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# Synthesis, in vitro and in vivo activity of benzophenone-based inhibitors of steroid sulfatase

Hatem A. M. Hejaz,[a] L. W. Lawrence Woo,[a] Atul Purohit,[b] Michael J. Reed[b] and Barry V. L. Potter[a,*]

[a]*Medicinal Chemistry, Department of Pharmacy and Pharmacology and Sterix Ltd., University of Bath, Bath BA2 7AY, UK*
[b]*Endocrinology and Metabolic Medicine and Sterix Ltd., Faculty of Medicine, Imperial College, St. Mary's Hospital, London W2 1NY, UK*

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**Abstract**—Steroid sulfatase (STS) is an important new therapeutic target in oncology. Attempts to design nonsteroidal STS inhibitors, because of the oestrogenicity of the original lead oestrone 3-*O*-sulfamate in rodents, have led to the discovery of benzophenone-4,4′-*O*,*O*-bis-sulfamate (BENZOMATE, **3**). The nonfused bicyclic BENZOMATE is a highly potent STS inhibitor in vitro, inhibiting STS activity in intact MCF-7 breast cancer cells by >70% at 0.1 μM and in placental microsomes by >98% at 10 μM. When MCF-7 cells were pre-treated with **3** at 1 μM and then washed to remove unbound inhibitor, the initial 94% inhibition was reduced to 89% suggesting that **3**, like other sulfamate-based STS inhibitors, inhibits the enzyme irreversibly. This agent also inhibits rat liver STS activity by 84% and 93% respectively 24 h after a single dose of 1 or 10 mg/kg, demonstrating that BENZOMATE possesses similar in vivo potency to the established potent nonsteroidal inhibitor 667COUMATE. Several modifications were made to BENZOMATE structurally and effects on in vitro activity were examined. These structure–activity relationship studies show that its carbonyl and bis-sulfamate groups are pivotal for activity, although conformational flexibility is not required. Two rigid anthraquinone-based sulfamate derivatives however showed inhibitory activity significantly better than BENZOMATE in the MCF-7 cell assay. BENZOMATE and related analogues therefore represent an important class of non-steroidal STS inhibitor and lead compounds for future drug design.


## 1. Introduction

Steroid sulfatase (STS) is a new therapeutic target in oncology. Since the discovery of oestrone 3-*O*-sulfamate (EMATE) as a highly potent irreversible inhibitor of STS, considerable progress has been made in developing a number of potent nonsteroidal/steroidal STS inhibitors. Highly potent steroidal analogues reported to date include some A-ring[1] and D-ring modified[2–5] derivatives of EMATE. Apart from the *N*-propyl- and *N*-(1′-pyridin-3′-ylmethyl)piperidinedione derivatives of EMATE,[5] as is common with other steroidal compounds, these EMATE derivatives are potentially oestrogenic in vivo in rodents.

The primary aim for developing an STS inhibitor has been for the treatment of hormone-dependent breast cancer (HDBC) as a stand-alone agent or in conjunction with an aromatase inhibitor. A recent development in this area has seen the validation of the concept of dual aromatase and sulfatase inhibition by a single agent.[6] In addition to its role as an anti-endocrine agent, the therapeutic potential of a STS inhibitor has now been widened, as recent evidence has suggested that inhibition of STS might have a clinical role in androgen-dependent skin diseases,[7] cognitive dysfunction[8,9] and also immune function.[10] For this reason, emphasis has been placed on the development of nonsteroidal inhibitors that either themselves, or through their metabolites, are unlikely to exert unwanted endocrinological effects.

Some impressive inhibitory activities have been observed with coumarin sulfamates, especially those compounds from the tricyclic series, with 667COUMATE[11] being shown to be some 3-fold more potent than EMATE against STS in a placental microsomes preparation. 667COUMATE retains all the essential features of EMATE such as oral activity and an

* Corresponding author. Tel.: +44-1225-386-639; fax: +44-1225-386-114; e-mail: prsbvlp@bath.ac.uk

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irreversible mechanism of enzyme inactivation, except that it lacks oestrogenic activity. Since it is expected that only a non-oestrogenic STS inhibitor will be of use in endocrine therapy, 667COUMATE has been selected as the first Phase I trial candidate for treating HDBC.

Because of the structural resemblance of the naturally occurring flavonoids to oestrogens, sulfamates of flavones, isoflavones and flavanones were prepared and shown to be moderate inhibitors of STS.[12] Further exploitation of this class of compound by others led to a series of chromenone- and thiochromenone-based sulfamates, of which 2-(1-adamantyl)-4*H*-thiochromen-4-on-6-*O*-sulfamate was found to be about 170-fold more potent than EMATE in vitro.[13]

Recent work by Poirier and co-workers[14] has shown that 1-(*p*-sulfamoyloxyphenyl)-5-(*p*-*tert*-butylbenzyl)-5-undecanol and related congeners are highly potent STS inhibitors in vitro. Simple *p*-sulfamoylated benzoic acid esters,[15] substituted phenyl sulfamates[16] and 4-sulfamoylated phenyl ketones[17] have been pursued as STS inhibitors, although none of these agents showed potent in vitro inhibitory activity. The $IC_{50}$ values for the best two inhibitors in these series of compounds were both reported to be 3.4 μM, some 7-fold higher than that (0.5 μM) reported for EMATE in the same assay.[17] It is interesting to note that this $IC_{50}$ value for EMATE reported by Ahmed et al. is much higher than those values (18–80 nM) obtained by other groups from similar placental microsomal preparations.[11,18,19]

Despite the structural diversity of irreversible STS inhibitors reported to date, they all share and support our proposed pharmacophore, that the minimal structural requirement for STS inhibition is a phenol sulfamate ester and that substituents, which exploit favourable hydrophobic interactions with the enzyme active site will confer higher potency to the inhibitors.[20] In addition, our work on coumarin sulfamates[11,20] and on the 4-nitro analogue of EMATE[1] demonstrated that sulfamoylated inhibitors that presumably are able to transfer more effectively their sulfamoyl group to an essential amino acid residue in the enzyme active site during the inactivation process are more potent STS inhibitors. It has been reasoned that the sulfamoyl group transfer ability of an aryl sulfamate, and hence its inhibitory activity of STS, is related to the leaving group ability of the parent phenol. A substantial decrease in the inhibitory activities of analogues was observed in our structure–activity relationship (SAR) studies on coumarin sulfamates[20] when the conjugated coumarin ring system was disrupted such as via the saturation of their C3–C4 double bond. The overall effect of these disruptions is an increase in the p*K*a value of the resulting phenols, rendering the parent coumarins poorer leaving groups and hence their sulfamates weaker inhibitors of STS.

Our initial studies to develop a non-steroidal STS inhibitor involved the sulfamoylation of diethylstilboestrol (DES), which has two nonfused aryl rings. The mono-(**1**, Fig. 1) and bis-sulfamate (**2**, Fig. 1) derivatives of DES[21]

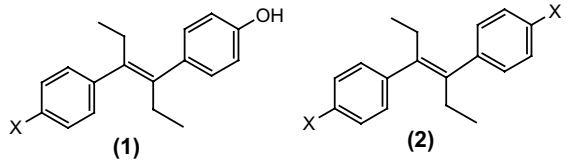


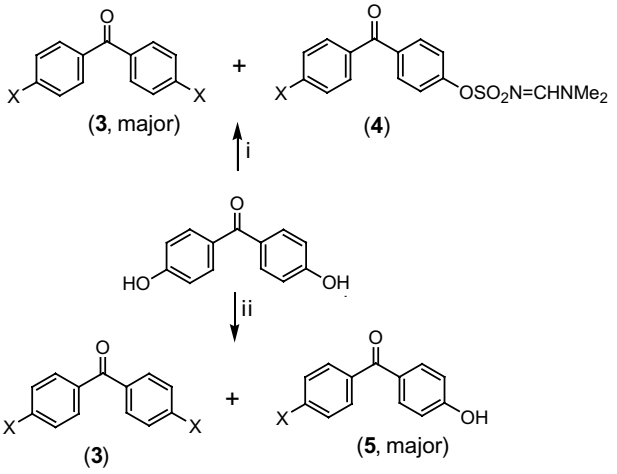

**Figure 1.** Structures of diethylstilboestrol mono-sulfamate (**1**) and diethylstilboestrol bis-sulfamate (**2**). $X = OSO_2NH_2$.

were considerably more potent inhibitors than the fused bicyclic tetrahydronaphthol (THN) sulfamates.[18]

DES-bis-sulfamate (**2**) was found to have an $IC_{50}$ value of 10 nM as assessed in intact MCF-7 breast cancer cells.[21] Despite these encouraging results, DES is a known potent oestrogen and toxic compound, which would restrict the use of its bis-sulfamoylated derivative in treating HDBC. However, the finding that it was not necessary to have a fused ring system for STS inhibition encouraged us to synthesise a series of sulfamate analogues with a non-fused ring structure. A prime objective of our design strategy is to introduce functionalities into such a structural system that would increase the sulfamoyl group transfer ability of the inhibitors. We now report that benzophenone sulfamates, and their analogues, are potent inhibitors of STS with in vivo activity. Structural modifications to the lead inhibitor have also been made in order to understand more fully the structure–activity relationships (SARs) for this class of nonsteroidal STS inhibitor. While this work was being prepared for publication, in vitro activities of some related sulfamates also reported here were published by other groups.[17,22]

## 2. Chemistry

The sulfamate derivatives of 4,4′-dihydroxybenzophenone (**3**–**5**, Scheme 1) were synthesised as follows. When 4,4′-dihydroxybenzophenone in *N*,*N*-dimethylformamide (DMF) was treated with 3 equiv of sodium hydride followed by an excess of sulfamoyl chloride,




**Scheme 1.** Synthesis of benzophenone derivatives **3**–**5**. Reagents and conditions: (i) 3NaH/DMF, excess $H_2NSO_2Cl$; (ii) NaH/DMF, excess $H_2NSO_2Cl$. $X = OSO_2NH_2$.

benzophenone-4,4′-*O*,*O*-bis-sulfamate (**3**, BENZOMATE), was obtained as the major product. From the same reaction, a minor product was also isolated (7%) and it was subsequently identified as the azomethine adduct **4**, which was formed between the bis-sulfamate **3** and the reaction medium DMF. A similar reaction between a sulfamate and DMF in the presence of a base and the possible mechanism involved has already been reported in the synthesis of 2-nitrophenol-*O*-sulfamate under related conditions.[20] When 4,4′-dihydroxybenzophenone was sulfamoylated after treating with only 1 equiv of sodium hydride, the mono-sulfamate (4′-hydroxybenzophenone-4-*O*-sulfamate **5**) was obtained as the major product and its separation from the bis-sulfamate (**3**) and the starting material, was achieved by preparative TLC.

