using these compounds, we have investigated the involvement of two physicochemical properties, namely logP and pK_a. The results of this study show that there was a strong correlation between the inhibitory activity and the logP of the parent compound. Within the series of compounds studied, hydrophobicity appears to be a more important factor than pK_a in determining the overall inhibitory activity. In a previous report, we showed that pK_a plays an important role in stabilizing the phenoxide ion resulting from the hydrolysis of the sulphamate group. Here, we propose that although pK_a is an important factor in determining the overall inhibitory activity when a wide range of compounds are considered, both hydrophobicity and pK_a need to be considered in the design of potential inhibitors of oestrone sulphatase.

School of Chemical and Pharmaceutical Sciences, Kingston University, Penrhyn Road, Kingston upon Thames, Surrey KT1 2EE, UK

Caroline Owen, Sabbir Ahmed

Institute of Cancer Research, Sutton, UK

Karen James

Novartis Pharma AG, CH-4002 Basel, Switzerland

Luther Sampson

Correspondence: S. Ahmed, School of Chemical and Pharmaceutical Sciences, Kingston University, Penrhyn Road, Kingston upon Thames, Surrey KT1 2EE, UK. E-mail: s.ahmed@kingston.ac.uk

Acknowledgement: The authors would like to thank: the EPSRC National Mass Spectrometry Service, at the University of Wales College of Swansea, for the determination of the high resolution mass spectral data.

Introduction

In the treatment of hormone-dependent breast cancer, extensive research has been undertaken to produce compounds which are both potent and selective inhibitors of the cytochrome P450 enzyme aromatase (Feutrie & Bonneterre 1999; Brodie & Njar 2000). However, the use of aromatase inhibitors does not result in the inhibition of all of the biosynthetic processes which lead to oestrogen formation. That is, the enzyme oestrone sulphatase converts the stored (sulphated) form of the oestrogens to the active (non-sulphated) forms (Figure 1), thereby allowing the stimulation of tumours via a non-aromatase pathway (which, in general, is not blocked by aromatase inhibitors). Furthermore, it has been proposed that androstenediol (derived from the hydrolysis of androstenediol sulphate, catalysed by oestrone sulphatase) is a major mitogen (although a poor binder to the oestrogen receptor). It is therefore thought to play a major role in the progression of breast cancer due to its high concentration in the plasma.

A number of steroidal inhibitors has been investigated as potent inhibitors of oestrone sulphatase (Reed et al 1996; Howarth et al 1997; Woo et al 1998), including oestrone-3-*O*-sulphamate (EMATE) – a time and concentration dependent irreversible inhibitor. However, this compound has been shown to possess potent oestrogenic properties, and as a result, the investigation into non-steroidal inhibitors has intensified. A particular series of non-steroidal inhibitors is based upon the coumarin structure, for example, 4-methylcoumarin-7-*O*-sulphamate (COUMATE) (Woo et al 1996a) which is a time- and concentration-dependent irreversible inhibitor. COUMATE has been further derivatized, resulting in a series of tricyclic compounds (Woo et al 2000) such as 667-, 668- and 669-COUMATE. In general, these potent steroidal and non-steroidal inhibitors contain an aminosulphonate moiety, which has been shown to be involved in the irreversible inhibition of oestrone sulphatase.

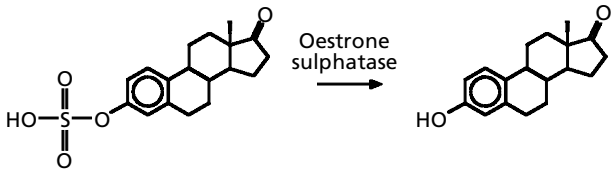

Figure 1 Action of the enzyme oestrone sulphatase on oestrone sulphate.

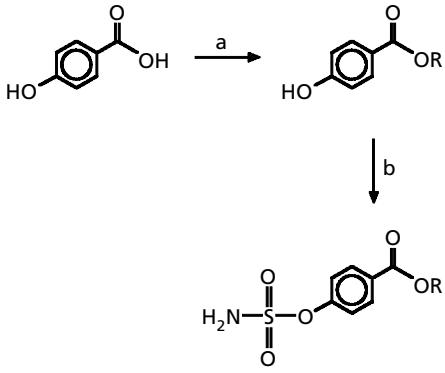

Figure 2 Synthesis of the 4-sulphamate derivative of the substituted benzoic acid (a = ROH/Δ/toluene; b = NaH/H_2NSO_2Cl/toluene).

From the consideration of the results obtained with the known sulphamate containing steroidal and non-steroidal inhibitors, a 'definitive model' was proposed where it was suggested that the most fundamental and basic requirements for inhibition was the phenolic ring, and a bridging oxygen atom joining the phenyl ring to the sulphamate group (Woo et al 1996b). We have recently shown that the requirement of the phenyl group is the stabilization of the phenoxide ion (Ahmed et al 2000, 2001) and that incorporation of electron-withdrawing groups within the phenyl ring can result in an increase in the inhibitory activity. However, the role of the carbon backbone is yet to be fully rationalized.

In an effort to overcome the lack of detailed information regarding the active site of oestrone sulphatase, to probe the nature of the proposed pharmacophore, and to rationalize the inhibitory activity of the aminosulphonate based compounds, we initiated a series of structure–activity relationship (SAR) determination studies. From the results of our initial molecular modelling study and a review of potential mechanisms for oestrone sulphatase, we concluded that logP may be an important factor in the inhibition of oestrone sulphatase. In particular, from the molecular modelling study we proposed that potential inhibitors did not have to bind to the oestrone sulphatase active site in a similar manner to the steroid backbone of oestrone sulphate (Ahmed et al 1998); however, potent inhibitors would be expected to closely mimic the physicochemical factors (more specifically logP) of the steroid backbone.

Previously, whilst studying the role of the stability of the phenoxide ion in the determination of the inhibitory activity (Ahmed et al 2000, 2001), we eliminated the role of any hydrophobic factor through the synthesis of sulphamate derivatives of phenols. Here, we report the results of our study to verify our previous hypothesis regarding the contribution of logP to the overall inhibition of oestrone sulphatase. We have undertaken the synthesis of a number of straight chain esters of 4-sulphamoylated benzoic acid and have carried out biochemical evaluation (the mode of inhibition was also investigated). The pK_a and logP values of the esters of 4-hydroxybenzoic acid have been determined in an effort to elucidate any relationship between these two physicochemical properties and the inhibitory activity.

Materials and Methods

Chemistry

In the synthesis of the 4-aminosulphonated derivatives of benzoic acid, modified literature procedure (Woo et al 1996a) was followed (Figure 2). The three standard compounds, namely EMATE, COUMATE and 667-COUMATE, were synthesized using reported literature procedures, and as such, the experimental method for these three compounds has not been considered within the current report.

