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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-sulfamated phenyl alkyl ketones as potential inhibitors of the enzyme estrone sulfatase. The results of the study show that these compounds are potent inhibitors, possessing greater inhibitory activity than COUMATE, but weaker activity than EMATE. Furthermore, the compounds are observed to be irreversible inhibitors. © 2001 Elsevier Science Ltd. All rights reserved.

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).[1,2] 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) form (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 inhibitors[3,4] have been investigated as potent inhibitors of this enzyme, including EMATE (a time- and concentration-dependent irreversible steroidal inhibitor) and COUMATE (an irreversible non-steroidal inhibitor) (Fig. 2). However, since EMATE has been shown to possess potent estrogenic properties, the investigation into non-steroidal inhibitors has intensified.

In general, the potent inhibitors contain an aminosulfonate moiety which is believed to be involved in the irreversible inhibition of ES. From the consideration of the results obtained with the known sulfamate 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 sulfamate group.[5] We have recently shown that the stabilisation of the phenoxide ion[6] and that the incorporation of electron-withdrawing groups within the phenyl ring can result in a significant increase in the inhibitory activity of compounds, thus alkyl sulfamates are poorer acids (in comparison to phenols) and are therefore weaker inhibitors of ES.[6]

In an effort to: overcome the lack of detailed information regarding the active site of ES; probe the nature of the proposed pharmacophore; and rationalise the inhibitory activity of the aminosulfonate based compounds, we initiated a series of SAR determination studies. From the results of our initial molecular modelling

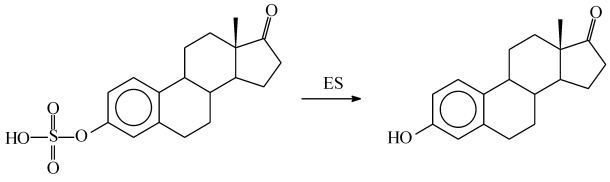

Figure 1. Action of the enzyme ES on estrone sulfate.

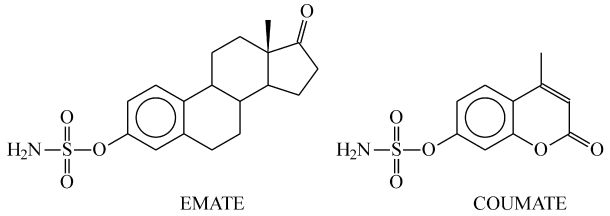

Figure 2. Structures of EMATE and COUMATE.

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study and a consideration of potential mechanisms for ES (Fig. 3), we concluded that logP may also be an important factor in the inhibition process. That is, we concluded that the role of the carbon backbone is to favour the formation of the RO^- ion (thus the requirement for the phenolic moeity) *and* the expulsion of the RO^- due to the high logP of carbon backbone within RO^-. We also hypothesised that as a result of the high logP requirement, the reaction catalysed by ES 'appears' to be an irreversible reaction.

With respect to the requirement of pK_a in the irreversible inhibition of ES,[6] we hypothesised that the sulfamic acid produced is the inhibitory moeity and that the carbon backbone aids the synthesis of sulfamic acid. That is, we took the above mechanism and postulated an inhibition mechanism involving the sulfamoyl moiety—that is, an attack by the NH_2 of sulfamic acid produced as a result of the desulfatation reaction results in the production of an imine type product (Fig. 4). We have recently shown that the lack of production of sulfamic acid results in the lack of irreversible inhibition—alkyl sulfamates in partcular show this trend.

In order to verify our hypothesis with respect to the importance of logP, we undertook a design process so as to incorporate the increasing logP whilst restricting the pK_a of the parent phenol. We concluded that sulfamated phenyl ketones would possess the appropriate characteristics. Here, we report the initial results of our study where we have undertaken: the synthesis of derivatives of 4-sulfamated phenyl ketones; the in vitro biochemical evaluation of the synthesised compounds; and the evaluation of the mode of action, i.e., reversible or irreversible inhibition.

Chemistry

In the synthesis of the 4-aminosulfonated derivatives of 4-hydroxyphenyl ketones, modified literature procedure[5,7] (Scheme 1) was followed and was found to proceed well and in good yield without any major problems. The synthesis of 4-nonanoylphenyl sulfamate is given as an example—it should be noted that since 4-hydroxynonanophenone is commercially available, it was not synthesised via the initial Friedel–Crafts acylation reaction.