A number of structural variants were designed to examine SAR parameters. The synthesis of compounds **6**–**11**, **14**–**16** and **20**–**21** (Fig. 2) was achieved by sulfamoylating the corresponding parent phenols in the usual manner and, where applicable, the mono- and bis-sulfamates were separated by flash chromatography.

Benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione-3-*O*-sulfamate (**13**, Scheme 2) was prepared from the parent compound **12**, which was obtained by reacting 2,3-dichloro-1,4-naphthoquinone with resorcinol in ethanolic sodium ethoxide solution.[23]

The 4,4′-dihydroxydiphenylmethane (**17**, Scheme 3) was prepared by catalytic hydrogenation of a solution of

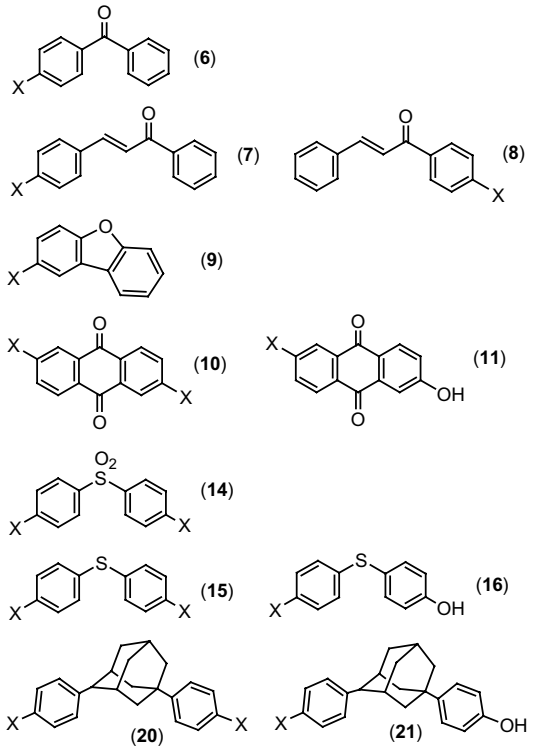
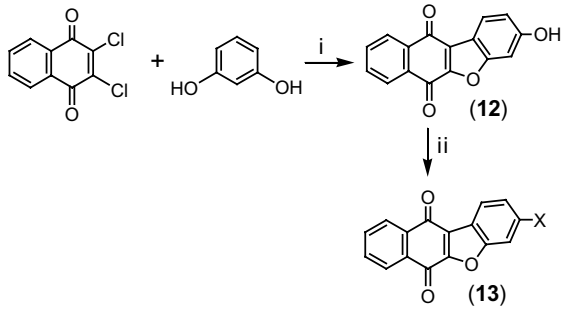


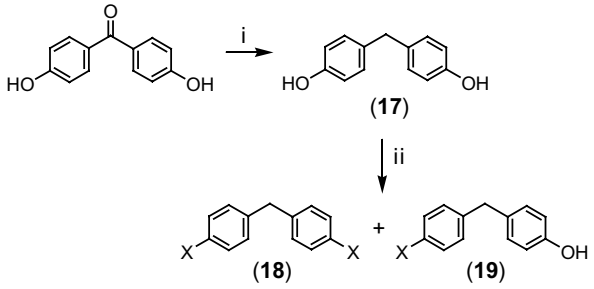

**Figure 2.** Structures of sulfamates **6**–**11**, **14**–**16** and **20**–**21**. X = $OSO_2NH_2$.



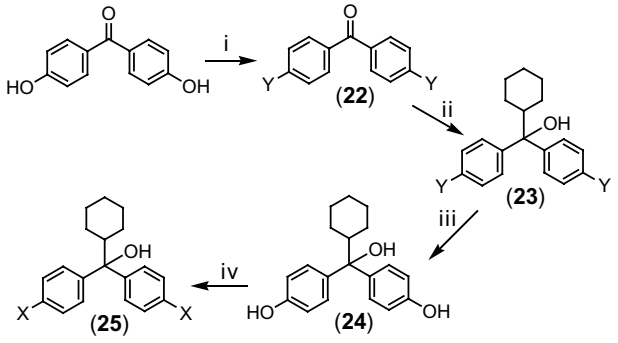

**Scheme 2.** Synthesis of benzo[*b*]naphtho[2,3-*d*]-furan-6,11-dione-3-*O*-sulfamate (**13**). Reagents and conditions: (i) NaOEt/ethanol, 12 h; (ii) NaH/DMF, $H_2NSO_2Cl$. X = $OSO_2NH_2$.

4,4′-dihydroxybenzophenone in ethanol in the presence of Pd–C (10%) at room temperature under balloon pressure for 6 h.[24] Sulfamoylation of compound **17** (Scheme 3) in the usual manner gave a mixture of compounds **18** and **19**, which were separated by flash chromatography.

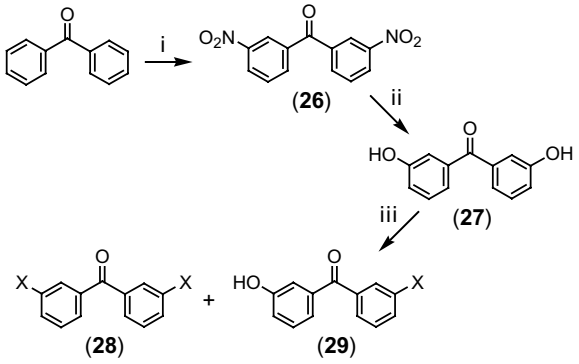
For the preparation of compound **25** (Scheme 4), 4,4′-dihydroxybenzophenone was first protected by two *tert*-butyldimethylsilyl (TBDMS) groups to give the disilylated **22**, which was reacted with cyclohexyl magnesium




**Scheme 3.** Synthesis of diphenylmethane sulfamates (**18** and **19**). Reagents and conditions: (i) Pd–C/96% ethanol, 6 h; (ii) NaH/DMF, $H_2NSO_2Cl$. X = $OSO_2NH_2$.




**Scheme 4.** Synthesis of 1-cyclohexyl-1,1-(4,4′-*O*,*O*-bis-sulfamoylphenyl)methanol (**25**). Reagents and conditions: (i) TBDMS–Cl/THF, imidazole, 3 h; (ii) cyclohexylmagnesium chloride/ether, 12 h; (iii) TBAF/THF, rt, 10 min; (iv) DBMP/$CH_2Cl_2$, $H_2NSO_2Cl$, 2 h. X = $OSO_2NH_2$, Y = OTBDMS.




**Scheme 5.** Synthesis of benzophenone-3′,3-*O*,*O*-bis-sulfamate (**28**) and 3′-hydroxybenzophenone 3-*O*-sulfamate (**29**). Reagents and conditions: (i) $H_2SO_4/HNO_3$, 75 °C, 30 min; (ii) (a) $SnCl_2/HCl$, 70 °C, 6 h; (b) $NaNO_2/H_2SO_4$, 0 °C to Δ; (iii) NaH/DMF, $H_2NSO_2Cl$. $X = OSO_2NH_2$.

chloride to give the substituted benzhydrol **23**. The TBDMS groups of compound **23** were cleaved by tetrabutylammonium fluoride (TBAF) in THF to give the trihydroxylated parent compound **24**, which, as a solution in dichloromethane, was sulfamoylated by reacting with sulfamoyl chloride in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DBMP) to give compound **25**. The $^1H$ NMR spectrum of compound **25** showed a downfield shift for the C3, C3′, C5 and C5′ protons in comparison with those of compound **24**, which is indicative of the presence of sulfamate group at both the 4- and 4′-position. The fact that compound **25** was isolated as the main product suggested that the tertiary alcohol was not sulfamoylated, as anticipated in the conditions adopted, because of steric hindrance and the use of a weak base.

Nitration of benzophenone with a mixture of nitric acid and sulfuric acid gave 3,3′-dinitrobenzophenone in 53% yield (**26**, Scheme 5). Reduction of compound **26** with stannous chloride in HCl followed by hydroxydeamination of the crude salt by sodium nitrite in $H_2SO_4$ gave 3,3′-dihydroxy-benzophenone (**27**, Scheme 5).[25] Sulfamoylation of compound **27** gave a mixture of the corresponding bis-(**28**) and mono-(**29**) sulfamate (Scheme 5).