Methyl 4-hydroxybenzoate (1)

Concentrated sulphuric acid (H_2SO_4) (3 mL, 10 M) was carefully added to a suspension of 4-hydroxybenzoic acid (3 g, 21.74 mmol) in methanol (20 mL) and the solution refluxed for 1 h. After cooling to room temperature, sodium hydroxide (NaOH) (~ 15 mL) was added to neutralize the solution. The resulting mixture was allowed to stand for 15 min, before being poured into a cool beaker, and made up to 500 mL with water. A white precipitate formed which was filtered, and dried (80°C), to give **1** (3.3 g, 99.9%) as a white crystalline solid (mp 112–115°C; R_f 0.47 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3263.0 (OH) 1688.2 (C=O). δ_H ($CDCl_3$): 7.95 (2H, d, J = 8 Hz, ArH), 6.89 (2H, d, J = 8 Hz, ArH), 6.06 (1H, s, OH), 3.90 (3H, s, CH_3). δ_c ($CDCl_3$): 167.2 (C=O), 160.0, 131.8, 122.3, 115.1 (CAr), 52.0 (CH_3). GCMS t_R 9.176 m/z 152 (M^+).

Methyl 4-[(aminosulphonyl)oxy]benzoate (2)

Sodium hydride (NaH) (60% dispersion in mineral oil, 0.16 g, 4 mmol) was added to a stirred solution of **1** (0.5 g, 3.29 mmol) in dimethyl formamide (DMF) (10 mL) under an atmosphere of nitrogen gas at 0°C. After evolution of hydrogen had ceased (30 min), aminosulphonyl chloride in toluene (10 mL, ~ 10 mmol) was added in one portion and the reaction allowed to stir for 10 h. The reaction was then quenched with saturated sodium bicarbonate ($NaHCO_3$) solution (50 mL), extracted into dichloromethane (DCM)

(2×50 mL), washed with water (3×30 mL) and dried over anhydrous magnesium sulphate ($MgSO_4$). The mixture was filtered and the solvent removed under vacuum to give a yellow oil which solidified on addition of water. The crude product was purified using flash chromatography to give **2** (0.24 g, 31.6%) as a white solid (mp 118–121°C; R_f 0.24 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3376.1, 3274.0 (NH_2), 1704.3 (C=O), 1376.7, 1156.9 (S=O). δ_H ($CDCl_3$): 8.08 (2H, d, J = 9 Hz, ArH), 7.41(2H, d, J = 9 Hz, ArH), 5.10 (2H, s, NH_2), 3.93 (3H, s, H_3C-). δ_c ($CDCl_3$): 165.2 (C=O), 154.0, 149.8, 131.6, and 121.9 (CAr), 52.4 (CH_3). MS m/z found: M^+ 231.0198, $(C_8H_9NO_5S)^+$ requires 231.0201.

Ethyl 4-hydroxybenzoate (3)

Compound **3** was synthesized following the same procedures as for **1** except conc. H_2SO_4 (3 mL) was added to 4-hydroxybenzoic acid (3 g, 21.74 mmol) in ethanol (20 mL) to give **3** (2.91 g, 81%) as a white solid (mp 122–124°C; R_f 0.66 diethyl ether/petroleum ether 40–60°C (70/30)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3219.5 (OH) 1672.6 (C=O). 90 MHz δ_H ($CDCl_3$): 6.9 (2H, d, J = 8 Hz, ArH), 8.0 (2H, d, 8 Hz, ArH), 6.5 (1H, s, OH), 4.3 (2H, q, J = 7 Hz, CH_2CH_3), 1.3 (3H, t, J = 7 Hz, CH_2CH_3). GCMS t_R 10.308 m/z 166 (M^+).

Ethyl 4-[(aminosulphonyl)oxy]benzoate (4)

Compound **4** was synthesized following the same procedures as for **2** except that NaH (60% dispersion in mineral oil, 0.2 g, 5 mmol) was added to a stirred solution of **3** (0.5 g, 3.01 mmol) in DMF (10 mL). Aminosulphonyl chloride in toluene (10 mL, ~ 10 mmol) was added after 30 min. Removal of the solvent under vacuum yielded a yellow oil, which was purified using flash chromatography to give **4** (0.29 g, 39.3%) as a white solid (mp 83–86°C; R_f 0.55 diethyl ether/petroleum ether 40–60°C (70/30)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3341.4, 3217.7 (NH_2), 1693.7 (C=O), 1383.1, 1174.7 (S=O). δ_H ($CDCl_3$): 8.08 (2H, d, J = 9 Hz, ArH), 7.40 (2H, d, J = 9 Hz, ArH), 5.21 (2H, s, NH_2), 4.33 (2H, q, J = 7 Hz, CH_2), 1.40 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 165.8 (C=O), 154.7, 131.5, 129.3, 121.8 (CAr), 61.4 (CH_2), 13.3 (CH_3). MS m/z 246 (MH^+).

Propyl 4-hydroxybenzoate (5)

Compound **5** was synthesized following the same procedures as for **1** except conc. H_2SO_4 (3 mL) was added to 4-hydroxybenzoic acid (3 g, 21.74 mmol) in propanol (20 mL) to give **5** (3.11 g, 79.5%) as a white crystalline solid (mp 88–90°C; R_f 0.48 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3285.8 (OH) 2967.0, 2866.3 (CH), 1679.1 (C=O). δ_H ($CDCl_3$): 7.95 (2H, d, J = 8 Hz, ArH), 6.91 (2H, d, J = 8 Hz, ArH), 6.71 (1H, s, OH), 4.26 (2H, t, J = 6 Hz, OCH_2), 1.78 (2H, m, OCH_2CH_2), 1.03 (3H, t, J = 6 Hz, CH_3). δ_c ($CDCl_3$): 167.1 (C=O), 160.3, 131.9, 122.5, 115.3 (CAr), 66.6 (OCH_2), 22.1 (OCH_2CH_2), 10.5 (CH_3). GCMS t_R 12.786 m/z 180 (M^+).

Propyl 4-[(aminosulphonyl)oxy]benzoate (6)

Compound **6** was synthesized following the same procedures as for **2** except that NaH (60% dispersion in mineral oil, 0.13 g, 4 mmol) was added to a stirred solution of **5** (0.5 g, 2.78 mmol) in DMF (10 mL). Aminosulphonyl chloride in toluene (10 mL, < 10 mmol) was added after 30 min. Removal of the solvent under vacuum yielded a yellow oil, which was purified using flash chromatography to give **6** (0.34 g, 47.2%) as a white solid (mp 58–60°C; R_f 0.35 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3352.5, 3265.7 (NH_2), 1702.4 (C=O), 1389.6, 1156.0 (S=O). δ_H ($CDCl_3$): 8.04 (2H, d, J = 9 Hz, ArH), 7.37 (2H, d, J = 9 Hz, ArH), 5.43 (2H, s, NH_2), 4.26 (2H, t, J = 7 Hz, OCH_2), 1.78 (2H, m, OCH_2CH_2), 1.01 (3H, t, J = 7 Hz, CH_3). 300 MHz δ_c ($CDCl_3$): 166.6 (C=O), 153.4, 131.5, 129.2, 122.0 (CAr), 67.0 (OCH_2), 22.0 (OCH_2CH_2), 10.5 (CH_3). MS m/z 259 (M^+).

