Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 605–609
Bioorganic & Medicinal Chemistry Letters

Inhibition of estrone sulfatase (ES) by alkyl and cycloalkyl ester derivatives of 4-[(aminosulfonyl)oxy] benzoic acid

Chirag K. Patel, Caroline P. Owen and Sabbir Ahmed*

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

Received 11 September 2003; revised 29 October 2003; accepted 20 November 2003

Abstract—In our search for potent inhibitors of the enzyme estrone sulfatase (ES), we have undertaken the synthesis and biochemical evaluation of a range of esters of 4-[(aminosulfonyl)oxy] benzoic acid. The results of the study show that the synthesised compounds possess potent inhibitory activity, indeed the cyclooctyl derivative was found to be more potent than 667-COUMATE, which is currently undergoing clinical trials.

In the treatment of hormone dependent breast cancer, extensive research has led to the synthesis of highly selective and potent inhibitors of the cytochrome P-450 enzyme aromatase (AR).[1] However, the enzyme estrone sulfatase (ES) provides another source of estrogens and is responsible for the catalysis of the conversion of the stored (sulfated) form of the estrogens to the active (non-sulfated) form (Fig. 1). The same enzyme has also been shown to catalyse the conversion of androgen sulfate (e.g., androstenediol sulfate) to the non-sulfated androgen (Fig. 1).

A number of aminosulfonate derivatives of both steroidal and non-steroidal inhibitors has been investigated (the sulfamate moiety is believed to be involved in the irreversible inhibition of ES). An example of a potent steroidal inhibitor is estrone-3-*O*-sulfamate (EMATE)[2]—a time and concentration dependent irreversible inhibitor. However, this compound has been shown to possess potent estrogenic properties, and as a result, the investigation into non-steroidal inhibitors has intensified. This search has resulted in the coumarin derived compound, namely, 4-methylcoumarin-7-*O*-sulfamate

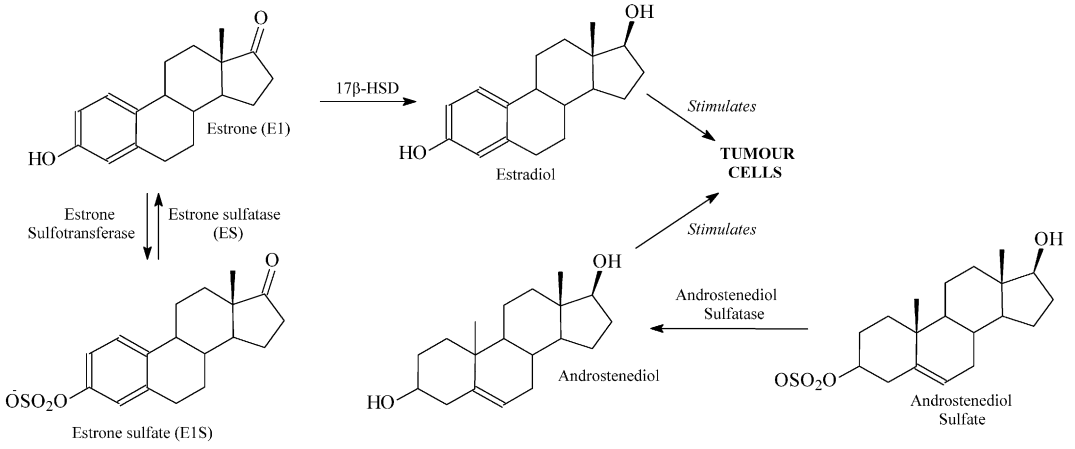


Figure 1. Action of the enzyme ES on estrone sulfate and androstenediol sulfate.

* Corresponding author. Tel.: +44-20-8547-2000; fax: +44-20-8547-7562; e-mail: s.ahmed@kingston.ac.uk

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.11.067

(COUMATE) and its derivatives, such as 667- and 669-COUMATE (Fig. 2).