## 3. Results and discussion

Our earlier programme of developing new classes of nonsteroidal STS inhibitors identified that the mono-(**1**, Fig. 1) and bis-sulfamate (**2**, Fig. 1) derivatives of diethylstilboestrol (DES) are moderate STS inhibitors.[21] These findings indicated that it is not necessary to have a fused ring system for STS inhibition. Although the reason for the inhibitory activities observed for the sulfamate derivatives of DES is not clear, it is possible that their conjugated stilbene system facilitates the inactivation of STS via sulfamoylation. Because of the toxicities associated with DES, which rule out further modifications to the structure for drug development, we replaced instead the alkene moiety of this compound with a carbonyl group to produce the sulfamate derivatives of 4,4′-dihydroxybenzophenone. The carbonyl group is chosen on the basis that the mesomeric effect of the benzophenone system, which is expected to lower the p*K*a values of the bis-phenol, will result in a more effective sulfamoyl group transferring agent. Indeed, the $pKa_1$ and $pKa_2$ values of 4,4′-dihydroxybenzophenone in a solution of 50% v/v aqueous methanol as determined by potentiometric titration were found to be 8.32 and 9.72, respectively. In contrast, the $pKa_1$ and $pKa_2$ values of its unconjugated congener, bis-(4-hydroxyphenyl)methane were found to be 10.44 and 11.38, respectively, under the same conditions [lit.[26] (48% v/v aq ethanol), $pKa_2 = 11.22$]. These observations prompted us to investigate if sulfamate derivatives of 4,4′-dihydroxybenzophenone will also show high potency against STS.

The inhibition of STS activity in intact MCF-7 breast cancer cells and in a placental microsomes preparation by sulfamates **3**–**5** are tabulated in Tables 1 and 2, respectively. The best compound in this series was BENZOMATE (**3**), which showed >70% inhibition of STS activity in intact MCF-7 breast cancer cells at 0.1 μM and >98% inhibition of placental microsomes STS at 10 μM. In one experiment, when MCF-7 cells were pre-treated with **3** at 1 μM for 2 h and then washed to remove unbound inhibitor, the initial 94% inhibition was reduced to 89% suggesting that **3**, like other sulfamate-based STS inhibitors, inhibited the enzyme irre-

**Table 1.** Inhibition of STS activity in intact MCF-7 breast cancer cells by sulfamates **3**–**16**, **18**–**21**, **25**, **28**–**29**

| Compound | % Inhibition of STS activity in MCF-7 cells | | |
|---|---|---|---|
| | 0.1 μM | 1 μM | 10 μM |
| **3** | 71.4 ± 6.9 | 95.6 ± 2.1 | 98.7 ± 2.8 |
| **4** | 46.3 ± 13.6 | 83.5 ± 1.9 | 93.9 ± 1.2 |
| **5** | 34.8 ± 1.7 | 83.8 ± 0.5 | 96.7 ± 1.0 |
| **6** | <10 | 33.0 ± 10.7 | 87.9 ± 2.0 |
| **7** | <10 | 24.3 ± 11.9 | 83.8 ± 0.2 |
| **8** | <10 | 33.0 ± 10.7 | 87.9 ± 2.0 |
| **9** | 21.3 ± 1.5 | 67.9 ± 1.9 | 97.1 ± 0.8 |
| **10** | 93.1 ± 2.1 | 98.6 ± 1.0 | 99.1 ± 0.1 |
| **11** | 99.3 ± 0.6 | 94.5 ± 1.6 | 90.1 ± 0.8 |
| **13** | 46.6 ± 4.5 | 93.9 ± 0.8 | 98.6 ± 0.3 |
| **14** | <10 | 50.6 ± 3.3 | 87.4 ± 2.7 |
| **15** | 67.2 ± 1.1 | 96.6 ± 1.4 | 99.7 ± 0.1 |
| **16** | 26.6 ± 4.3 | 83.9 ± 1.2 | 97.7 ± 0.4 |
| **18** | 22.2 ± 0.1 | 50.01 ± 0.1 | 93.3 ± 0.1 |
| **19** | <10 | 39.3 ± 0.1 | 92.4 ± 0.1 |
| **20** | 16.1 ± 4.0 | 32.7 ± 2.3 | 90.1 ± 0.7 |
| **21** | <10 | 16.3 ± 1.5 | 64.1 ± 1.1 |
| **25** | <10 | 27.4 ± 0.8 | 75.1 ± 2.8 |
| **28** | 75.3 ± 0.8 | 98.4 ± 0.3 | 99.9 ± 0.1 |
| **29** | <10 | 30.7 ± 5.6 | 87.2 ± 1.8 |

Monolayers of intact MCF-7 cells in 25 $cm^3$ flasks were incubated for 20 h at 37 °C with [$^3$H]oestrone sulfate (2 nM) and sulfamates at various concentrations. STS activity was determined by measuring the total amount of $^3$H-labelled oestrone and oestradiol formed. STS activity in untreated cells was 100–120 fmol/20 h/$10^6$ cells. Each value represents the mean ± SD of triplicate measurements. Both EMATE and 667COUMATE showed >99% inhibitions at all concentrations.

**Table 2.** Inhibition of STS activity in placental microsomes by sulfamates **3**–**16**, **18**–**21**, **25**, **28**–**29**

| Compound | % Inhibition of STS activity in placental microsomes | | | |
|---|---|---|---|---|
| | 10 μM | 25 μM | 50 μM | 100 μM |
| **3** | 98.2 ± 0.1 | ND | 99.0 ± 0.1 | 99.2 ± 0.0 |
| **4** | 85.3 ± 1.4 | 92.1 ± 0.0 | 94.7 ± 0.4 | 96.6 ± 0.1 |
| **5** | 62.1 ± 1.4 | 82.0 ± 0.4 | 88.1 ± 0.3 | 92.8 ± 0.2 |
| **6** | 76.7 ± 2.5 | ND | 88.0 ± 0.8 | 92.3 ± 0.4 |
| **7** | 47.4 ± 0.1 | ND | 75.1 ± 1.4 | 82.8 ± 0.2 |
| **8** | 71.3 ± 2.4 | ND | 75.8 ± 2.1 | 80.5 ± 1.9 |
| **9** | 28.8 ± 3.2 | 48.5 ± 0.5 | 62.0 ± 2.8 | 75.8 ± 1.2 |
| **10** | 91.3 ± 0.7 | 95.7 ± 0.5 | 97.2 ± 0.3 | 97.7 ± 0.1 |
| **11** | 86.1 ± 0.5 | 91.2 ± 0.2 | 94.4 ± 0.5 | 95.6 ± 0.1 |
| **13** | ND | ND | ND | ND |
| **14** | 65.0 ± 1.8 | 85.5 ± 0.6 | 92.3 ± 0.3 | 95.4 ± 0.2 |
| **15** | 65.3 ± 0.9 | 79.4 ± 0.9 | 87.4 ± 0.9 | 91.9 ± 0.4 |
| **16** | 33.1 ± 1.7 | 49.5 ± 0.1 | 64.7 ± 0.9 | 76.8 ± 0.4 |
| **18** | 22.1 ± 0.6 | 29.4 ± 0.1 | 38.7 ± 4.9 | 53.1 ± 0.1 |
| **19** | 11.7 ± 2.3 | 17.3 ± 2.7 | 22.8 ± 1.4 | 34.3 ± 1.0 |
| **20** | 31.4 ± 1.6 | 41.9 ± 2.3 | 62.5 ± 7.5 | 69.0 ± 4.8 |
| **21** | <10 | 15.9 ± 0.9 | 32.5 ± 1.1 | 60.1 ± 3.4 |
| **25** | <10 | 20.0 ± 1.6 | 33.4 ± 2.2 | 44.7 ± 0.4 |
| **28** | 51.1 ± 3.7 | 73.6 ± 1.9 | 84.1 ± 1.9 | ND |
| **29** | 18.8 ± 1.3 | 30.7 ± 3.4 | 43.7 ± 2.6 | 58.3 ± 0.1 |

[$^3$H]Oestrone sulfate ($4\times10^5$ dpm), adjusted to 20 μM with unlabelled substrate, with or without the inhibitor at various concentrations, was incubated with placental microsomes (125 μg of protein/mL) for 30 min. The product formed was isolated by extraction into toluene with [4-$^{14}$C]oestrone ($7\times10^3$ dpm) being used to monitor procedural losses. Each value represents the mean ± SD of triplicate measurements. ND = not determined. 667COUMATE and EMATE showed 92% and 91% inhibitions at 1 μM, respectively; and >99% at 10 μM.

versibly. The mono-sulfamate derivative **5** was slightly less potent than **3** suggesting that a sulfamate group at both the 4- and 4′-positions of benzophenone is required for maximising the inhibitory activity for this type of STS inhibitor. We observed in related work that the azomethine adduct of 667COUMATE (cf. **4**) is inactive as an STS inhibitor (unpublished result). The STS inhibitory activity observed for compound **4** is thus anticipated to be the result of its mono-sulfamate group inactivating the enzyme via a sulfamoyl group transfer.