Butyl 4-hydroxybenzoate (7)

Concentrated H_2SO_4 was added to a stirred solution of 4-hydroxybenzoic acid (4 g, 29.9 mmol) and butanol (2.7 mL, 30 mmol) in toluene (50 mL), and the solution refluxed using Dean and Stark apparatus until it became clear (2–4 h). The toluene was removed under vacuum and the remaining residue quenched with saturated $NaHCO_3$. The organic product was extracted into DCM (2×30 mL), washed with water (2×30 mL), dried ($MgSO_4$), and the solvent removed under vacuum to give **7** (2.76 g, 47.4%) as a white solid (mp 64–66°C; R_f 0.52 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3385.0 (OH) 2955.1, 2873.6 (CH), 1679.5 (C=O). δ_H ($CDCl_3$): 7.95 (2H, d, J = 8 Hz, ArH), 6.91 (2H, d, J = 8 Hz, ArH), 6.92 (1H, s, OH), 4.31 (2H, t, J = 6 Hz, OCH_2), 1.74 (2H, m, OCH_2CH_2), 1.47 (2H, m, $-CH_2CH_3$), 0.97 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 167.3 (C=0), 160.4, 131.9, 122.4, 115.3 (CAr), 64.9 (OCH_2), 30.5 (OCH_2CH_2), 19.3 ($O(CH_2)_2CH_2$) 13.8 (CH_3). GCMS t_R 12.786 m/z 194 (M^+).

Butyl 4-[(aminosulphonyl)oxy]benzoate (8)

Compound **8** was synthesized following the same procedures as for **2** except that NaH (60% dispersion in mineral oil, 0.05 g, 1.25 mmol) was added to a stirred solution of **7** (0.17 g, 0.88 mmol) in DMF (10 mL). Aminosulphonyl chloride in toluene (10 mL, ~ 10 mmol) was added after 30 min. Removal of the solvent under vacuum yielded a yellow oil, which was purified using flash chromatography to give **8** (0.06 g, 25%) as a white solid (mp 71–74°C; R_f 0.31 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3362.3, 3264.6 (NH_2), 1703.4 (C=O), 1387.7, 1156.1 (S=O). δ_H ($CDCl_3$): 8.04 (2H, d, J = 9 Hz, ArH), 7.39 (2H, d, J = 9 Hz, ArH), 5.37 (2H, s, NH_2), 4.31

(2H, t, J = 6 Hz, OCH_2), 1.74 (2H, m, OCH_2CH_2), 1.47 (2H, m, CH_2CH_3), 0.98 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 165.7 (C═O), 153.2, 131.4, 129.2, 121.9 (CAr), 65.3 (OCH_2), 30.6 (OCH_2CH_2), 19.1 ($O(CH_2)_2CH_2$), 13.7 (CH_3). MS m/z 274 (MH^+).

Pentyl 4-hydroxybenzoate (9)

Compound **9** was synthesized following the same procedures as for **7** except that concentrated H_2SO_4 (1 mL) was added to 4-hydroxybenzoic acid (4.5 g, 33 mmol) and pentanol (3.4 mL, 33 mmol) in toluene (50 mL). Compound **9** was obtained as a clear oil (2.78 g, 41.1%) (R_f 0.57 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3276.4 (OH) 2954.3, 2872.0 (CH), 1682.9 (C═O). δ_H ($CDCl_3$): 7.95 (2H, d, J = 8 Hz, ArH), 6.91 (2H, d, J = 8 Hz, ArH), 7.78 (1H, s, OH), 4.31 (2H, t, J = 6 Hz, OCH_2), 1.77 (2H, m, OCH_2CH_2), 1.40 (4H, m, -$(CH_2)_2CH_3$), 0.92 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 167.9 (C═O), 161.1, 132.0, 121.8, 115.8 (CAr), 65.5 (OCH_2), 28.4 (OCH_2CH_2), 28.2 ($O(CH_2)_2CH_2$) 22.365 ($O(CH_2)_3CH_2$), 13.993 (CH_3). GCMS t_R 14.009 m/z 208 (M^+).

Pentyl 4-[(aminosulphonyl)oxy]benzoate (10)

Compound **10** was synthesized following the same procedures as for **2** except that NaH (60% dispersion in mineral oil, 0.12 g, 3 mmol) was added to a stirred solution of **9** (0.5 g, 2.40 mmol) in DMF (10 mL). Aminosulphonyl chloride in toluene (10 mL, ~ 10 mmol) was added after 30 min. Removal of the solvent under vacuum yielded a yellow oil, which was purified using flash chromatography to give **10** (0.34 g, 49.4%) as a white solid (mp 48–49°C; R_f 0.31 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3362.3, 3264.1 (NH_2), 1704.2 (C═O), 1386.6, 1156.5 (S═O). δ_H ($CDCl_3$): 8.00 (2H, d, J = 9 Hz, ArH), 7.37 (2H, d, J = 9 Hz, ArH), 5.59 (2H, s, NH_2), 4.28 (2H, t, J = 6 Hz, OCH_2), 1.74 (2H, m, OCH_2CH_2), 1.38 (4H, m, $(CH_2)_2CH_3$), 0.92 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 165.9 (C═O), 153.4, 131.5, 129.2, 122.0 (CAr), 65.7 (OCH_2), 28.3 (OCH_2CH_2), 28.1 ($O(CH_2)_2CH_2$), 22.3 ($O(CH_2)_3CH_2$), 14.0 (CH_3). MS m/z 288 (MH^+).

Hexyl 4-hydroxybenzoate (11)

Compound **11** was synthesized following the same procedures as for **7** except that conc. H_2SO_4 (1 mL) was added to 4-hydroxybenzoic acid (4.5 g, 33 mmol) and hexanol (4.05 mL, 33 mmol) in toluene (50 mL). Compound **11** was obtained as a clear oil (3.9 g, 53.2%) (R_f 0.57 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3281.4 (OH) 2959.0, 2958.1 (CH), 1682.8 (C═O). δ_H ($CDCl_3$): 7.94 (2H, d, J = 8 Hz, ArH), 6.92 (2H, d, J = 8 Hz, ArH), 7.78 (1H, broad s, OH), 4.30 (2H, t, J = 6 Hz, OCH_2), 1.75 (2H, m, OCH_2CH_2), 1.38 (6H, m, -$(CH_2)_3CH_3$), 0.89 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 167.5 (C═O), 160.7, 131.9, 122.2, 115.4 (CAr), 65.3 (OCH_2), 31.5 (OCH_2CH_2), 28.7 ($O(CH_2)_2CH_2$) 25.7 ($O(CH_2)_3CH_2$), 22.6 ($O(CH_2)_4CH_2$), 14.1 (CH_3). GCMS t_R 5.114 m/z 222 (M^+).