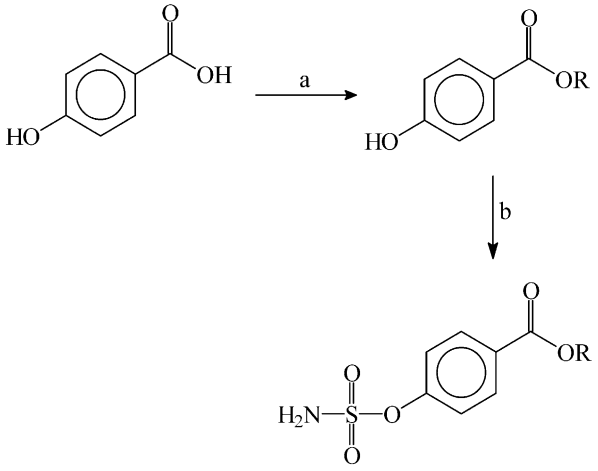
We have previously shown that the requirement of the phenyl group within both the steroidal and, in particular, the non-steroidal inhibitors, is necessary for the cleavage of the S–O–Ar bond resulting in the formation of the phenoxide ion[3–5] and sulfamic acid (the latter is therefore the inhibiting moiety which results in the formation of an irreversibly bound imine species at the active site of ES[6,7]). The requirement of logP and the nature of the role of this physicochemical property in the inhibition of ES has also been rationalised by us[6] and from the consideration of the potential mechanism for the de-sulfatation reaction catalysed by ES,[7] we proposed that the role of logP was to aid the exit of the carbon backbone from the active site.

Here, we report the initial results of a study in an attempt to consider the contribution of size (and therefore steric hindrance) to the overall inhibitory activity of ES inhibitors. We have therefore undertaken: the synthesis of a number of straight chain and cyclic esters of 4-sulfamated benzoic acid; biochemical evaluation (including the mode of action, i.e., reversible or irreversible); the determination of physicochemical properties (such as logP, pK_a, as well as the size of the overall molecule), and the rationalisation of the inhibitory activity involving the superimposing of the inhibitors onto the estrone sulfate backbone of the transition-state of the reaction catalysed by ES.

In the synthesis of the 4-aminosulfonated derivatives of benzoic acid, modified literature procedure[7] (Scheme 1) was followed and was found to proceed well and in good yield without any major problems. The syntheses of methyl 4-hydroxybenzoate (**1**)[8] and methyl 4-[(aminosulfonyl)oxy]benzoate (**2**)[9] are described as examples. The synthesised compounds were then evaluated for ES inhibition using standard literature method to determine the initial screening inhibition and IC_{50} values,[10] whilst the mode of action was determined using a method involving dialysis of bound/unbound inhibitor.[11]

In the calculation of the logP (Table 1) of the aminosulfonated compounds, we discovered that very little was known about the contribution of the sulfamate group towards the overall logP of the molecule. In an effort to simplify our logP calculations and therefore remove any potential problems with the calculated values, we used the parent *non-sulfamated* compounds as a guide to the determination of the potential optimum logP (using Projectleader^{TM}). The determination of the pK_a (Table 1) of the starting phenols involved a spectroscopic technique[12] that considered the change in UV absorption by the phenolic group under: acidic (pH 2); pH9, and; basic (pH 11) conditions.

Experimentally determined IC_{50} and pK_a values are summarised in Table 1 together with the calculated logP of the non-sulfamated starting phenol. From an initial consideration of the inhibitory activity, we observe that the compounds synthesised possess potent inhibitory activity, for example, heptyl 4-aminosulfonyl benzoate (**14**) is the most potent straight chain containing compound possessing greater inhibitory activity than COUMATE but lower inhibitory activity than EMATE and 667-COUMATE. Cyclooctyl 4-aminosulfonyl benzoate (**28**) is found to be the most potent cyclic ester based compound and is more potent than 667-COUMATE, which is currently undergoing clinical trials. The



Scheme 1. Synthesis of the 4-*O*-sulfamate derivative of the alkyl and cycloalkyl esters of benzoic acid [a = ROH/Δ/toluene; b = NaH/H_2NSO_2Cl/DMF or DMA] (R = Me to Dec or cyclobutyl to cyclooctyl).

