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The design, synthesis, and biochemical evaluation of derivatives of biphenyl sulfamate-based compounds as novel inhibitors of estrone sulfatase

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Abstract

We report the initial results of our study into the use of a potential transition state (TS) of the reaction catalyzed by the enzyme estrone sulfatase (ES) in the design of a series of simple 4′-*O*-sulfamoyl-4-biphenyl-based compounds as novel inhibitors of ES. The results of the study show that these compounds are: potent inhibitors, possessing greater inhibitory activity than 4-methylcoumarin-7-*O*-sulfamate (COUMATE); weaker inhibitors than the tricyclic derivative of COUMATE, namely 667-COUMATE and the steroidal inhibitor estrone-3-*O*-sulfamate (EMATE), and irreversible inhibitors of ES.

In the treatment of hormone-dependent breast cancer, extensive research has been undertaken to produce compounds that are both potent and selective inhibitors of the enzyme aromatase (AR) [1]. The enzyme estrone sulfatase (ES) provides an alternative route to estrogens, that is, ES converts the stored form of the estrogens to the active forms (Fig. 1) thereby allowing stimulation of tumors via a non-AR pathway.

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 derivatized 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 [4–6]. 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 [R–H, –CN, –COOR′ (Fig. 3)], may undergo hydrogen bonding with any potential group(s) within the active site of ES. Here, we report the initial results of our study where we have undertaken: the design (involving the derivation of a potential TS of the reaction catalyzed by ES and modelling of the potential inhibitors onto the TS); synthesis of a number of derivatives of 4′-*O*-sulfamoyl-4-biphenyl; and the in vitro biochemical evaluation of the synthesized compounds in an effort to study any H-bonding interaction about the area corresponding to the steroid C(17) = O. The compounds were also evaluated to determine their mode of action, i.e., reversible or irreversible inhibition.

Experimental

Molecular modelling. In the construction of the probable transition state as a representation of the ES active site, the structures of the substrate, formylglycine (FGly69), and histadine (N229) residues (proposed by von Bulow et al. [7] to exist the active site), were all

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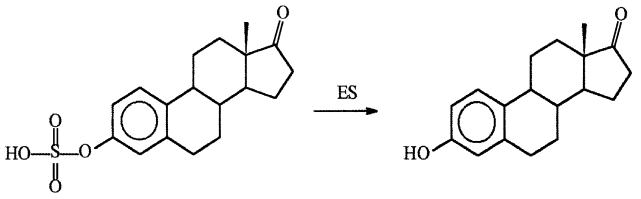


Fig. 1. Action of the enzyme ES on estrone sulfate.

constructed within the CACHE [8] molecular modelling software and then refined in mechanics using augmented MM2 [9], followed by a geometry optimization in Mopac [10] using AM1 parameters [11]. To determine the TS, reactant and product files were constructed and the initial structures were minimized using the MM2 and Mopac/AM1 routines. The saddle point for the reaction was then calculated and the resulting TS was refined by performing a gradient minimization calculation using Mopac and AM1 parameters. The molecule's vibrational transitions were determined to 'verify' the transition state (within Mopac using AM1 parameters). A single negative vibration ($-502.21\,cm^{-1}$) was observed in the final structure of the TS. The potential biphenyl-based inhibitors were all constructed and the completed structures were subjected to a minimization using the conjugate-gradient algorithm, prior to conformational analysis being undertaken. On the assumption that the shape of estrone sulfate would reflect the nature of the binding site of ES, the lowest energy conformers of the inhibitors were superimposed by specification of three or more points on the sulfonate group of the substrate onto the TS—the superimpositioning points were chosen since the sulfamate group is postulated to mimic the natural substrate's sulfonate group in binding to the ES active site.

Chemistry. In the sulfamoylation of derivatives of 4′-hydroxy-4-biphenyl, modified literature procedure [12] (Scheme 1) was followed and found to proceed well and in good yield without any major problems. In the synthesis of the ester derivatives of 4′-hydroxy-4-biphenyl carboxylate, 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, both the esterification and the sulfamoylation steps resulted in extremely poor yields, as such, the higher alkyl chain containing compounds were not synthesised. The syntheses of 1,1′-biphenyl-4-yl sulfamate (**1**) are given as examples.

*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 dimethylformamide (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 was 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 was 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 [m.p.146–149 °C (literature 165 °C, Hedayatullah, 1975); R_f 0.31 diethyl ether/petroleum ether 40–60 °C (50/50)]. $\nu_{(max)}$ (Film) cm^{-1}: 3421.3, 3301.9 (NH), 1382.0, 1177.9 (S=O). $\delta_H(CDCl_3)$ 7.85–7.15 (9H, m, ArH), 7.12 (2H, s, NH_2). $\delta_C(CDCl_3)$: 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.

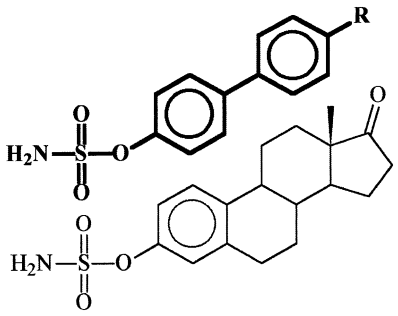
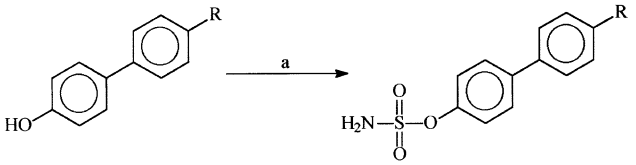


Fig. 3. Diagram to show the rationale for the synthesis of biphenyl sulfamates.



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) is shown.

