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Design, Synthesis and Biochemical Evaluation of AC Ring Mimics as Novel Inhibitors of the Enzyme Estrone Sulfatase (ES)

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Abstract—We report the initial results of our study into a series of simple 4′-*O*-sulfamoyl-4-biphenyl based compounds as novel inhibitors of the enzyme estrone sulfatase (ES). The results of the study show that these compounds are potent inhibitors, possessing greater inhibitory activity than COUMATE, but weaker inhibitory activity than EMATE or the tricyclic derivative of COUMATE, namely 667-COUMATE. Furthermore, the compounds are observed to be irreversible inhibitors.

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 P-450 enzyme aromatase (AR).[1] However, the use of AR inhibitors does not result in the inhibition of all of the biosynthetic processes which lead to estrogen formation. That is, the enzyme estrone sulfatase (ES) converts the stored (sulfated) form of the estrogens to the active (non-sulfated) forms (Fig. 1), thereby allowing the stimulation of tumours via a non-AR pathway (which, in general, is not blocked by AR inhibitors).

A number of steroidal and non-steroidal sulfamate containing compounds[2] have been investigated as potent inhibitors of this enzyme, including estrone-3-*O*-sulfamate (EMATE) ($IC_{50} = 65$ pM) (a time- and concentration-dependent irreversible steroidal inhibitor) and 4-methylcoumarin-7-*O*-sulfamate (COUMATE) (an irreversible non-steroidal inhibitor) (Fig. 2). COUMATE has been further derivatised since it has been shown to lack significant levels of estrogenicity, and a series of tricyclic compounds such as 667-, 668- and 669-COUMATE[3] (Fig. 2 shows 667-COUMATE) has resulted. In general, the sulfamate moiety is believed to be involved in the irreversible inhibition of ES.

In an effort to overcome the lack of detailed information regarding the active site of ES, we initiated a series of structure–activity relationship (SAR) determination studies.[4–5–6] From the results of our initial molecular modelling study and a review of potential mechanisms for ES, we concluded that compounds such as EMATE (and other steroidal compounds) are not able to undergo interaction via the C(17) carbonyl group due to a lack of hydrogen bonding group(s) in this area. We therefore undertook a design process so as to incorporate the results of our SAR and molecular modelling studies (including the derivation of a potential transition-state for the reaction catalysed by ES). As a result, we concluded that sulfamated biphenyl compounds may be an important set of compounds which would allow us to mimic the A and C rings of the steroid backbone whilst the functional group R (Figs. 3 and 4) may undergo hydrogen bonding with any potential H-bonding group(s) within the active site of ES—the biphenyl moiety has previously been extensively studied as a steroid mimic.[7] Here, we report the initial results of our study where we have undertaken the design and synthesis of a number of derivatives of 4′-*O*-sulfamoyl-4-biphenyl, and the in vitro biochemical evaluation of the synthesised compounds in an effort to study any potential hydrogen bonding interaction about the area corresponding to the steroidal C(17) = O. The compounds were also evaluated to determine their mode of action, i.e., reversible or irreversible.

In the sulfamoylation of derivatives of 4′-hydroxy-4-biphenyl, modified literature procedure[8] (Scheme 1) was followed and was found to proceed well and in good yield without any major problems. In the synthesis of the ester derivatives of 4′-hydroxy-4-biphenyl carboxyl-

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ate, the carboxylic acid was refluxed with the appropriate alcohol, and in general, the reactions proceeded well and in good yield. However, for esters containing alkyl chains greater than butyl, the esterification step resulted in extremely poor yields. Furthermore, the sulfamoylation (for esters containing carbon chains greater than butyl) was found to be difficult and, in general, did not give the desired compound, presumably due to steric factors. The syntheses of 1,1′-biphenyl-4-yl sulfamate[9] and the starting ethyl-4′-hydroxy-4-biphenyl carboxylate[10] (**5**) (from 4′-hydroxy-4-biphenyl) and ethyl-4′-*O*-sulfamoyl-4-biphenyl carboxylate[11] (**6**) are given as examples.

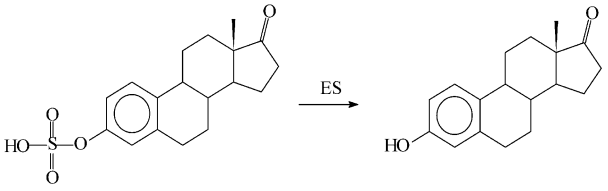


Figure 1. Action of the enzyme ES on estrone sulfate.