Hexyl 4-[(aminosulphonyl)oxy]benzoate (12)

Compound **12** was synthesized following the same procedures as for **2** except that NaH (60% dispersion in mineral oil, 0.12 g, 3 mmol) was added to a stirred solution of **11** (0.5 g, 2.25 mmol) in DMF (10 mL). Aminosulphonyl chloride in toluene (10 mL, ~ 10 mmol) was added after 30 min. Removal of the solvent under vacuum yielded a yellow oil, which was purified using flash chromatography to give **12** (0.24 g, 35.4%) as a white solid (mp 37–39°C; R_f 0.37 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3365.4, 3265.1 (NH_2), 1703.0 (C═O), 1389.2, 1181.7 (S═O). δ_H ($CDCl_3$): 8.01 (2H, d, J = 9 Hz, ArH), 7.37 (2H, d, J = 9 Hz, ArH), 5.58 (2H, s, NH_2), 4.28 (2H, t, J = 6 Hz, OCH_2), 1.74 (2H, m, OCH_2CH_2), 1.35 (6H, m, $(CH_2)_3CH_3$), 0.90 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 165.8 (C═O), 153.3, 131.4, 129.1, 121.9 (CAr), 65.6 (OCH_2), 31.4 (OCH_2CH_2), 28.5 ($O(CH_2)_2CH_2$), 25.6 ($O(CH_2)_3CH_2$), 22.5 ($O(CH_2)_4CH_2$), 13.9 (CH_3). MS m/z 302 (MH^+).

Heptyl 4-hydroxybenzoate (13)

Compound **13** was synthesized following the same procedures as for **7** except that concentrated H_2SO_4 (0.5 mL) was added to 4-hydroxybenzoic acid (2 g, 14.5 mmol) and heptanol (2.1 mL, 14.6 mmol) in toluene (50 mL). Compound **13** was obtained as a clear oil (1.45 g, 42.4%) (R_f 0.57 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3348.6 (OH) 2956.6, 2929.9 (CH), 1682.5 (C═O). δ_H ($CDCl_3$): 7.94 (2H, d, J = 8 Hz, ArH), 6.92 (2H, d, J = 8 Hz, ArH), 7.90 (1H, broad s, OH), 4.30 (2H, t, J = 6 Hz, OCH_2), 1.75 (2H, m, OCH_2CH_2), 1.37 (8H, m, -$(CH_2)_4CH_3$), 0.88 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$):167.7 (C═O), 161.0, 132.0, 121.9, 115.4 (CAr), 65.4 (OCH_2), 31.7 (OCH_2CH_2), 29.0 ($O(CH_2)_2CH_2$) 28.7 ($O(CH_2)_3CH_2$), 26.0 ($O(CH_2)_4CH_2$), 22.6 ($O(CH_2)_5CH_2$), 14.1 (CH_3). GCMS t_R 16.236 m/z 236 (M^+).

Heptyl 4-[(aminosulphonyl)oxy]benzoate (14)

Compound **14** was synthesized following the same procedures as for **2** except that NaH (60% dispersion in mineral oil, 0.05 g, 1.25 mmol) was added to a stirred solution of **13** (0.18 g, 0.74 mmol) in DMF (10 mL). Aminosulphonyl chloride in toluene (10 mL, ~ 10 mmol) was added after 30 min. Removal of the solvent under vacuum yielded a yellow oil, which was purified using flash chromatography to give **14** (0.06 g, 25.7%) as a white solid (mp 54–56°C; R_f 0.37 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3362.3, 3272.0 (NH_2), 1704.4 (C═O), 1388.1, 1156.6 (S═O). δ_H ($CDCl_3$): 8.02 (2H, d, J = 9 Hz, ArH), 7.39 (2H, d, J = 9 Hz, ArH), 5.41 (2H, s, NH_2), 4.28 (2H, t, J = 6 Hz, OCH_2), 1.75 (2H, m, OCH_2CH_2), 1.36

(8H, m, $(CH_2)_4CH_3$), 0.89 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 165.8 (C═O), 153.3, 131.5, 129.3, 122.0 (CAr), 65.7 (OCH_2), 31.7 (OCH_2CH_2), 28.9 ($O(CH_2)_2CH_2$), 28.6 ($O(CH_2)_3CH_2$), 26.0 ($O(CH_2)_4CH_2$), 22.6 ($O(CH_2)_5CH_2$), 14.1 (CH_3). MS m/z 333 (MNH_4^+).

Octyl 4-hydroxybenzoate (15)

Compound **15** was synthesized following the same procedures as for **7** except that conc. H_2SO_4 (0.3 mL) was added to 4-hydroxybenzoic acid (1 g, 7.24 mmol) and octanol (1.2 mL, 7.6 mmol) in toluene (50 mL). The crude oil was purified using flash chromatography to give **15** as a clear oil (1.05 g, 58.0%) (R_f 0.61 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3349.7 (OH) 2924.7, 2854.5 (CH), 1684.2 (C═O). δ_H ($CDCl_3$):7.94 (2H, d, J = 8 Hz, ArH), 6.89 (2H, d, J = 8 Hz, ArH), 6.09 (1H, broad s, OH), 4.29 (2H, t, J = 6 Hz, OCH_2), 1.75 (2H, m, OCH_2CH_2), 1.36 (10H, m, $-(CH_2)_5CH_3$), 0.85 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$):166.9 (C═O), 160.1, 132.0, 123.0, 115.3 (CAr), 65.2 (OCH_2), 31.9 (OCH_2CH_2), 29.8 ($O(CH_2)_2CH_2$) 29.3 ($O(CH_2)_3CH_2$), 28.9 ($O(CH_2)_4CH_2$), 26.2 ($O(CH_2)_5CH_2$), 27.8 ($O(CH_2)_6CH_2$), 14.2 (CH_3). GCMS t_R 17.206 m/z 250 (M^+).