Having identified BENZOMATE as a potent STS inhibitor, we prepared a range of compounds in order to establish the SARs for this compound. These analogues include benzophenone-4-*O*-sulfamate, where one of the two phenyl rings is not substituted (**6**, Fig. 2); *trans*-chalcone sulfamates (**7** and **8**, Fig. 2), where the distance between the two nonfused-rings is extended; dibenzofuran-2-*O*-sulfamate (**9**, Fig. 2), 2,6-anthraquinone bis-(**10**, Fig. 2) and mono-(**11**) sulfamate and benzo[*b*]-naphtho[2,3-*d*]-furan-6,11-dione-3-*O*-sulfamate (**13**, Scheme 2), where the molecules are conformationally restricted; sulfonyl-diphenyl-4,4′-*O*,*O*-bis-sulfamate (**14**, Fig. 2), and thiodiphenyl bis-(**15**, Fig. 2) and mono-(**16**) sulfamate, where the carbonyl group is replaced by other functionalities; diphenylmethane 4,4′-*O*,*O*-bis-sulfamate (**18**, Scheme 3) and 4′-hydroxydiphenylmethane 4-*O*-sulfamate (**19**, Scheme 3), where the carbonyl group is removed; (1,3-adamantanediyl)diphenyl bis-(**20**, Fig. 2) and mono-(**21**) sulfamate, and 1-cyclohexyl-1,1-(4,4′-bis-sulfamoyloxy-phenyl)methanol (**25**, Scheme 4), where extra aliphatic ring(s) are introduced; benzophenone 3,3′-*O*,*O*-bis-sulfamate (**28**, Scheme 5) and 3′-hydroxybenzophenone-3-*O*-sulfamate (**29**, Scheme 5), where the sulfamate group(s) are relocated.

Upon examination of the abilities of these BENZOMATE analogues (**6**–**11**, **13**–**16**, **18**–**21**, **25** and **28**–**29** to inhibit STS activity (Tables 1 and 2), almost all of them were found to be less effective STS inhibitors than BENZOMATE, with the exception of the sulfamate derivatives (**10** and **11**, Fig. 2) of 2,6-anthraquinone and benzophenone-3,3′-bis-sulfamate **28** (Scheme 5), which were found to inhibit STS stronger than or to a similar extent to BENZOMATE in MCF-7 breast cancer cells. Although compounds **10** and **11** were again found to be similar in potency to BENZOMATE as STS inhibitors in placental microsomes, compound **28** was clearly less potent than BENZOMATE in this assay. This latter finding, which is in agreement with that of Nussbaumer et al.,[22] further provides evidence to support our reasoning that BENZOMATE is a highly potent STS inhibitor because its sulfamate group(s) are more efficiently transferred in the presence of the carbonyl group in a position *para* to them. Such an effect would be diminished by the relocation of its sulfamate groups to the 3,3′-position as in compound **28** rendering the compound a weaker STS inhibitor.

The in vitro results here also show that most compounds, which poorly inhibit STS in intact MCF-7 cells (Table 1) also perform poorly when examined in placental microsomes (Table 2). In addition, bis-sulfamates are in general more potent than their corresponding mono-sulfamates, for example, **3** versus **5**, **3** versus **6** (a similar observation has been made[22]), **15** versus **16** and **18** versus **19**. Although it is still unclear why such a pattern exists, this further demonstrates that the sulfamate group is the key chemical structural requirement for potent STS inhibition.

The sulfamate derivatives (**7** and **8**, Fig. 2) of *trans*-chalcone are analogues of compound **6** (Fig. 2) where the distance between the two nonfused phenyl rings has been extended without disrupting the conjugation between the ring system bearing the sulfamate and the carbonyl group. Both sulfamates **7** and **8** show similar activities in both enzyme systems at higher concentrations, although compound **8** inhibits placental microsomes STS more strongly at 10 μM suggesting that the positioning of the mono-sulfamate group *para* to the carbonyl group might be preferred to a larger separation between the two moieties, as in compound **7**. Although compound **8** shares similar potency to compound **6** in our studies, Ahmed et al.[17] reported an $IC_{50}$ value for compound **8** in placental microsomes being some 4-fold lower than that of compound **6**. The much weaker inhibition than BENZOMATE observed for the chalcone sulfamates **7** and **8** show the importance of the benzophenone scaffold for the biological activities of BENZOMATE.

Replacement of the carbonyl group of BENZOMATE by a methylene unit (**18**, Scheme 3), or a divalent sulfur (**15**, Fig. 2) or a sulfonyl group (**14**, Fig. 2) or aliphatic rings (**20**, Fig. 2) or substitution with a cyclohexyl ring (**25**, Scheme 4) significantly reduces inhibitory activity. The lower potency observed for bis-sulfamates **15** and **18** can be attributed to their weaker sulfamoyl group transferring potential as a result of the higher p*K*a values of their parent bis-phenol (p$Ka_1$ and p$Ka_2$ values of bis-(4-hydroxyphenyl)methane were found to be 10.44 and 11.38, respectively, vide supra; p$Ka_2$ value of 4,4′-thiodiphenol was reported to be 10.80[26]). However, it is surprising to find that bis-sulfamate **14** is a much weaker STS inhibitor than BENZOMATE since the p$Ka_2$ value of 4,4′-sulfonyldiphenol was reported to be 9.41,[26] which is lower than that of 4,4′-dihydroxybenzophenone (9.72, vide supra) determined in similar conditions. The fact that the carbonyl group of BENZOMATE is required for potent activity suggests that this moiety might be involved in extra binding to the enzyme active site in addition to conjugation with the phenyl ring with activation of its sulfamate groups. Substituting the carbonyl group with a bulky ring (adamantanediyl, **20**, Fig. 2; cyclohexyl, **25**, Scheme 4) attenuates the inhibitory activities of the BENZOMATE analogue. These results thus highlight the presumed limited tolerance of the enzyme to these bulky rings. It is possible that these rings may shift the sulfamate groups at the 4- and 4′-positions away from the position occupied by the sulfamate groups of BENZOMATE in the binding site, and hence the analogue might not be activated effectively for the sulfamoylation of the enzyme. These rings may of course simply be too large, so that the molecule cannot fit into the enzyme active site.

Anthraquinone derivatives and 3-hydroxybenzo[*b*]-naphtha-[2,3-*d*]furan-6,11-dione (**12**, Scheme 2) are known intercalating cytostatic agents and have antitumour properties. However, we considered that introduction of a sulfamate group into the structure of these compounds, might significantly engender STS inhibitory activity and, might provide a new structural class of STS inhibitor. Such anthraquinone derivatives are of course conformationally restricted analogues of BENZOMATE, and could be used to explore details of the potential active site binding mode. The bis-(**10**) and mono-(**11**) sulfamates (Fig. 2) of 2,7-dihydroxyanthraquinone were prepared and were examined in MCF-7 cells and placental microsomes for STS inhibition. They are potent STS inhibitors (Tables 1 and 2) with potency comparable to or better than BENZOMATE. Thus, compounds **10** and **11** inhibit STS activity in MCF-7 cells by 93% and >99% at 0.1 μM, respectively (Table 1) and in placental microsomes by 91% and 86% at 10 μM, respectively (Table 2). These findings suggest that the conformational flexibility of BENZOMATE might not be a prerequisite for its potent biological activity. For comparison, 667COUMATE inhibited the STS activity in placental microsomes by 91% and >99% at 1 and 10 μM, respectively,[11] showing that compounds **10** and **11** are weaker STS inhibitors than 667COUMATE in vitro. While the tetracyclic 3-*O*-sulfamoylbenzo[*b*]-naphtha[2,3-*d*]furan-6,11-dione (**13**, Scheme 2) inhibits STS activity in MCF-7 cells potently and more strongly than the tricyclic dibenzofuran-2-*O*-sulfamate (**9**, Fig. 2), it is less potent than BENZOMATE at 0.1 μM (Table 1). However, compound **13** also kills MCF-7 cells, indicating that it might be cytotoxic and have additional biological activities. The question obviously arises whether their inhibition of STS results from the sulfamoyl group alone or is due to their other properties. This remains to be elucidated precisely, but the following observation was noted: as expected, the corresponding starting material of **13** (i.e., **12**, Scheme 2) shows no significant inhibitory activity against STS when examined in MCF-7 cells at 10 μM (data not shown) while sulfamate **13** inhibited STS by 99% at the same concentration (Table 1). When the percentage growth inhibition of MCF-7 breast cancer cells by compound **12** was examined, it was found to be 12%, 20% and 56% at 0.1, 1 and 10 μM, respectively, compared to sulfamate **13**, which inhibited the growth of these cells by 41% and 44% at 1 and 10 μM, respectively, and was inactive at 0.1 μM, indicating that both the phenol **12** and the corresponding sulfamate **13** have the same property of inhibition of the growth of MCF-7 breast cancer cells.