4-Nonanoylphenyl sulfamate (7)

Sodium hydride (NaH) (60% dispersion in mineral oil, 0.18 g, 4.5 mmol) was added to a stirred solution of 4-hydroxynonophenone (1 g, 4.27 mmol) in dimethyl formamide (DMF) (10 mL) under an atmosphere of

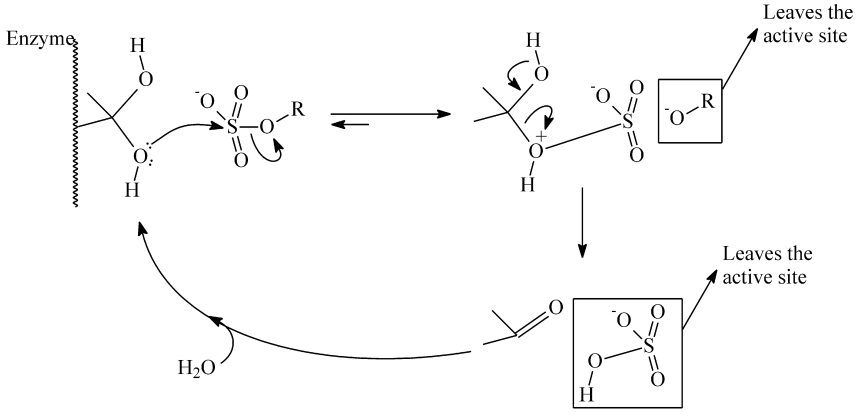


Figure 3. Mechanism of action of ES on estrone sulfate.

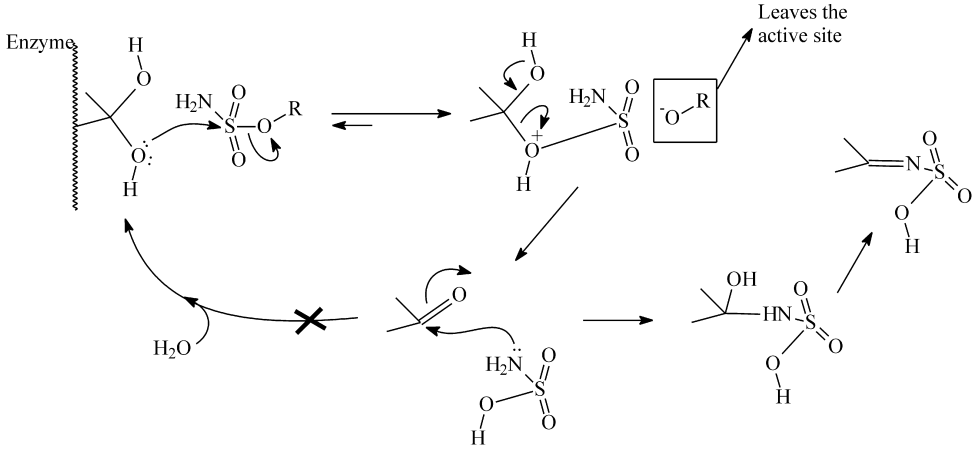
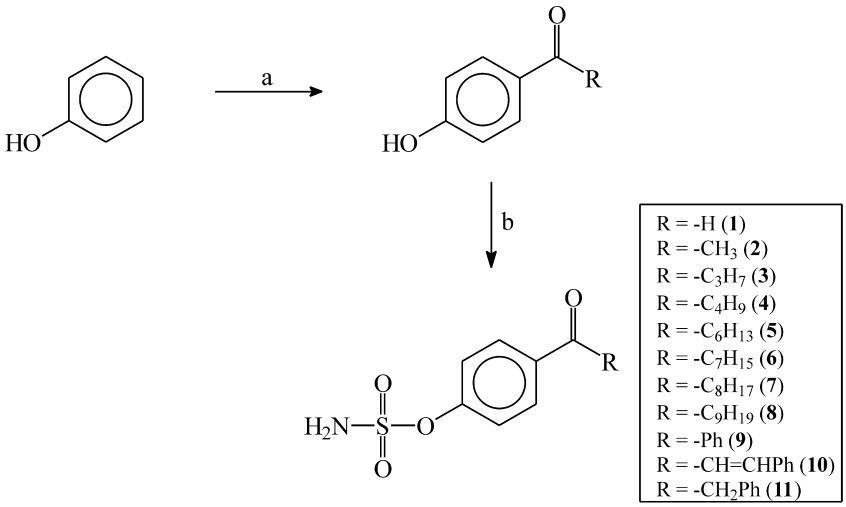


Figure 4. Mechanism for the irreversible inhibition of ES.