Biochemistry. The synthesized 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 [13], whilst the mode of action (reversible or irreversible inhibition) was determined using a method involving dialysis of bound/unbound inhibitor [14]. The results of the biochemical evaluation are shown in Table 1, together with the calculated log *P* of the parent phenolic structure.

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 was 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 were placed on ice. The quenched samples were vortexed for 45 s and centrifuged (3000 rpm, 10 min). One ml toluene was removed and added to 5 ml scintillation cocktail (TRITONX). The

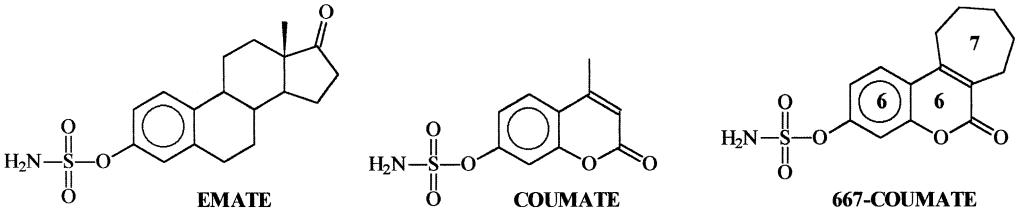


Fig. 2. Potent inhibitors of ES.

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 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 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.

Results and discussion

Consideration of the derived TS shows that the amino acid residues postulated to be involved in the reaction mechanism are positioned close to the sulfonate and the C(3) position of the steroidal backbone, such that the C(2) is hindered [the nearest amino acid atom to steroid C(2) being 2.1 Å (Fig. 4)]. However, consideration of the C(4) position show that it is slightly less hindered than the C(2)—the nearest amino acid atom to steroid C(4) being 3.5 Å. This observation is therefore consistent with experimental data [15] that shows that the 2-nitro derivatives of EMATE are poorer inhibitors than the 4-nitro-substituted derivatives. On superimpositioning of the biphenyl sulfamate-based compounds onto the TS, it was observed that where the R group is a carboxy moiety, interaction with potential H-bonding groups at the active site is possible [Fig. 4 shows the superimpositioning of methyl-4′-*O*-sulfamoyl-4-biphenyl carboxylate (**4**) onto the derived TS].

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. Compound **8** is ~3 times more potent than this coumarin-based compound. Furthermore, the determination of the mode of action of all of the synthesized 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 dialyzed with time.

From consideration of the initial inhibition data of the compounds considered within the present study, we are able to conclude that these compounds may be able to undergo polar–polar or H-bonding interactions with the active site. That is, the R group (Fig. 4) may be able to undergo favorable interaction(s) that result(s) in an increase in the binding ability of these compounds compared to COUMATE, resulting in good inhibitory activity. For example, from the consideration of compound **1**, we observed that this compound does not possess a group (R) which is able to undergo some form of polar–polar interaction and is some 7.6 times weaker than COUMATE and some 22 times weaker than compound **8**. As such, the current study suggests that group(s) may exist at the active site corresponding to the C(17) area of the steroid backbone and that interaction with the active site about this area may enhance inhibitory activity. Since the compounds were designed using the derived TS of the desulfatation reaction, the results of our study add further support to the accuracy of the derived TS.

An initial consideration of the physicochemical properties of these compounds, in particular, the alkyl esters, shows that within the small number of ester derivatives considered, there appears to be a good correlation between log *P* and inhibitory activity (IC_{50} value) (Fig. 5), an 'optimum' log *P* appears to exist at approximately 3.7—it is important to note that the calculated log *P* of estrone is observed to be 3.8. This similarity in log *P* requirement is intriguing and we hypothesize that hydrophobicity of the carbon backbone plays an important role in destabilizing the anion resulting from the hydrolysis

Table 1
The inhibition data for the synthezised compounds, EMATE, COUMATE, and 667-COUMATE (log *P* was calculated for the parent non-sulfamated phenol)

Compound (R=)	Compound number	% Inhibition	IC_{50} (μM/Tube)	log *P*
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[c]	0.1 ± 0.01	3.870

[a] [I] of 5 μM.
[b] [I] of 10 μM.
[c] [I] of 0.01 μM.

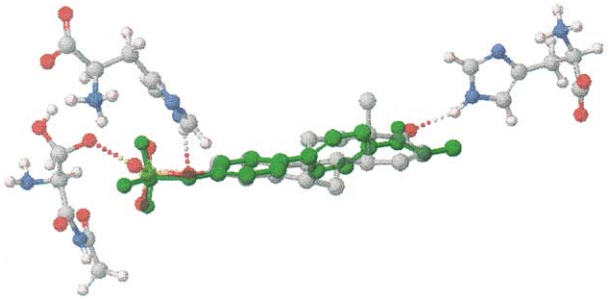


Fig. 4. Superimposition of one of the potential inhibitors (in green) on the derived transition state for the reaction catalyzed by ES.

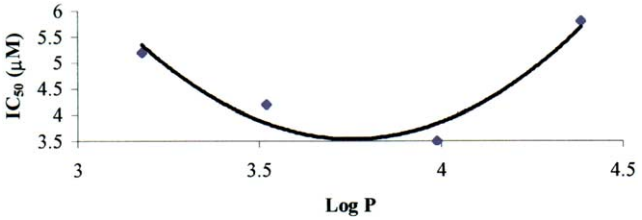


Fig. 5. Plot of IC_{50} versus log P to show the relationship between the two parameters.

reaction thereby aiding the loss of the carbon backbone of the inhibitor from the active site.

In conclusion, we have successfully designed and synthesized a range of compounds so as to mimic the steroid backbone of estrone. Furthermore, from consideration of the inhibitory data, we have been able to propose the existence of a potential H-bonding group at an area corresponding to the C(17) area of the steroid backbone and have added further support to the calculated TS.

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