Having identified that benzophenone-4,4′-*O*,*O*-bis-sulfamate (BENZOMATE, Scheme 1, **3**) is a potent inhibitor, this compound was tested in vivo in rats. The liver STS activity was found to be inhibited by 84% and 93% 24 h after a respective single oral dose of the agent at 1 or 10 mg/kg (Fig. 3). In comparison, 667COUMATE inhibited rat liver STS activity by 91% and 93% at 1 and 10 mg/kg, respectively.[27] These results demonstrate that BENZOMATE is orally active and a highly potent nonsteroidal STS inhibitor with comparable potency to 667COUMATE in vivo. The oestrogenicity of BENZOMATE has not been studied in this work. However, it has been reported that 4,4′-dihydroxybenzophenone binds to oestrogen receptor with a rela-

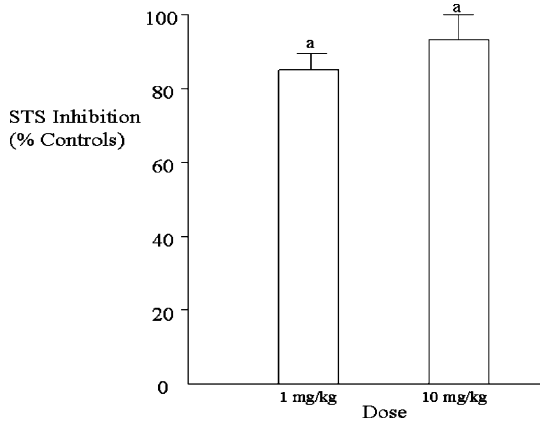



**Figure 3.** In vivo effects of BENZOMATE (**3**) on STS activity in rat liver tissue preparations. Female Wistar rats (Harlan Olac, Bicester, Oxon, United Kingdom) were treated with vehicle (propylene glycol) or **3** at 1 or 10 mg/kg with animals receiving a single dose p.o. Samples of liver tissue were collected for assay of STS activity 24 h after administration of the dose. Results are expressed as the percentage of STS inhibition compared with that in control animals (mean ± SD, $n = 3$). a, $P < 0.001$.

tive binding affinity $3\times10^{-5}$-fold of that of 17β-oestradiol.[28] This finding clearly suggests that neither BENZOMATE nor its putative metabolite(s) formed after enzyme inactivation is likely to present oestrogenicity complications.

We have observed from our experience in the handling of many structurally diversified phenol sulfamate esters that while these sulfamoylated compounds are perfectly stable as solids, some of them when in solution degrade over time to their corresponding phenolic compounds as the result of the hydrolysis of the sulfamate group. Like their inhibitory activity against STS in vitro, the instability of some phenol sulfamate esters in solution apparently, and not surprisingly, also relates to the p*K*a value of their corresponding phenols. Hence, highly potent STS inhibitors such as 2-nitro- and 4-nitroestrone 3-*O*-sulfamates,[1] whose respective phenolic parent compound has a p*K*a value of 8.85 and 7.77 in ethanol/water as measured spectrophotometrically,[29] exhibit a much higher degree of degradation in solutions than EMATE over the same period of time, particularly when they are in solution in highly polar solvents such as DMSO. To assess the stability of BENZOMATE in solution, the disappearance of BENZOMATE, as analysed by UV spectroscopy, over time under conditions similar to those used for the in vitro assay was performed. Hence, a freshly prepared methanolic solution of BENZOMATE was added to phosphate buffer saline (PBS) containing 0.25 M sucrose to give a final concentration of methanol of either 1% or 10%. The half-life of BENZOMATE at both methanolic concentrations incubated at 37 °C was found to be about 60 min (Fig. 4),[12b] demonstrating the instability of this compound in solution.

When the above degradation was studied by LC/MS, BENZOMATE was found to break down to its monosulfamate derivative, that is compound **5** and 4,4′-dihydroxybenzophenone (Fig. 5). It appears that this degradation is sequential with the hydrolysis of one sulfamate group at a time. There was still some BENZOMATE detected after 130 min into the experiment but complete disappearance of BENZOMATE was observed after 240 min (data not shown). While such

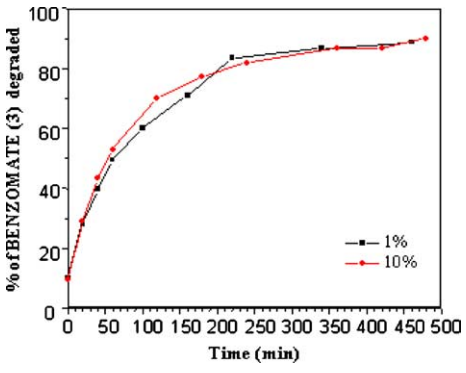



**Figure 4.** Stability study of BENZOMATE (**3**) in PBS containing 0.25 M sucrose and 1% or 10% of methanol at 37 °C as monitored by UV spectroscopy. Points are mean of triplicate determinations.

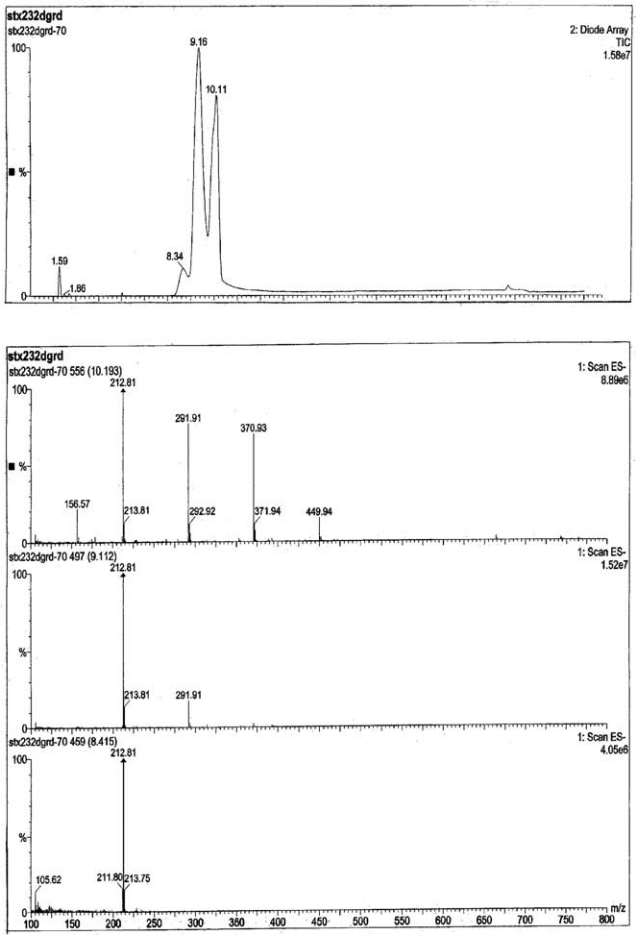



**Figure 5.** Degradation of BENZOMATE in PBS containing 0.25 M sucrose and methanol (10%) at 37 °C after 70 min of incubation as monitored by LC/MS. The peaks at $t_R = 10.11$, 9.16 and 8.34 min were assigned by mass spectrometry as BENZOMATE (top), the monosulfamate **5** (middle) and 4,4′-dihydroxybenzophenone (bottom spectrum), respectively.

instability of BENZOMATE may present problems in the pharmaceutical development of this class of STS inhibitor, the fact that it inhibits so potently the STS activity in MCF-7 cells after an incubation period of 20 h suggests that the inactivation of STS by BENZOMATE is rapid and highly effective. The high potency against liver STS activity observed for BENZOMATE in vivo when administered orally to rats suggests also that this apparent instability of BENZOMATE in solution might not be as problematic as it seems. In particular, it is thought that aryl sulfamates in vivo are transported in red blood cells and avoid first pass metabolism.[30] The carrier protein that mediates this is apparently carbonic anhydrase II (hCAII). Aryl sulfamates bind effectively to hCAII using a coordination of the sulfamate anion to the active site zinc atom.[31,32] We have recently determined the X-ray crystal structure of EMATE bound to hCAII.[33] It seems highly likely that BENZOMATE would also be transported in vivo via hCAII and any intrinsic chemical instability of this agent could be markedly attenuated in vivo by this effect.

## 4. Conclusions

Attempts to design and synthesise non-steroidal STS inhibitors, in the light of the potent oestrogenicity of EMATE in rodents, have led to the discovery of BENZOMATE (**3**), a significant improvement over initial lead non-steroidal candidates such as THN-2-*O*-sulfamate and bicyclic coumarin sulfamates. BENZOMATE, a non-fused bicyclic bis-sulfamate, is a highly potent inhibitor of STS in vitro. This agent inhibits in vivo rat liver STS activity by 84% and 93%, respectively, at a single oral dose (1 and 10 mg/kg), showing potency of a similar order of magnitude to 667COUMATE, the established potent non-steroidal STS inhibitor, which is currently in Phase I trial. Several modifications were made to the structure of BENZOMATE. These SAR studies conducted on BENZOMATE show that its carbonyl and bis-sulfamate groups are pivotal for inhibition of STS although conformational flexibility is apparently not required. BENZOMATE represents an important lead compound and new class of STS inhibitor. Despite the apparent instability of BENZOMATE in solution, it is anticipated that BENZOMATE or its optimised derivatives might subsequently be developed for therapeutic use in the treatment of HDBC.