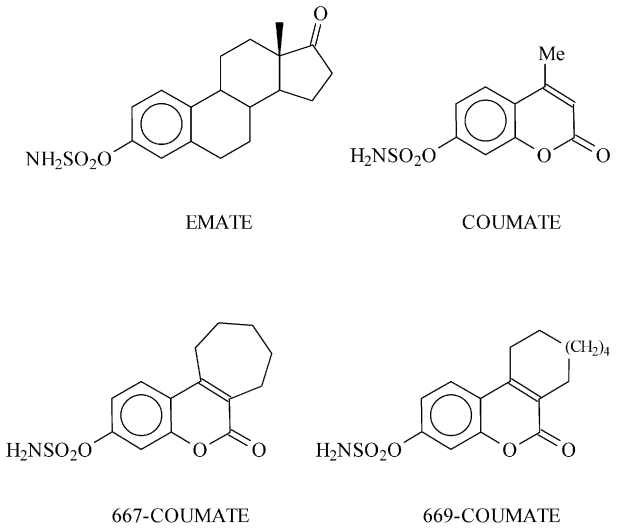


Figure 2. Structures of EMATE, COUMATE and two tricyclic derivatives of COUMATE.

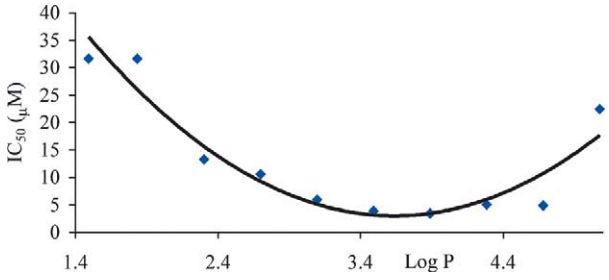


Figure 3. Plot of IC_{50} versus logP of the synthesised straight chain containing compounds.

synthesised compounds were found to block ES in an irreversible manner similar to EMATE, COUMATE and 667-COUMATE.

A more detailed consideration of pK_a and IC_{50} data from the current study shows that within the series of compounds synthesised in this study, the pK_a of all of the benzoic acid based compounds fall within a relatively narrow range, that is, the values range from approximately 8.0–8.9 and that there is no correlation between IC_{50} and pK_a within this range of compounds.

Consideration of the logP (for the parent phenolic compound) and IC_{50} data shows that a good correlation exists between these two parameters (Fig. 3), as such, within the series of compounds based on the straight chain esters, an 'optimum' logP value is observed between 3.5 and 4.3. It is interesting to note that the calculated logP of the carbon backbone of a number of the known potent inhibitors of ES is also close to the optimum observed within the current study, for example, EMATE and 669-COUMATE are calculated to possess logP values of 3.8 and 3.4, respectively.

In an effort to rationalise the inhibitory activity of the straight chain containing esters and the cyclic moiety containing esters, we superimposed compounds **14** and **28** (the most potent compound from each range) onto the transition-state for the reaction catalysed by ES (Fig. 4) — the derivation and use of the transition-state

Table 1. Showing the calculated logP and the pK_a of the non-sulfamated derivatives, the inhibitory activity and the mean IC_{50} values ($n = 3$) of the synthesised compound

Compd	R	logP	pK_a	Initial screening (% inhibition)	IC_{50}/μM
2	CH_3	1.49	8.28±0.07	25.4[a]	31.6±1.23
4	C_2H_5	1.84	8.22±0.09	25.6[a]	31.6±1.95
6	C_3H_7	2.30	8.03±0.11	42.6[a]	13.2±0.4
8	C_4H_9	2.70	8.07±0.09	48.3[a]	10.5±0.28
10	C_5H_{11}	3.10	8.47±0.13	64.4[a]	5.9±0.44
12	C_6H_{13}	3.49	8.52±0.15	72.6[a]	3.8±0.16
14	C_7H_{15}	3.89	8.27±0.10	69.5[a]	3.4±0.25
16	C_8H_{17}	4.29	8.09±0.13	63.0[a]	5.0±0.26
18	C_9H_{19}	4.68	8.39±0.09	76.2[a]	4.8±0.17
20	$C_{10}H_{21}$	5.08	8.09±0.13	36.5[a]	22.4±0.48
22	$CycloC_5H_9$	2.68	8.73±0.02	62[b]	9.3±0.07
24	$CycloC_6H_{11}$	3.08	8.81±0.10	84[b]	1.7±0.07
26	$CycloC_7H_{13}$	3.47	8.88±0.08	87[b]	0.5±0.028
28	$CycloC_8H_{15}$	3.87	8.09±0.01	82[b]	0.17±0.007
EMATE	—	3.80	8.37±0.05	68.6[a]	0.5±0.001
COUMATE	—	1.60	8.00±0.14	47.6[c]	13.8±0.07
667-COUMATE	—	2.65	8.27±0.05	56[d]	0.21±0.01