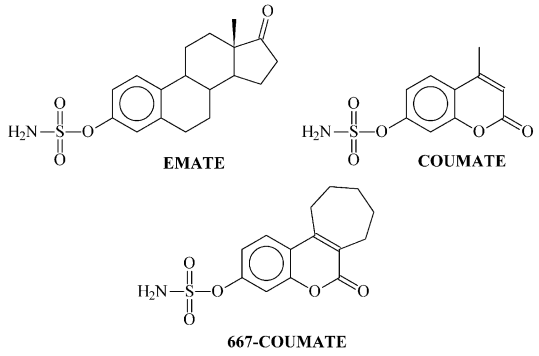


Figure 2. Potent inhibitors of ES.

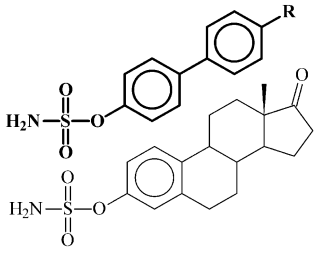


Figure 3. Diagram to show the rationale for the synthesis of biphenyl sulfamates.

The synthesised compounds were then evaluated for inhibitory activity against ES using standard literature method so as to determine the initial screening inhibition and IC_{50} values,[12] whilst the mode of action (reversible or irreversible inhibition) was determined using a method involving dialysis of bound/unbound inhibitor.[13] The results of the biochemical evaluation are shown in Table 1, together with the calculated logP of the parent phenolic structure.

Consideration of the inhibitory data (Table 1) shows that all of the compounds are weaker than EMATE and the recently reported tri-cyclic derivative of COUMATE, namely, 667-COUMATE. However, with the exception of **1**, all of the compounds within the current study possess more potent inhibitory activity when compared to COUMATE. Indeed, compound **8** is ~3 times more potent than this coumarin based compound. Furthermore, the determination of the mode of action of all of the synthesised compounds showed them to be time-dependent irreversible inhibitors of ES, i.e., the compounds were found to bind irreversibly to the ES active site and could not be dialysed with time.

From the consideration of the inhibition data of the biphenyl based compounds considered within the present study, we are able to conclude that these compounds may be able to undergo polar–polar or hydrogen bonding interactions with the active site. That is, the R group (Fig. 3) may indeed be able to undergo favourable interaction which results in an increase in the binding ability of these compounds compared to COUMATE, resulting in good inhibitory activity. As such, the current study suggests that hydrogen bonding groups may exist at the active site correponding to the C(17) area of the steroid backbone. Since the compounds were designed using the derived transition-state

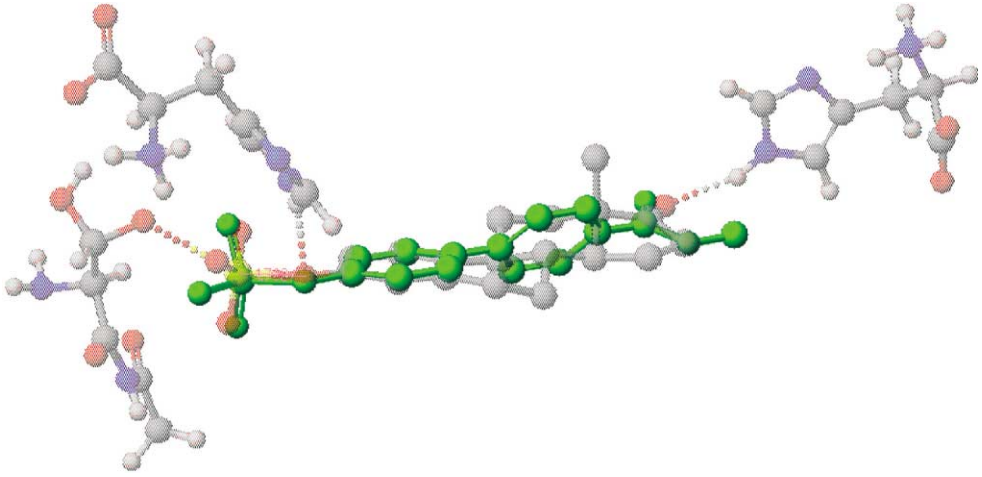
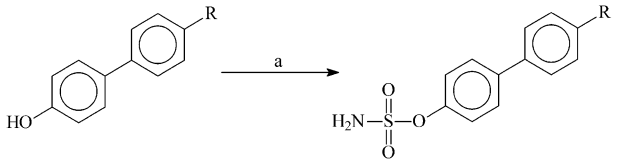


Figure 4. Superimposition of one of the potential inhibitors (in green) on the derived transition-state for the reaction catalysed by ES.