## 5. Experimental

### 5.1. Materials and methods

All reagents and solvents employed were of general purpose or analytical grade unless otherwise stated, and purchased from either Aldrich or Sigma Chemicals or Lancaster Synthesis. They were stored away from moisture and light and dried before use where necessary. Silica gel refers to Merck silica gel grade 60. Product(s) and starting material were detected by either viewing under UV light or treating with a methanolic solution of phosphomolybic acid followed by heating. NMR spectra were determined using acetone-$d_6$, $CDCl_3$ or DMSO-$d_6$ as solvent and TMS as internal standard, unless otherwise stated. The $^1$H NMR spectra were recorded on a Jeol GX 270 or on a Jeol EX 400 NMR spectrometer. Chemical shifts are given in $\delta$ units. The following abbreviations are used to describe resonances in $^1$H NMR spectra: s, singlet; d, doublet; br, broad; t, triplet; m, multiplet and combination such as dd, doublet of doublets. IR spectra were determined as KBr discs, using a Perkin–Elmer 782 Infrared Spectrophotometer. UV spectra were determined using a Perkin–Elmer Lambda 3B (UV–vis) spectrophotomer. Melting points were determined on a Reichert-Jung Kofler Block and were uncorrected. Mass spectra were recorded on VG 7070 and VG Autospec instruments at the Mass Spectrometry Service at the University of Bath. FAB−mass spectra were carried out using *m*-nitrobenzyl alcohol (*m*-NBA) as the matrix. CHN analysis was determined using gas chromatography at the Microanalysis Service at the University of Bath. LC/MS was carried out using Waters 2790 Alliance, ZQ MicroMass spectrometer and PDA detector under the following conditions, Ionisation technique: electrospray; Column: Waters 'Symmetry' C18 (packing: 3.5 μm), 4.6×100 mm; elution: gradient (flow rate): 5:95 MeOH/$H_2O$ (0.5 mL/min) to 95:5 MeOH/$H_2O$ (1.0 mL/min) over 22 min.

### 5.2. Determination of the acidity constant (p*K*a) of 4,4′-dihydroxybenzophenone and bis-(4-hydroxyphenyl)methane

The general procedure of Albert and Serjeant[34] was used for the study. A 5 mM solution of 4,4′-dihydroxybenzophenone or bis-(4-hydroxyphenyl)methane in methanol/water (1:1, 50 mL) was prepared and its pH at room temperature read (WPA Linton Cambridge UK, CCMD625 pH meter). The titrant (aqueous NaOH, 100 mM) was then added in equal portions. The pH was recorded after each addition of titrant when equilibrium was reached (after stirring). Titrations were carried out in duplicate and the average p*K*a value was presented. The p$Ka_1$ and p$Ka_2$ values of the compound were taken as the respective pH of the mixture after 1.25 and 3.75 mL of titrant was added and were uncorrected.

### 5.3. Stability study of BENZOMATE

**5.3.1. As monitored by UV spectroscopy.** To select the optimum wavelength for monitoring the degradation of BENZOMATE, the UV spectrum of BENZOMATE and its putative degradation products, that is compound **5** (Scheme 1) and 4,4′-dihydroxybenzophenone, in PBS (freshly prepared by dissolving one tablet of PBS in 100 mL of distilled water) containing 0.25 M sucrose was individually determined. The $\lambda_{max}$ for BENZOMATE, compound **5**, 4,4′-dihydroxybenzophenone and PBS containing 0.25 sucrose was found to be 260, 295, 296 and 374 nm, respectively. The UV spectra of compound **5** and 4,4′-dihydroxybenzophenone are similar.

Approximately 5 mg of BENZOMATE was dissolved in 1 mL of HPLC grade methanol. Of this freshly prepared methanolic solution, 30 and 300 μL were, respectively, added to 2970 and 2700 μL of PBS containing 0.25 M sucrose in a cuvette. The mixtures were kept in a water bath at 37 °C during the course of the experiment. Each cuvette was removed from the water bath and stirred magnetically before absorbance was determined at intervals between 0 and 8 h. The absorbance of the mixture at 325 nm was determined, since at this wavelength the absorbance of BENZOMATE is minimal in contrast to its putative degradation products, which still absorb UV strongly. Blanks were prepared without the sample. The decomposition percentage versus time was plotted (Fig. 4).

**5.3.2. As monitored by LC/MS.** To follow the course of the breakdown of BENZOMATE, a mixture of BENZOMATE in PBS containing 0.25 M sucrose and 10% methanol was prepared as described above and was kept at 37 °C in a water bath throughout the experiment.

Aliquots were removed at intervals between 0 and 5 h and assessed by LC/MS. The retention time of 4,4′-dihydroxybenzophenone, compound **5** and BENZOMATE was found to be around 8–9, 9–10 and 10–11 min, respectively. The results at $t = 70$ min are shown in Figure 5.

### 5.4. Biological assay of sulfamates

In vitro studies in placental microsomal (100,000 g) preparations or in intact MCF-7 breast cancer cells, and in vivo studies in rats were performed essentially as described previously.[35] For details, see legends of individual figure and tables.

### 5.5. General method for sulfamoylation

Starting with the parent compound, the sulfamate derivatives were prepared essentially as previously described.[36] In this regard, a solution of the appropriate parent compound in anhydrous DMF (or another solvent as specified) was treated with sodium hydride [60% dispersion; 1.2 and 2.5 equiv. for monohydroxyl and dihydroxyl compounds, respectively, unless stated otherwise] at 0 °C under an atmosphere of $N_2$. After evolution of hydrogen had ceased, a freshly concentrated solution of sulfamoyl chloride in toluene[36] [excess, ca. 5–6 equiv] was added and the reaction mixture poured into brine after warming to room temperature overnight. Ethyl acetate was added and the organic fraction that separated was washed exhaustively with brine, dried ($MgSO_4$), filtered and evaporated under reduced pressure. In general, the crude product obtained was purified by flash chromatography or preparative TLC followed by recrystallisation to give the corresponding sulfamate. All new compounds were characterised by spectroscopic and combustion analysis.

### 5.6. Synthesis of sulfamates

**5.6.1. Benzophenone-4,4′-*O*,*O*-bis-sulfamate (3) and azomethine derivative (4).** Upon sulfamoylation, 4,4′-dihydroxybenzophenone (1.0 g, 4.67 mmol) gave a crude product (1.63 g), which was fractionated by flash chromatography (chloroform/acetone gradient). The band at $R_f$ 0.46 (chloroform/acetone, 2:1) gave a creamy residue (1.17 g), which was further purified by recrystallisation from acetone/chloroform (1:2) to give **3** as white crystals (730 mg, 43%); mp 182–184 °C; $\nu_{max}$ (KBr) 3340 ($NH_2$), 1660 (C=O), 1370 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 7.36 (4H, br s, ex. with $D_2O$, $2\times OSO_2NH_2$), 7.51 (4H, d, $J$ 8.8 Hz, ArH) and 7.9 (4H, d, $J$ 8.4 Hz, ArH); MS $m/z$ (FAB+) 372.9 [100, $(M+H)^+$], 293.0 [30, $(M+H-SO_2NH_2)^+$], 213.1 [10, $(M+H-2\times SO_2NH_2)^+$]; MS $m/z$ (FAB−) 370.9 [100, $(M-H)^-$], 291.9 [82, $(M-SO_2NH_2)^-$], 213.0 [20, $(M+H-2\times SO_2NH_2)^-$]; Acc. MS (FAB+) 373.0177, $C_{13}H_{13}N_2O_7S_2$ requires 373.0164. Found C, 41.9; H, 3.24; N, 7.50; $C_{13}H_{12}N_2O_7S_2$ requires C, 41.93; H, 3.25; N, 7.52.

The band at $R_f$ 0.57 (chloroform/acetone, 2:1) gave a beige residue (183 mg), which was further purified by recrystallisation from acetone/hexane (1:2) to give **4** as white crystals (130 mg, 7%); mp 158–160 °C; $\nu_{max}$ (KBr) 3340–3240 ($NH_2$), 1650 (C=O), 1370 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 3.09 (3H, s, $NCH_3$), 3.3 (3H, s, $NCH_3$), 7.36 (2H, br s, ex. with $D_2O$, $OSO_2NH_2$), 7.48 (2H, d, $J$ 8.79 Hz, ArH), 7.52 (2H, d, $J$ 8.8 Hz, ArH) 7.82 (2H, d, $J$ 8.8 Hz, ArH), 7.84 (2H, d, $J$ 8.8 Hz, ArH) and 8.14 (1H, s, $SO_2N=CH$); MS $m/z$ (FAB+) 427.9 [100, $(M+H)^+$], 349.0 [20, $(M+H-SO_2NH)^+$], 294.1 [10, $(M+2H-SO_2N=CH-N(Me)_2)^+$]; MS $m/z$ (FAB−) 425.9 [100, $(M-H)^-$], 347.0 [40, $(M-SO_2NH_2)^-$], 293.0 [10, $(M+H-SO_2N=CH-N(Me)_2)^-$]; Acc. MS (FAB+) 428.0582, $C_{16}H_{18}N_3O_7S_2$ requires 428.0586.

**5.6.2. 4′-Hydroxybenzophenone-4-*O*-sulfamate (5).** Upon sulfamoylation using 1 equiv of NaH, 4,4′-dihydroxybenzophenone (300 mg, 1.40 mmol) gave a crude product (420 mg) of which 100 mg was fractionated on preparative TLC (chloroform/acetone, gradient). The white residue that isolated (46 mg) was further purified by recrystallisation from ethyl acetate/hexane (1:2) to give **5** as white crystals (32 mg, 32%); mp > 152 °C (dec); TLC (chloroform/acetone, 4:1 and 2:1): $R_f$s 0.27 and 0.41, respectively; $\nu_{max}$ (KBr) 3500–3000 ($NH_2$ and OH), 1630 (C=O), 1590, 1380 ($SO_2$) $cm^{-1}$, $^1H$ NMR (270 MHz, acetone-$d_6$) 7.0 (2H, d, $J$ 8.8 Hz, C3′-H, and C5′-H), 7.3 (2H, br s, ex. with $D_2O$, $OSO_2NH_2$), 7.48 (2H, d, $J$ 8.4 Hz, C3-H, and C5-H), 7.77 (2H, d, $J$ 8.4 Hz, ArH), 7.8 (2H, d, $J$ 8.42 Hz, ArH) and 8.1 (1H, br s, ex. with $D_2O$, OH); MS $m/z$ (FAB+) 447.1 [10, $(M+H+NBA)^+$], 294.0 [100, $(M+H)^+$], 215.1 [10, $(M+H-SO_2NH)^+$]; MS $m/z$ (FAB−) 446.1 [20, $(M+NBA)^-$], 292.1 [100, $(M-H)^-$], 213.1 [30, $(M-SO_2-NH_2)^-$]. Found C, 53.1; H, 3.9; N, 4.55; $C_{13}H_{11}NO_5S$ requires C, 53.24; H, 3.78; N, 4.78.