Octyl 4-[(aminosulphonyl)oxy]benzoate (16)

Compound **16** was synthesized following the same procedures as for **2** except that NaH (60% dispersion in mineral oil, 0.5 g, 12.5 mmol) was added to a stirred solution of **15** (2.38 g, 9.52 mmol) in DMF (10 mL). Aminosulphonyl chloride in toluene (20 mL, ~ 20 mmol) was added after 30 min. Removal of the solvent under vacuum yielded a yellow oil, which was purified using flash chromatography to give **16** (1.01 g, 32.2%) as a white solid (mp 62–64°C; R_f 0.45 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3364.3, 3267.7 (NH_2), 1704.2 (C═O), 1391.2, 1157.0 (S═O). δ_H ($CDCl_3$): 7.99 (2H, d, J = 9 Hz, ArH), 7.35 (2H, d, J = 9 Hz, ArH), 5.16 (2H, s, NH_2), 4.26 (2H, t, J = 7 Hz, OCH_2), 1.73 (2H, m, OCH_2CH_2), 1.35 (10H, m, $(CH_2)_5CH_3$), 0.87 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 165.9 (C═O), 153.4, 131.5, 129.1, 122.0 (CAr), 65.7 (OCH_2), 31.8 (OCH_2CH_2), 29.0 ($O(CH_2)_2CH_2$), 28.6 ($O(CH_2)_3CH_2$), 26.0 ($O(CH_2)_4CH_2$), 25.4 ($O(CH_2)_5CH_2$), 22.6 ($O(CH_2)_6CH_2$), 14.1 (CH_3). MS m/z 347 (MNH_4^+).

Nonyl 4-hydroxybenzoate (17)

Compound **17** was synthesized following the same procedures as for **7** except that conc. H_2SO_4 (0.5 mL) was added to 4-hydroxybenzoic acid (2 g, 14.5 mmol) and nonanol (2.5 mL, 14.4 mmol) in toluene (50 mL). The crude oil was purified using flash chromatography to give **17** as a clear oil (0.95 g, 24.8%) (R_f 0.57 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3352.2 (OH) 2926.6, 2855.8 (CH), 1682.3 (C═O). δ_H ($CDCl_3$): 7.93 (2H, d, J = 8 Hz, ArH), 6.94 (2H, d, J = 8 Hz, ArH), 7.11 (1H, broad s, OH), 4.30 (2H, t, J = 6 Hz, OCH_2), 1.76 (2H, m, OCH_2CH_2), 1.36 (12H, m, $-(CH_2)_6CH_3$), 0.88 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 167.7 (C═O), 161.0, 132.0, 121.9, 115.4 (CAr), 65.4 (OCH_2), 32.4 (OCH_2CH_2), 29.5 ($O(CH_2)_2CH_2$) 29.3 ($O(CH_2)_3CH_2$), 28.7 ($O(CH_2)_4CH_2$), 26.0 ($O(CH_2)_5CH_2$), 25.7 ($O(CH_2)_6CH_2$), 22.7 ($O(CH_2)_7CH_2$), 14.1 (CH_3). GCMS t_R 18.406 m/z 264 (M^+).

Nonyl 4-[(aminosulphonyl)oxy]benzoate (18)

Compound **18** was synthesized following the same procedures as for **2** except that NaH (60% dispersion in mineral oil, 0.2 g, 5 mmol) was added to a stirred solution of **17** (0.5 g, 1.90 mmol) in DMF (10 mL). Aminosulphonyl chloride in toluene (10 mL, ~ 10 mmol) was added after 30 min. Removal of the solvent under vacuum yielded a yellow oil, which was purified using flash chromatography to give **18** (0.07 g, 10.7%) as a white solid (mp 58–61°C; R_f 0.24 diethyl ether/petroleum ether 40–60°C (30/70)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3362.3, 3264.2 (NH_2), 1704.3 (C═O), 1391.8, 1181.8 (S═O). δ_H ($CDCl_3$): 8.02 (2H, d, J = 9 Hz, ArH), 7.38 (2H, d, J = 9 Hz, ArH), 5.46 (2H, s, NH_2), 4.28 (2H, t, J = 6 Hz, OCH_2), 1.74 (2H, m, OCH_2CH_2), 1.31 (12H, m, $(CH_2)_6CH_3$), 0.88 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 165.7 (C═O), 153.3, 131.5, 129.2, 122.0 (CAr), 65.7 (OCH_2), 31.8 (OCH_2CH_2), 29.5 ($O(CH_2)_2CH_2$), 29.3 ($O(CH_2)_3CH_2$), 29.2 ($O(CH_2)_4CH_2$), 28.6 ($O(CH_2)_5CH_2$), 26.0 ($O(CH_2)_6CH_2$), 22.7 ($O(CH_2)_7CH_2$), 14.1 (CH_3). MS m/z found: MNH_4^+ 361.1805, $(C_{16}H_{25}NO_5S)NH_4^+$ requires 361.1797.

Decyl 4-hydroxybenzoate (19)

Compound **19** was synthesized following the same procedures as for **7** except that conc. H_2SO_4 (0.5 mL) was added to 4-hydroxybenzoic acid (2 g, 14.5 mmol) and decanol (2.8 mL, 14.7 mmol) in toluene (50 mL). The crude oil was purified using flash chromatography to give **19** as a white solid (1.06 g, 26.3%) (mp 30–34°C; R_f 0.57 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3354.7 (OH) 2924.2, 2854.1 (CH), 1683.7 (C═O). δ_H ($CDCl_3$): 7.94 (2H, J = 8 Hz, ArH), 6.93 (2H, d, J = 8 Hz, ArH), 6.37 (1H, broad s, OH), 4.30 (2H, t, J = 6 Hz, OCH_2), 1.76 (2H, m, OCH_2CH_2), 1.36 (14H, m, $-(CH_2)_7CH_3$), 0.88 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 167.5 (C═O), 160.7, 131.9, 122.2, 115.3 (CAr), 65.3, 31.9, 29.5, 29.3, 28.7, 26.0, 22.7, 14.1 (only 8 aliphatic carbons visible). GCMS t_R 19.195 m/z 278 (M^+).

Decyl 4-[(aminosulphonyl)oxy]benzoate (20)

Compound **20** was synthesized following the same procedures as for **2** except that NaH (60% dispersion in mineral oil, 0.1 g, 2.5 mmol) was added to a stirred solution of **19** (0.5 g, 1.8 mmol) in DMF (10 mL). Aminosulphonyl chloride in toluene (10 mL, ~ 10 mmol) was added after 30 min. Removal of the solvent under vacuum yielded a

yellow oil, which was purified using flash chromatography to give **20** (0.21 g, 32.7%) as a white solid (mp 48–51°C; R_f 0.41 diethyl ether/petroleum ether 40–60°C (50/50)).