Table 1. The inhibition data for the synthesised compounds, EMATE COUMATE and 667-COUMATE (logP was calculated for the parent non-sulfamated phenol) (a = [I] of 5 μM; b = [I] of 10 μM; c = [I] of 0.01 μM)

Compd (R =)	Compd number	% Inhibition	IC_{50} (μM/tube)	LogP
H	**1**	5.5[a]	76.1 ± 0.9	3.447
CN	**2**	44.2[a]	6.7 ± 0.01	3.483
COOMe	**4**	44.2[a]	5.2 ± 0.07	3.177
COOEt	**6**	53.4[a]	4.2 ± 0.01	3.519
COOPr	**8**	60.5[a]	3.5 ± 0.01	3.988
COOBu	**10**	48.4[a]	5.8 ± 0.02	4.384
COUMATE	—	47.6[b]	10 ± 0.3	1.698
667-COUMATE	—	86.8[a]	0.21 ± 0.01	2.651
EMATE	—	16.4[d]	0.1 ± 0.01	3.870



Scheme 1. The sulfamoylation of 4′-*O*-sulfamoyl-4-biphenyl (a = NaH; H_2NSO_2Cl; anhydrous toluene) (R = H, −CN, −COOR′ where R′ is an alkyl group).

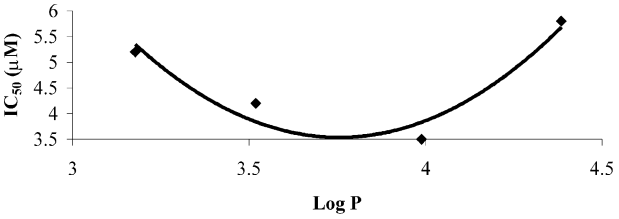


Figure 5. Plot of IC_{50} versus logP to show the relationship between the two parameters.

of the desulfatation reaction, the results of our study add further support to the accuracy of the derived transition-state.

A detailed consideration of the physicochemical properties of these compounds, in particular, the alkyl esters, shows that within the ester derivatives, there appears to be a good correlation between logP and inhibitory activity (IC_{50} value) (Fig. 5). Further detailed consideration of Figure 5 shows that the 'optimum' logP appears to be at approximately 3.7. We have previously rationalised[14] the requirement of the hydrophobic requirement as being an important factor in the determination of the inhibitory activity of compounds against ES. That is, through the consideration of the SAR determination study of a large number of alkyl and phenyl sulfamate based compounds, we proposed that the hydrophobic nature of the carbon backbone of the inhibitors is necessary to destablise the phenoxide ion resulting from the desulfatation reaction, which results in the loss of the carbon backbone of the inhibitor (phenoxide ion containing moiety) from the active site.[14] From our studies, we have also hypothesised that compounds which mimicked the estrone backbone with respect to hydrophobicity (calculated logP of estrone is 3.8) would possess good inhibition (assuming the pK_a factor was in the range of 7.7 to 8.2). As can be observed, the optimum logP within the present study is similar to that of the phenoxide ion of estrone, as such, the results of this study therefore add further support to our previous work.

In conclusion, we have successfully designed and synthesised a range of compounds so as to mimic the steroid backbone of estrone. Furthermore, from the consideration of the inhibitory data, we have been able to propose the existence of a potential hydrogen bonding group at an area corresponding to the C(17) area of the steroid backbone and have added further support to the accuracy of the derived transition-state (for the desulfatation reaction catalysed by ES). These compounds therefore are good lead compounds in the mimicking of the steroid backbone and thus allow us the opportunity to derivatise the biphenyl structure in order to increase potency.