**5.6.3. Benzophenone-4-*O*-sulfamate (6).** Upon sulfamoylation, 4-hydroxybenzophenone (1.0 g, 5.05 mmol) gave a crude product (1.45 g), which was fractionated by flash chromatography (chloroform/acetone, 8:1). The creamy residue that isolated (716 mg) was further purified by recrystallisation from ethyl acetate/hexane (1:2) to give **6** as white crystals (495 mg, 35%); mp 134–136 °C [lit.[17] 140–142 °C]; TLC (chloroform/acetone, 4:1 and 8:1): $R_f$s 0.68 and 0.35, respectively; $\nu_{max}$ (KBr) 3360 ($NH_2$), 1390 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 7.35 (2H, br s, ex. with $D_2O$, $SO_2NH_2$), 7.5 (2H, d, $J$ 8.8 Hz, C3-H and C5-H), 7.55–7.75 (3H, m, C3′-H, C4′-H and C5′-H), 7.84 (2H, m, C2′-H and C6′-H), 7.88 (2H, d, $J$ 8.8 Hz, C2-H and C6-H); MS $m/z$ (FAB+) 278.0 [100, $(M+H)^+$], 198.0 [10, $(M+H-SO_2NH_2)^+$]; MS $m/z$ (FAB−) 430.0 [15, $(M+NBA)^-$], 276.0 [100, $(M-H)^-$], 197.0 [25, $(M-SO_2NH_2)^-$]; Acc. MS $m/z$ (FAB+) 278.0503, $C_{13}H_{12}NO_4S$ requires 278.0487. Found C, 56.1; H, 4.01; N, 5.12; $C_{13}H_{11}NO_4S$ requires C, 56.31; H, 4.0; N, 5.05.

**5.6.4. *trans*-Chalcone-4-*O*-sulfamate (7).** Upon sulfamoylation, *trans*-4-hydroxychalcone (1.0 g, 4.46 mmol) gave a crude product (1.44 g), which was fractionated by flash chromatography (chloroform/acetone, 8:1). The creamy residue that obtained (719 mg) was further purified by recrystallisation from ethyl acetate/hexane (1:2) to give **7** as white crystals (464 mg, 34%); mp 136–138 °C; TLC (chloroform/acetone, 8:1, 4:1 and 2:1): $R_f$s 0.24, 0.48 and 0.66, respectively; $\nu_{max}$ (KBr) 3500–3220 ($NH_2$), 1670 (C=O), 1390 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 7.27 (2H, s, ex. with $D_2O$, $OSO_2NH_2$), 7.42 (2H, d, $J$ 8.79 Hz, C3-H and C5-H), 7.62 (2H, m, C3′-H, C4′-H and C5′-H), 7.85 (2H, d, $J$ 14.7 Hz, CH=CH), 7.95 (5H, d, $J$ 6.2 Hz, C2-H and C6-H) and 8.18 (2H, d, $J$ 7.7 Hz, C2′-H and C6′-H); MS $m/z$ (FAB+) 304.0 [100, $(M+H)^+$], 224.1 [10, $(M+H-SO_2NH_2)^+$]; MS $m/z$ (FAB−) 302.0 [100, $(M-H)^-$], 223.0 [40, $(M-SO_2NH_2)^-$]; Acc. MS (FAB+) 304.0646, $C_{15}H_{14}NO_4S$ requires 304.0644. Found C, 59.1; H, 4.29; N, 4.64 $C_{15}H_{13}NO_4S$ requires C, 59.40; H, 4.32; N, 4.62.

**5.6.5. *trans*-Chalcone-4′-*O*-sulfamate (8).** Upon sulfamoylation, *trans*-4′-hydroxychalcone (1.0 g, 4.46 mmol) gave a crude product (1.5 g), which was fractionated by flash chromatography (chloroform/acetone, 8:1). The creamy residue that obtained (723 mg) was further purified by recrystallisation from ethyl acetate/hexane (1:2) to give **8** as white crystals (425 mg, 31%); mp 187–189 °C [lit.[17] 190–193 °C]; TLC (chloroform/acetone, 8:1, 4:1 and 2:1): $R_f$s 0.17, 0.26 and 0.62, respectively; $^1H$ NMR (270 MHz, acetone-$d_6$) 7.34 (2H, s, ex. with $D_2O$, $OSO_2NH_2$), 7.49 (5H, m, C-3′, 5′, 3, 4 and C5-H), 7.88 (4H, m, CH=CH and C2-H, C6-H), 8.25 (2H, d, $J$ 8.8 Hz, C2′-H and C6′-H); MS $m/z$ (FAB+) 304.0 [100, $(M+H)^+$], 225.1 [10, $(M+2H-SO_2NH_2)^+$]; MS $m/z$ (FAB−) 302.0 [100, $(M-H)^-$], 223.1 [35, $(M-SO_2NH_2)^-$]; Acc. MS (FAB+) 304.0654, $C_{15}H_{14}NO_4S$ requires 304.0644. Found C, 59.1; H, 4.29; N, 4.65; $C_{15}H_{13}NO_4S$ requires C, 59.40; H, 4.32; N, 4.62.

**5.6.6. Dibenzofuran-2-*O*-sulfamate (9).** Upon sulfamoylation, 2-hydroxydibenzofuran (1.0 g, 5.43 mmol) gave a crude product (1.31 g), which was fractionated by flash chromatography (chloroform/acetone, 8:1). The beige residue that obtained (561 mg) was further purified by recrystallisation from ethyl acetate/hexane (1:2) to give **9** as white crystals (319 mg, 23%); mp > 120 °C (dec); TLC (chloroform/acetone, 8:1, 4:1 and 2:1): $R_f$s 0.31, 0.55 and 0.70, respectively; $\nu_{max}$ (KBr) 3400 ($NH_2$), 1600 (C=O), 1370 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 7.18 (2H, br s, ex. with $D_2O$, $SO_2NH_2$), 7.46 (2H, m, C4-H and C5-H), 7.56 (1H, t, $J$ 7.7 Hz, C7-H), 7.7 (2H, t, $J$ 7.3 Hz, C3-H and C6-H), 8.07 (1H, d, $J$ 2.2 Hz, C1-H) and 8.16 (1H, d, $J$ 7.7 Hz, C8-H); MS $m/z$ (FAB+) 417.0 [13, $(M+H+NBA)^+$], 263.1 [100, $(M)^+$], 183.1 [60, $(M-SO_2NH_2)^+$]; MS $m/z$ (FAB−) 416.0 [15, $(M+NBA)^-$], 262.0 [100, $(M-H)^-$]. Found C, 54.6; H, 3.46; N, 5.22; $C_{12}H_9NO_4S$ requires C, 54.75; H, 3.42; N, 5.32.

**5.6.7. Anthraquinone-2,6-*O*,*O*-bis-sulfamate (10) and 2-hydroxyanthraquinone-6-*O*-sulfamate (11).** Upon sulfamoylation, 2,6-dihydroxyanthraquinone (1.0 g, 4.16 mmol) gave crude products (1.41 g) of which a 100 mg sample was fractionated on preparative TLC (chloroform/acetone, gradient). The fraction at $R_f$ 0.39 (chloroform/acetone, 2:1) gave a yellow residue (32 mg), which was further purified by recrystallisation from acetone/hexane (1:2) to give **10** as yellow crystals (24 mg, 20%); mp > 220 °C (dec); TLC (chloroform/acetone, 4:1): $R_f$ 0.26; $\nu_{max}$ (KBr) 3380–3260 ($NH_2$), 1680 (C=O), 1390 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 7.57 (4H, br s, ex. with $D_2O$, $2\times SO_2NH_2$), 7.84 (2H, dd, $J$ 2.4 and 8.4 Hz, C3-H and C7-H), 8.17 (2H, d, $J$ 2.6 Hz, C1-H and C5-H) and 8.39 (2H, d, $J$ 8.4 Hz, C4-H and C8-H); MS $m/z$ (FAB+) 398.0 [40, $(M)^+$], 240.1 [30, $(M-2\times SO_2NH)^+$]; MS $m/z$ (FAB−) 551.1 [25, $(M+NBA)^-$], 398.1 [100, $(M)^-$], 239.1 [70, $(M+H-2\times SO_2NH_2)^-$]; Acc. MS $m/z$ (FAB+) 397.9887, $C_{14}H_{10}N_2O_8S_2$ requires 397.9879.