[a] [I] of 10 μM.
[b] [I] of 20 μM.
[c] [I] of 12 μM.
[d] [I] of 0.25 μM.

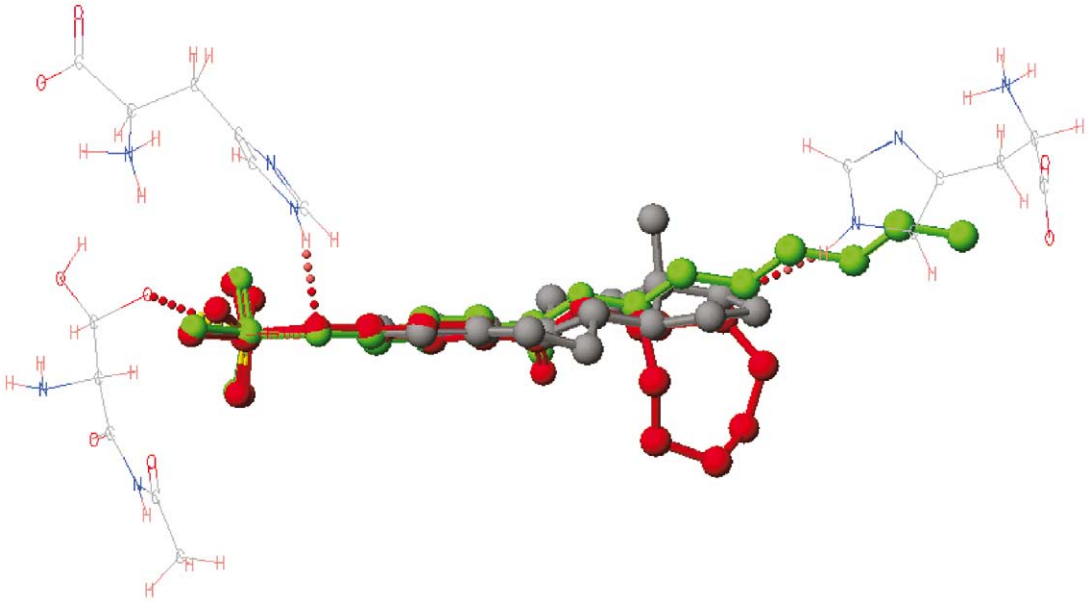


Figure 4. Superimpositioning of compounds **14** (in green) and **28** (in red) onto the transition-state of the reaction catalysed by ES (the hydrogen bonding groups are shown as lines and the estrone sulfate backbone in grey).

(TS) in the rationalisation of the inhibitory activity of a range of inhibitors of ES has been previously reported by us and as such will not be discussed here in detail.[13] In summary, the construction of the probable TS involved in the construction of the residues which have been proposed to exist at the active site within the CaChe[14] molecular modelling software on an IBM PC compatible microcomputer. The completed structures were then refined performing a pre-optimisation calculation, followed by a geometry optimisation. The oxygen atom of the formylglycine residue was then attached to the sulfonate group of estrone sulfate via a weak bond whilst another hydrogen atom from a histadine residue was attached to the phenolate oxygen atom of the substrate. The saddle point for the reaction was then calculated and the resulting TS structure was further refined by performing a minimise gradient calculation. The molecule's vibrational transitions were calculated in order to 'verify' the transition-state.