Acknowledgements

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9. **1,1′Biphenyl-4-yl sulfamate (1):** Sodium hydride (NaH) (60% dispersion in mineral oil, 0.15 g, 3.75 mmol) was added to a stirred solution of 4-phenylphenol (0.5 g, 2.94 mmol) in dimethyl formamide (DMF) (20 mL) under an atmosphere of nitrogen gas at 0 °C. After evolution of hydrogen had ceased, aminosulfonyl chloride in toluene (20 mL, ~20 mmol) was added after 30 min 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 (**1**) (0.12 g, 16.0%) as a pure white solid [mp 146–149 °C (literature 165 °C, Hedayatullah, 1975); R_f 0.31 diethyl ether/petroleum ether 40–60 °C (50/50)]. ν_{max} (Film) cm^{-1}: 3421.3, 3301.9 (NH), 1382.0, 1177.9 (S=O). δ_H ($CDCl_3$) 7.85–7.15 (9H, m, ArH), 7.12 (2H, s, NH_2). δ_C ($CDCl_3$): 1129.603, 128.734, 127.529, 123.402 (CAr). MS *m/z* found: MNH_4^+ 267.0798, $(C_{12}H_{11}NO_3S)NH_4^+$ requires 267.0803.

10. **Ethyl-(4′-hydroxy)-1,1′-biphenyl-4-carboxylate (5):** Conc. H_2SO_4 (1 mL) was added to a suspension of 4-hydroxybiphenyl carboxylic acid (0.5 g, 2.34 mmol) in ethanol (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 (**5**) (0.46 g, 81.23%) as a white solid [mp 141–143 °C; R_f 0.63 diethyl ether/petroleum ether 40–60 °C (70/30)]. ν_{max} (Film) cm^{-1}: 3335.9 (OH), 1681.4 (C=O). δ_H ($CDCl_3$): 8.00 (2H, d, J=8 Hz, ArH), 7.60 (2H, d, J=8 Hz, ArH), 7.53 (2H, d, J=8 Hz, ArH), 6.95 (2H, d, J=8 Hz, ArH), 5.81 (1H, s, OH), 4.41 (2H, q, J=7 Hz, $\underline{CH_2}CH_3$), 1.42 (3H, t, J=7 Hz, $CH_2\underline{CH_3}$). δ_C ($CDCl_3$): 167.2 (C=0), 157.3, 145.6, 132.5, 130.1, 128.6, 128.4, 126.4, 115.9 (CAr), 61.1 ($O\underline{CH_2}CH_3$), 14.3 ($OCH_2\underline{CH_3}$). GCMS t_R 19.223 *m/z* 242 (M^+).

11. **Ethyl-4′-[(aminosulfonyl)oxy]-1,1′-biphenyl-4-carboxylate (6):** Compound **6** was synthesised following the same procedure as for compound **1** except that NaH (60% dispersion in mineral oil, 0.05 g, 1.25 mmol) was added to a stirred solution of **60** (0.2 g, 0.83 mmol) in DMF (10 mL). Aminosulfonyl chloride in toluene (10 mL, ~10 mmol) was added after 30 min. Removal of the solvent under vacuum produced a solid, which was purified using flash chromatography to give **6** (0.08 g, 30.0%) as a pure white solid [mp 171.2–173.5 °C; R_f 0.30 petroleum ether 40–60 °C: ethyl acetate (65/35)]. ν_{max} (Film) cm^{-1}: 3343.7, 3222.2 (NH), 1693.9 (C=O) 1401.2, 1162.5 (S=O). δ_H ($CDCl_3$): 8.12 (2H, d, J=9 Hz, ArH), 7.80 (2H, d, J=9 Hz, ArH), 7.79 (2H, d, J=9 Hz, ArH), 7.44 (2H, d, J=9 Hz, ArH), 5.03 (2H, s, NH_2), 4.41 (2H, q, J=7 Hz, CH_2–), 1.42 (3H, t, J=7 Hz, $CH_2\underline{CH_3}$). δ_c (d_6-Acetone): 199.7 (C=O), 154.2, 135.0, 131.4, 130.3, 122.7, 115.2 (C-Ar), 32.0 (OCH_2), 8.2 (CH_3). MS *m/z* 321 (M^+).

12. **ES assay:** The total assay volume was 1 mL. ^{3}H-estrone sulfate (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 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.

13. **Irreversible ES assay:** The irreversible inhibition was determined using EMATE (10 μM), COUMATE (100 μM), **6** (700 μM) and **8** (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.

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