$\upsilon_{(max.)}$ (film) cm^{-1}: 3394.0, 3276.2 (NH_2), 1715.9 (C═O), 1377.2, 1181.7 (S═O). δ_H ($CDCl_3$): 8.04 (2H, d, J = 9 Hz, ArH), 7.39 (2H, d, J = 9 Hz, ArH), 5.39 (2H, s, NH_2), 4.29 (2H, t, J = 6 Hz, OCH_2), 1.75 (2H, m, OCH_2CH_2), 1.34 (14H, m, $(CH_2)_7CH_3$), 0.88 (3H, t, J = 7 Hz, CH_3). δ_c ($CDCl_3$): 165.7 (C═O), 153.3, 131.5, 129.3, 121.9 (CAr), 65.7, 31.9, 29.5, 29.3, 28.6, 26.0, 22.7, 14.1 (only 8 aliphatic peaks visible). MS m/z found: MNH_4^+ 375.1961, $(C_{17}H_{27}NO_5S)NH_4^+$ requires 375.1954.

Human placental microsomal oestrone sulphatase assay

In the biochemical evaluation, the standard literature method was used (Selcer et al 1996). The total assay volume was 1 mL. [^{3}H]Oestrone sulphate (25 μL, 20 mM/tube; 300 000 dpm) and the inhibitors (50 μM/tube) dissolved in ethanol were added to a 10-mL assay tube, and the ethanol removed with a stream of nitrogen. Tris-HCl buffer (0.05 M, pH 7.2, 0.2 mL) was added to each tube. Placental microsomes were then diluted with Tris-HCl buffer (115 $\mu g\ mL^{-1}$). The microsomes and assay tubes were pre-incubated for 5 min at 37°C in a shaking water bath before the addition of the microsomes (0.8 mL) to the tubes. After 20-min incubation (at 37°C), toluene (4 mL) was added to quench the assay, and the tubes placed on ice. The quenched samples were vortexed for 45 s and centrifuged (3000 rev min^{-1}, 10 min). Toluene (1 mL) was removed and added to 5 mL scintillation fluid (Tritonx). The samples were counted for 3 min. All samples were run in triplicate. Control samples with no inhibitor were incubated simultaneously. Blank samples were obtained by incubating with boiled microsomes. In the determination of the IC50 values, linear regression analysis by the least-square method was used to produce the line of best fit; as such, we considered correlation coefficients greater than 0.95 to suggest a linear relationship between the inhibitory activity and log of the concentration of inhibitor. The resulting best fit equation was then used to determine the IC50 value.

Irreversible oestrone sulphatase assay

The irreversible inhibition was determined using the procedure described by Purohit et al (1998) using EMATE (10 μM), COUMATE (100 μM), **12** (700 μM) and **14** (700 μM). Placental microsomes (18 mg mL^{-1}, 55 μL) were incubated with each of the inhibitors (25 μL in ethanol, removed with a stream of nitrogen) in Tris-HCl buffer (50 mM, pH 7.2, 945 μL) at 37°C for 10 min. A control tube with no inhibitor was incubated simultaneously (100% tubes). A 100-μL sample, in triplicate, was taken from each sample and tested for oestrone sulphatase activity using the procedure above, except that 900 μL Tris-HCl buffer was added to the assay tubes. A second sample (100 μL), in triplicate, was subjected to dialysis at 4°C for 16 h, with regular changes of Tris-HCl buffer. The microsomes were then removed from the dialysis tubing and tested for oestrone sulphatase activity as described above.

Calculation of logP

logP was calculated using the procedures available within CaChe molecular modelling suite of programs. In the calculation of the logP of the aminosulphonated compounds, we discovered that little was known about the contribution of the sulphamate group towards the overall logP of the molecule. In an effort to simplify our calculations (and remove any potential problems with the calculated values), we utilized the parent non-sulphamated compounds as a guide to the determination of the optimum logP. That is, the contribution to the overall logP by the sulphamate group is expected to be constant for all of the compounds considered within this study, the only difference structurally, and thus from a hydrophobicity point of view, is the carbon backbone attached to the sulphamate group. It is therefore reasonable to presume that any relationship that exists between logP and the inhibitory activity can be correlated between the backbone and the inhibitory activity of the compounds synthesized.

Determination of pK_a

Although the pK_a values for some of the phenol compounds were available in the literature, the values for the majority of phenolic compounds were lacking. As such, we concluded that the determination of values for all of the parent compounds would be a better and more consistent approach. The evaluation of the pK_a of the starting phenols involved a spectroscopic technique (Harwood & Moody 1989) which considered the change in UV absorption by the phenolic group under acidic, buffer (pH 9) and basic conditions. That is, a solution of borax buffer was prepared using sodium tetraborate decahydrate (9.54 g in 1 L), to which hydrochloric acid (92 mL, 0.1 M) was added.

Approximately 3–4 mg of the phenolic compound was added to 100 mL borax buffer. The solution was mixed thoroughly and the UV spectra determined (between 350 and 250 nm). The absorbance value was adjusted to approximately 1 involving the addition of buffer or the phenolic compound. The solution was filtered (to remove any undissolved phenol) and 20 mL of the stock solution was made up to 25 mL with either HCl (2 M), borax buffer, or NaOH (2 M). The UV spectrum of each solution was determined and using the absorption values at a single chosen wavelength, the mole fraction (x) was calculated. The pK_a was then calculated using the equation: $pK_a = pH + [(1 - x)/x]$.

Results and Discussion

The syntheses of the various esters were attempted using the conditions outlined in Figure 2. The reactions were found to proceed well and in good yield without any major problems.

Table 1 pK_a of parent phenols of 4-hydroxy benzoic acid.

Compound	R	pK_a
1	CH_3	8.28±0.07
3	C_2H_5	8.22±0.09
5	C_3H_7	8.03±0.11
7	C_4H_9	8.07±0.09
9	C_5H_{11}	8.47±0.13
11	C_6H_{13}	8.52±0.15
13	C_7H_{15}	8.27±0.1

Values given are mean±s.e.m.

The results of the determination of the pK_a values for the ester derivatives of 4-hydroxybenzoic acid are shown below in Table 1. As can be observed from the experimental data, the parent phenolic compounds possessed a small range of pK_a values. However, the inhibitory activity of the sulphamate derivatives within these compounds varied considerably (Table 2). (It should be noted that except for the recently reported tricyclic derivatives of COUMATE, these compounds were observed to possess some of the most potent inhibition shown by any series of non-steroidal inhibitors of oestrone sulphatase.) A plot of the IC50 vs pK_a showed an extremely poor relationship with a correlation coefficient value less than 0.4. This initial result appeared to contradict our previous reports, where we had shown a very strong correlation between pK_a and IC50 values for a range of simple substituted phenyl sulphamates (Ahmed et al 2000, 2001), and which we postulated to be associated with the stability of the phenoxide ion. However, we suggest that the results observed within this study were not a contradiction but a clarification of the involvement of this physicochemical factor within a given series of phenolic derivatives. We propose that whilst the pK_a of the parent phenol plays a critical role in determining the extent of inhibition of oestrone sulphatase by sulphamate containing compounds, within a given series of compounds (in which therefore the range of pK_a values may be expected to be small), the role of pK_a is diminished. The importance of the R group in the stability of the RO^- ion can be observed in the difference in inhibitory activity between compounds where R is an alkyl group (for example in ethyl sulphamate) as opposed to an aryl moiety; indeed the alkyl sulphamates were found to possess no inhibitory activity whatsoever (Ahmed et al 2001). Furthermore, within the substituted phenyl sulphamate based compounds, electron-donating groups attached to the phenyl ring were observed to possess IC50 values greater than 10 mM whilst compounds containing electron-withdrawing groups were found to possess extremely low IC50 values (see Table 3 for examples of IC50 values for a small range of sulphamated compounds).