From the modelling study, in particular, the superimpositioning of the most potent cycloalkyl and alkyl inhibitors onto the steroid backbone within the TS, we can clearly observe that the larger straight chain containing compounds [such as **14** (shown in green)] are able to undergo steric interaction with the area of the active site which would normally undergo hydrogen bonding with the estrone sulfate C(17) = O group. From the superimpositioning of **28** [Fig. 4 (shown in red)], we observe that the cyclooctyl moiety is far removed from the hydrogen bonding group and therefore does not undergo steric interactions with the protein backbone of the active site. The enzyme–inhibitor complex for the cyclic derivatives therefore appear to be more stable than for the straight chain containing compounds, resulting in the latter compounds possessing less potent inhibitory activity.

In conclusion, the results of the present study show that whilst hydrophobicity is an important factor in determining the overall inhibitory activity of the sulfamate based inhibitors of ES, the overall size of the molecule is also important and that the combination of pK_a, logP and overall inhibitor length can result in the production of highly potent inhibitors such as **28**.

Acknowledgements

The high resolution mass spectra were undertaken by the EPSRC National Mass Spectrometry centre at the University of Wales College Swansea and the elemental analysis were undertaken at the School of Pharmacy, University College London.

References and notes

1. Feutrie, M. L.; Bonneterre, J. *Bull. Cancer* **1999**, *86*, 821.
2. Woo, L. W. L.; Howarth, N. M.; Purohit, A.; Hejaz, H. A. M.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1998**, *41*, 1068.
3. Ahmed, S.; James, K.; Owen, C. P.; Patel, C. K.; Patel, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 899.
4. Ahmed, S.; James, K.; Patel, C. K. *Biochem. Biophys. Res. Commun.* **2000**, *272*, 583.
5. Owen, C. P.; James, K.; Sampson, L.; Ahmed, S. *J. Pharm. Pharmacol.* **2003**, *55*, 85.
6. Ahmed, S.; James, K.; Owen, C. P.; Patel, C. K.; Patel, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2525.
7. Ahmed, S.; James, K.; Owen, C. P. *J. Steroid Biochem. Mol. Biol.* **2002**, *82*, 425.
8. Methyl-4-hydroxybenzoate (**1**): Concd H_2SO_4 (3 mL) was added to a suspension of 4-hydroxy benzoic acid (3 g, 21.74 mmol) in methanol (20 mL) and the solution refluxed for 1 h. After cooling to room temperature, NaOH (~15 mL) was added to neutralise 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. The white precipitate 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)]. $\nu_{(max.)}$ (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^+).
9. Methyl 4-[(aminosulfonyl)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) (20 mL) under an atmosphere of nitrogen gas at 0 °C. After evolution of hydrogen had ceased (after 30 min), aminosulfonyl 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 sulfate ($MgSO_4$). The mixture was filtered and the solvent removed under vacuum to give a yellow oil which was purified using flash chromatography to give **2** (0.24 g, 31.6%) as a pure white solid [mp 118–121 °C; R_f 0.24 diethyl ether/petroleum ether 40–60 °C (50/50)]. $\nu_{(max)}$ (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.
10. *ES assay*: The total assay volume was 1 mL. ^{3}H-estrone sulfate (25 μL, 20 μM/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 μg/mL). The microsomes and assay tubes were pre-incubated for 5 min at 37 °C in a shaking water bath prior to 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 rpm, 10 min). 1 mL of toluene was removed and added to 5 mL scintillation cocktail (TRITONX). The aliquots 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. It should be noted that EMATE and COUMATE were synthesised within our laboratories using published

procedures and used within our assays as the standard compounds.

11. *Irreversible ES assay*: The irreversible inhibition was determined using EMATE (10 μM), COUMATE (100 μM), **12** (700 μM) and **14** (700 μM). Placental microsomes (18 mg/mL, 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). An aliquot (100 μL) in triplicate, was taken from each sample and tested for ES activity using the procedure above, except that 900 μL of Tris–HCl buffer was added to the assay tubes. A second aliquot (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 ES activity as described above.
12. Harwood, L. M.; Moody C. J. In *Experimental Organic Chemistry*; Blackwell, 1989; p 716.
13. Ahmed, S.; James, K.; Owen, C. P.; Patel, C. K.; Patel, M. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 3001.
14. CaChe is a trademark of Oxford Molecular Ltd, Oxford Science Park, Oxford, UK.