Scheme 1. Synthesis of the 4-sulfamate derivative of the substituted benzoic acid (a = acid chloride/$AlCl_3$/DCM; b = NaH/H_2NSO_2Cl/toluene).

nitrogen gas at 0 °C. After evolution of hydrogen had ceased, 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 solidified on addition of water. The crude product was purified using flash chromatography to give (**7**) (0.32 g, 23.9%) as a white solid (mp 102–104 °C; R_f 0.57 ether/petroleum ether 40–60 °C (70:30)).

$\nu_{(max)}$ (Film) cm^{-1}: 3389.0, 3289.0 (NH_2), 1682.3 (C=O), 1377.9, 1181.8 (S=O). δ_H ($CDCl_3$): 7.99 (2H, d, J = 9 Hz, ArH), 7.42 (2H, d, J = 9 Hz, ArH), 5.17 (2H, s, NH_2), 2.94 (2H, t, J = 7 Hz, $COC\underline{H}_2CH_2$), 1.72 (2H, m, $COCH_2\underline{CH}_2CH_2$), 1.35 (10H, m, $COCH_2CH_2[\underline{CH}_2]_5$-$CH_3$), 0.88 (3H, t, J = 7 Hz, -$C\underline{H}_3$). δ_C (d_6-Acetone): 154.6 (C=O), 138.3, 136.1, 130.4, 122.8 (C-Ar) 38.8, 32.3, 29.7, 23.0 (CH_2), 14.1 (CH_3). MS m/z obtained MH^+ 314.1422, $(C_{15}H_{23}NO_4S)H^+$ requires 314.1426.

ES assay

In the biochemical evaluation, the standard literature method was used.[8] The total assay volume was 1 mL. ^{3}H-estrone sulfate (25 μL, 50 μM/tube; 750,000 dpm) and the inhibitors (various concentrations) 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.

Irreversible ES assay

The irreversible inhibition was determined using the procedure described by Purohit et al. (1998)[9] using EMATE (10 μM), COUMATE (100 μM) and sulfamated phenyl ketones (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, pH7.2, 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.

The results of the biochemical evaluation of the synthesised sulfamated phenyl ketones (as well as EMATE and COUMATE within our assays for comparison) are shown in Table 1 together with the relative potencies against the latter two compounds. Consideration of the results show that these compounds are potent inhibitors of ES, with compound **7** being only 6.8 times weaker than EMATE. In comparison to COUMATE, we observe that a number of the synthesised compounds are equipotent or are much stronger inhibitors than COUMATE, indeed compounds 5, 6 and 7 are 2.4, 2.1 and 3.5 times more potent, respectively. These three compounds would therefore appear to be some of the most potent non-steroidal compounds known to date. The synthesised compounds were further evaluated to determine their mode of action. It was discovered that

Table 1. Inhibition data for compounds **1**–**11** and the relative potencies of some of the synthesised compounds in comparison to EMATE and COUMATE

Compound	R	IC_{50} (μM)	Potency w.r.t EMATE	Potency w.r.t COUMATE
1	H	254±10.1	0.0019	0.0472
2	CH_3	302±6.7	0.0016	0.0397
3	C_3H_7	39.8±1.4	0.0126	0.3015
4	C_4H_9	20.9±0.38	0.0239	0.5742
5	C_6H_{13}	5.0±0.36	0.1000	2.4000
6	C_7H_{15}	5.6±0.19	0.0893	2.1429
7	C_8H_{17}	3.4±0.13	0.1471	3.5294
8	C_9H_{19}	13±0.05	0.0385	0.9231
9	Ph	63±1.83	0.0079	0.1905
10	CH=CHPh	263±5.5	0.0019	0.0456
11	CH_2Ph	33±1.1	0.0152	0.3636
EMATE	—	0.5±0.001	1	24
COUMATE	—	12±0.16	0.0417	1

the enzyme did not recover after the incubation with the synthesised compounds, i.e., the compounds are irreversible inhibitors of ES.

From the consideration of the results within Table 1, it would appear that an optimum chain length appears to be C_6 to C_8. Furthermore, the data supports our hypothesis that the carbon backbone of the inhibitors plays a crucial role in the mechanism of inhibition by sulfamated compounds, which are then able to release sulfamic acid as the inhibiting moiety involving the attack of an aldehydic group by the NH_2 group at the active site (as proposed in Fig. 4).

In conclusion, we have synthesised a range of sulfamated phenyl ketones, which have proved to be some of the most potent non-steroidal compounds known to date, only the recently reported 667-COUMATE is known to be of greater potency. The compounds are therefore good lead compounds in the search for potent non-steroidal inhibitors and have allowed us further insight into the structural features required for increased inhibitory activity.

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