The results of the calculation of the logP of the parent phenol and IC50 values are shown in Table 2. (It should be noted that the logP values of the non-aminosulphonated compounds were determined due to the lack of data for the aminosulphonate group.) The relationship between logP and IC50 was mathematically modelled using a quadratic relationship and the validity of this relationship was confirmed using an analysis of variance ($P < 0.05$ denoted significance). Consideration of the plot of the logP vs IC50 (Figure 3) showed that a strong correlation existed between the hydrophobicity of the carbon backbone and the inhibitory activity of the sulphamated compound. Furthermore, we observed that the inhibitory activity of these compounds increased to C_7, after which the inhibitory activity began to decrease (within the series of compounds considered in this study, an 'optimum' logP value of 3.5 was observed).

Table 2 The synthesized sulphamate compounds and the calculated logP (calculated for the non-sulphamated 4-hydroxybenzoate 'backbone' and not for the final sulphamated compound) and IC50 values.

Compound	R	Calculated logP	Percentage inhibition %		IC50 (μM)
			[I] = 50 μM	[I] = 10 μM	
2	CH_3	1.492	74.7±0.3	25.4±0.1	31.6±1.23
4	C_2H_5	1.835	63.4±0.2	25.6±0.1	31.6±1.95
6	C_3H_7	2.303	76.9±0.2	42.6±0.2	13.2±0.4
8	C_4H_9	2.7	ND	48.3±0.1	10.5±0.28
10	C_5H_{11}	3.096	86.2±0.5	64.4±0.5	5.9±0.44
12	C_6H_{13}	3.492	ND	72.6±0.4	3.8±0.16
14	C_7H_{15}	3.889	83.7±0.4	69.5±0.4	3.4±0.25
16	C_8H_{17}	4.285	80.4±0.4	63.0±0.5	5±0.26
18	C_9H_{19}	4.681	76.2±0.1	ND	4.8±0.17
20	$C_{10}H_{21}$	5.077	ND	36.5±0.1	22.4±0.48
–	COUMATE	1.698	ND	ND	12±0.16
–	667-COUMATE	2.651	ND	ND	0.25±0.02
–	EMATE	3.870	ND	ND	0.5±0.01

Values given are mean±s.e.m. ND, not determined at this concentration.

Table 3 Examples of the IC50 values of a small range of substituted phenylsulphamate-based compounds to show the importance of the stability of the RO^- ion.

Group	Substitution	% inhibition	IC50 (μM)	pK_a (experimental)	pK_a (literature)
H	–	29.7[a]±0.5	>10000	9.86±0.21	9.89
CH_3	3	39.5[a]±0.2	>10000	10.0±0.12	10.01
F	3	79.6[a]±0.1	2089±50	9.16±0.12	NA
Cl	3	62.0[b]±0.2	537±21.2	9.00±0.08	8.85
Br	3	79.2[b]±0.8	257±6.3	8.94±0.1	NA
CN	3	84.3[b]±0.4	190.5±4.3	8.54±0.11	NA
NO_2	3	90.4[b]±0.4	120±3.9	8.66±0.23	8.28

Values given are mean±s.e.m. [a]At inhibitor concentration of 10000 μM. [b]At inhibitor concentration of 1000 μM. NA, not available.

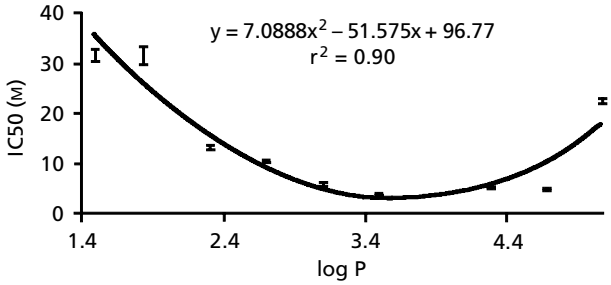

Figure 3 Plot of IC50 vs logP of the non-sulphamated backbone of the synthesized inhibitors.

As previously mentioned, from our molecular modelling study, we hypothesized that whilst the non-steroidal inhibitors would not be expected to closely mimic the binding of the steroidal backbone of oestrone sulphate, the potential inhibitors would, however, be expected to mimic a number of the physicochemical factors associated with the steroid backbone, such as hydrophobicity. It was therefore interesting to observe that the calculated logP of oestrone (the non-sulphamated backbone of EMATE) was 3.9, which was found to be close to the optimum observed within our compounds in the study. Another potent inhibitor of oestrone sulphatase, 669-COUMATE, was also found to possess a logP value close to the optimum observed within this study (logP of non-sulphamated backbone of 669-COUMATE was calculated to be 3.4).

We believe that the hydrophobicity requirement of the carbon backbone of inhibitors of oestrone sulphatase was pivotal in the mechanism of oestrone sulphatase (Figure 4). That is, this enzyme appeared to be an 'irreversible enzyme' in that once the de-sulphatation reaction had occurred, the reverse sulphatation reaction 'could not' then reoccur. This point has always intrigued us since other 'irreversible enzymes', such as aromatase, involve the cleavage of much stronger bonds (for example, carbon–carbon bonds). We believe that the reaction catalysed by oestrone sulphatase is reversible; however, the relatively high hydrophobicity requirement of the R group (such as oestrone in EMATE) causes the resulting anion to be expelled out of the active site, thereby preventing the re-formation of the sulphate to

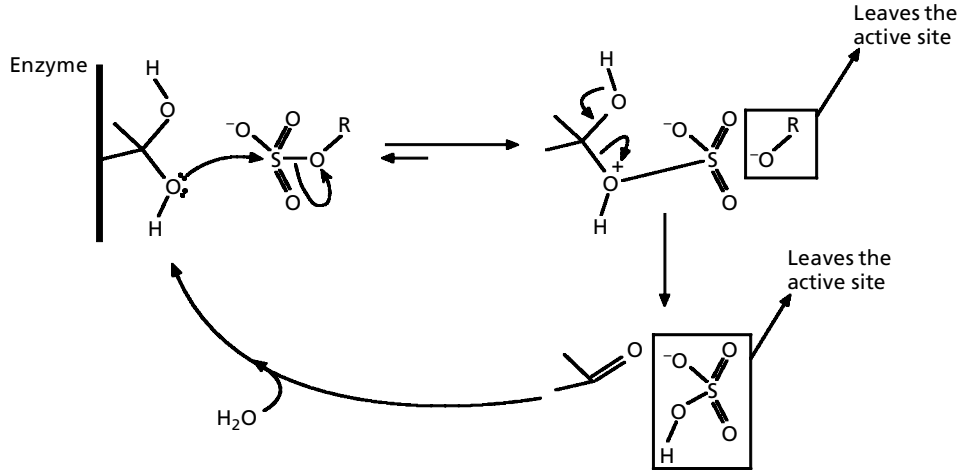

Figure 4 Proposed mechanism of oestrone sulphatase involving the attack of the sulphonate by the diol.

steroid backbone bond (in oestrone sulphate) and thereby it appears an irreversible process. Where the carbon backbone is of lower hydrophobicity, the anion may be somewhat stabilized within the active site and thus exist for a longer period of time. We suggest that in the case of compounds containing a less hydrophobic backbone, the re-formation of the sulphur oxygen bond is possible, thereby reversing the de-sulphatation reaction.

Inhibitors of oestrone sulphatase containing an aminosulphonate group are also believed to undergo a similar process. Recently, we reported a mechanism for the inhibition of oestrone sulphatase by sulphamate containing compounds where we proposed that the cleavage of the S-OR bond (and thus pK_a) was a crucial step in the inhibition process so as to produce sulphamic acid and the anion containing the carbon backbone (RO^-) (Ahmed et al 2002). The destabilization of the RO^- (due to the relatively high hydrophobicity of the R group) would therefore appear to aid the overall inhibitory process. We therefore suggest that whilst the carbonyl group of the carboxy moiety of the compounds studied within this report were pivotal in the inhibition process (since they were electron-withdrawing and as such favoured the initial, and crucial, cleavage of the S-OR bond through the stabilization of the phenoxide ion), the length of the alkyl chain played an important role also. A detailed consideration of the plot of IC50 vs logP showed that the inhibitory activity of these compounds increased to C_7, after which the inhibitory activity began to decrease. Although this was initially unexpected (since a high logP would further aid the expulsion of RO^-), the decrease in inhibitory activity could be rationalized through the involvement of unfavourable interaction with the enzyme active site, presumably due to the presence of as yet unknown components within the active site.

Conclusion

Results of our previous studies have shown that the overall stability of the RO^- ion of the parent phenol (which is therefore related to pK_a) is a major factor in the overall inhibition of oestrone sulphatase. Furthermore, we proposed that the stability of the phenoxide ion played a critical role in the cleavage of the S-OAr bond, and that without such factors compounds would lack inhibitory activity against oestrone sulphatase. However, the results of this study showed that there was a strong correlation between the inhibitory activity and the logP of the parent compound and that for a series of phenolic derivatives (in which therefore the range of pK_a values may be expected to be small), the logP of the inhibitors was an important factor also. As such, this is the first report to suggest that these two factors can be combined in the design of highly potent inhibitors of oestrone sulphatase.

References

Ahmed, S., James, K., Sampson, L. (1998) Molecular modelling study of inhibitors of the enzyme oestrone sulphatase (ES). *Pharm. Pharmacol. Commun.* **4**: 481–483

Ahmed, S., James, K., Patel, C. K. (2000) First report of the investigation into the importance of pKa in the inhibition of estrone sulfatase by sulfamate containing compounds. *Biochem. Biophys. Res. Commun.* **272**: 583–585

Ahmed, S., James, K., Owen, C. P., Patel, C. K. (2001) Acid dissociation constant, a potential physicochemical factor in the inhibition of the enzyme estrone sulfatase (ES). *Bioorg. Med. Chem. Lett.* **11**: 899–902

Ahmed, S., James, K., Owen, C. P., Patel, C. K., Sampson, L. (2002) Evidence for the mechanism of the irreversible inhibition of oestrone sulphatase (ES) by aminosulphonate based compounds. *J. Steroid Biochem. Mol. Biol.* **80**: 429–440

Brodie, A. M. H., Njar, V. C. O. (2000) Aromatase inhibitors and their application in breast cancer treatment. *Steroids* **65**: 171–179

Feutrie, M. L., Bonneterre, J. (1999) Aromatase inhibitors. *Bull. Cancer* **86**: 821–827

Harwood, L. M., Moody, C. J. (1989) In: *Experimental organic chemistry*, pp 716–719

Howarth, N. M., Purohit, A., Reed, M. J., Potter, B. L. V. (1997) Estrone sulfamates – potent inhibitors of estrone sulfatase with therapeutic potential. *Steroids* **62**: 346–350

Purohit, A., Potter, B. V. L., Parker, M. G., Reed, M. J. (1998) Steroid sulphatase: expression, isolation and inhibition for active-site identification studies. *Chemico-Biological Interactions* **109**: 183–193

Reed, M. J., Purohit, A., Woo, L. W. L., Potter, B. V. L. (1996) The development of steroid sulphatase inhibitors. *Endocrine-Related Cancer* **3**: 9–23

Selcer, K. W., Jagannathan, S., Rhodes, M. E., Li, P.-K. (1996) Inhibition of placental estrone sulfatase activity and MCF-7 breast cancer cell proliferation by estrone-3-amino derivatives. *J. Steroid Biochem. Mol. Biol.* **59**: 83–91

Woo, L. W. L., Purohit, A., Reed, M. J., Potter, B. V. L. (1996a) Active site directed inhibition of estrone sulfatase by nonsteroidal coumarin sulfamates. *J. Med. Chem.* **39**: 1349–1351

Woo, L. W. L., Lighttowler, M., Purhoit, A., Reed, M. J., Potter, B. V. L. (1996b) Heteroatom-substituted analogues of the active-site directed inhibitor estra-1,3,5(10)-trien-17-one-3-sulphamate inhibit estrone sulphatase by a different mechanism. *J. Steroid Biochem. Mol. Biol.* **57**: 79–88

Woo, L. W. L., Howarth, N. M., Purohit, A., Hejaz, H. A. M., Reed, M. J., Potter, B. V. L. (1998) Steroidal and nonsteroidal sulfamates as potent inhibitors of steroid sulfatase. *J. Med. Chem.* **41**: 1068–1083

Woo, L. W. L., Purohit, A., Malini, B., Reed, M. J., Potter, B. V. L. (2000) Potent active site-directed inhibition of steroid sulphatase by tricyclic coumarin-based sulphamates. *Chem. Biol.* **7**: 773–791