The fraction at $R_f$ 0.46 (chloroform/acetone, 2:1) gave a white residue (46 mg), which was further purified by recrystallisation from ethyl acetate/hexane (1:2) to give **11** as white crystals (31 mg, 21%); mp > 285 °C (dec); TLC (chloroform/acetone, 4:1): $R_f$ 0.3; $\nu_{max}$ (KBr) 3500–3000 (OH, $NH_2$), 1670 (C=O) and 1390 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 7.34 (1H, dd, $J$ 2.6 and 8.4 Hz, C3-H), 7.53 (2H, br s, ex. with $D_2O$, $SO_2NH_2$), 7.65 (1H, d, $J$ 2.6 Hz, C1-H), 7.77 (1H, dd, $J$ 2.4 and 8.4 Hz, C7-H), 8.13 (1H, d, $J$ 2.6 Hz, C5-H), 8.2 (1H, d, $J$ 8.4 Hz, C4-H), 8.33 (1H, d, $J$ 8.4 Hz, C8-H) and 9.98 (1H, s, ex. with $D_2O$, OH); MS $m/z$ (FAB+) 320.0 [100, $(M+H)^+$], 240.1 [30, $(M+H-SO_2NH_2)^-$], 225.1 (15); MS $m/z$ (FAB−) 473.2 [20, $(M+H+NBA)^-$], 318.1 [100, $(M-H)^-$], 239.1 [90, $(M-SO_2NH_2)^-$]; Acc. MS (FAB+) 320.0224, $C_{14}H_{10}NO_6S$ requires 320.0229. Found C, 52.5; H, 2.84; N, 4.25; $C_{14}H_9NO_6S$ requires C, 52.67; H, 2.84; N, 4.39.

**5.6.8. 3-Hydroxybenzo[*b*]naphtho[2,3-*d*]furan-6,11-dione (12).** Resorcinol (6.6 g, 60 mmol) in ethanol (80 mL) was added dropwise to a stirred ethanolic sodium ethoxide solution (prepared by dissolving 3.8 g of sodium metal in 120 mL of absolute ethanol) containing 2,3-dichloro-1,4-naphtho-quinone (6.9 g, 30 mmol) at 0 °C. After stirring at room temperature overnight, the black reaction mixture was acidified with 5 N HCl at 0 °C. The yellow solid that precipitated was collected by filtration, washed successively with water, methanol, diethyl ether and air dried to give crude **12** as a deep yellow solid (7.1 g, 88%); mp > 310 °C [lit.[23] >300 °C]; TLC (chloroform/acetone, 8:1): $R_f$ 0.47; $\nu_{max}$ (KBr) 3400 (OH), 1670 and 1620 (C=O) $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) 7.05 (1H, d, $J$ 8.5 Hz, C4-H), 7.16 (1H, s, C2-H), 7.2–7.7 (2H, m, C8-H and C9-H), 7.95 (1H, d, $J$ 8.5 Hz, C5-H), 8.2–8.7 (2H, m, C7-H and C10-H) and 10.53 (1H, br s, ex. with $D_2O$, C3-OH); MS $m/z$ (FAB+) 265.1 [70, $(M+H)^+$], 255.3 (75), 243.2 (30), 173.2 (100); MS $m/z$ (FAB−) 263.1 [100, $(M-H)^-$], 242.1 (20), 210.1 (25), 198.1 (45), 181.1 (35); Acc. MS (FAB+) 265.0502, $C_{16}H_9O_4$ requires 265.0501.

**5.6.9. Benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione-3-*O*-sulfamate (13).** Upon sulfamoylation, **12** (500 mg, 1.89 mmol) gave a crude product (655 mg), which was fractionated by flash chromatography (chloroform/acetone, gradient). The yellow residue that obtained (510 mg) was further purified by recrystallisation from acetone/hexane (1:3) to give **13** as white crystals (450 mg, 69%); mp > 270 °C (dec); TLC (chloroform/acetone, 8:1): $R_f$ 0.28; $\nu_{max}$ (KBr) 3280, 3380 ($NH_2$), 1680 (C=O), 1380 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, DMSO-$d_6$) 7.52 (1H, dd, *J* 2.0 and 8.4 Hz, C4-H), 7.85–8.0 (3H, m, ArH), 8.1–8.2 (2H, m, ArH), 8.25 (2H, br s, ex. with $D_2O$, $SO_2NH_2$) and 8.27 (1H, d, *J* 8.4 Hz, ArH); MS *m/z* (FAB+) 344.1 [100, $(M+H)^+$], 263.1 [30, $(M-SO_2NH_2)^+$]; MS *m/z* (FAB−) 242.2 [100, $(M-H)^-$]; 264.2 [20, $(M-SO_2NH)^-$]; Acc. MS (FAB+) 344.0218, $C_{16}H_{10}NO_6S$ requires 344.0229. Found C, 55.83; H, 2.68; N, 3.96; $C_{16}H_9NO_6S$ requires C, 55.98; H, 2.64; N, 4.08.

**5.6.10. Sulfonyldiphenyl-4,4′-*O*,*O*-bis-sulfamate (14).** Upon sulfamoylation, 4,4′-sulfonyldiphenol (1.0 g, 4.00 mmol) gave a brown residue (1.56 g). A sample of this crude material (150 mg) was fractionated on preparative TLC (chloroform/acetone, gradient). The pale white residue that obtained (117 mg) was further purified by recrystallisation from acetone/hexane (1:2) to give **14** as white crystals (94 mg, 67%); mp 174–176 °C; TLC (chloroform/acetone, 4:1 and 2:1): $R_f$s 0.22 and 0.43, respectively; $\nu_{max}$ (KBr) 3380–3240 ($NH_2$), 1380 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 7.41 (4H, br s, ex. with $D_2O$, 2×$OSO_2NH_2$) 7.57 (4H, d, *J* 8.8 Hz, C3-H, C5-H, C3′-H and C5′-H) and 8.12 (4H, d, *J* 8.8 Hz, C2-H, C6-H, C2′-H and C6′-H); MS *m/z* (FAB+) 562.1 [10, $(M+H+NBA)^+$], 409.0 [60, $(M+H)^+$], 330.1 [50, $(M+H-SO_2NH)$]; MS *m/z* (FAB−) 407.1 [100, $(M-H)^-$], 328.1 [25, $(M-SO_2NH_2)^-$], 249.1 [25, $(M+H-2\times SO_2NH_2)^-$]; Acc. MS *m/z* (FAB−) 406.9675, $C_{12}H_{11}N_2O_8S_3$ requires 406.9678. Found C, 35.4; H, 3.0; N, 6.71; $C_{12}H_{12}N_2O_8S_3$ requires C, 35.29; H, 2.96; N, 6.86.

**5.6.11. Thiodiphenyl-4,4′-*O*,*O*-bis-sulfamate (15) and 4′-hydroxythiodiphenyl-4-*O*-sulfamate (16).** Upon sulfamoylation, 4,4′-thiodiphenol (760 mg, 3.48 mmol) gave a crude product (1.09 g), which was fractionated by flash chromatography (chloroform/acetone, gradient). The fraction at $R_f$ 0.42 (chloroform/acetone, 2:1) gave a beige residue that (889 mg), which was further purified by recrystallisation from acetone/hexane (1:2) to give **15** as white crystals (665 mg, 50%); mp 142–144 °C; TLC (chloroform/acetone, 4:1): $R_f$ 0.25; $\nu_{max}$ (KBr) 3400–3220 ($NH_2$), 1590, 1390 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 7.22 (4H, br s, ex. with $D_2O$, 2×$OSO_2NH_2$), 7.34 (4H, d, *J* 8.8 Hz, C3-H, C5-H, C3′-H and C5′-H) 7.44 (4H, d, *J* 6.6 Hz, C2-H, C6-H, C2′-H and C6′-H); MS *m/z* (FAB+) 530.1 [10, (M+H+NBA)] 376.0 [100, $(M)^+$], 297.0 [40, $(M+H-SO_2NH_2)^+$]; 217.1 [20, $(M+H-2\times SO_2NH_2)^+$]; MS *m/z* (FAB−) 529.2 [10, $(M+NBA)^-$], 375.1 [100, $(M-H)^-$], 296.1 [40, $(M-SO_2NH_2)^-$]; 216.1 [10, $(M-2SO_2NH_2)^-$]; Acc. MS (FAB+) 376.9905, $C_{12}H_{13}N_2O_6S_3$ requires 376.9936. Found C, 38.8; H, 3.24; N, 7.37; $C_{12}H_{12}N_2O_6S_3$ requires C, 38.29; H, 3.21; N, 7.44.

The fraction at $R_f$ 0.57 (chloroform/acetone, 2:1) gave a white residue (123 mg), which was further purified by recrystallisation from ethyl acetone/hexane (1:4) to give **16** as white crystals (110 mg, 10%); mp 170–172 °C (dec); TLC (chloroform/acetone, 4:1): $R_f$ 0.35; $\nu_{max}$ (KBr) 3500–3000 ($NH_2$ and OH), 1390 ($SO_2$) $cm^{-1}$; $^1H$ NMR (400 MHz, acetone-$d_6$) 6.73 (2H, br s, ex. with $D_2O$, $OSO_2NH_2$), 6.95 (2H, d, *J* 8.5 Hz, C3′-H and C5′-H), 7.18 (2H, d, *J* 8.8 Hz, ArH), 7.25 (2H, d, *J* 8.8 Hz, ArH), 7.42 (2H, d, *J* 8.5 Hz, ArH) and 8.97 (1H, br s, ex. with $D_2O$, OH); MS *m/z* (FAB+) 297.0 [100, $(M)^+$], 217.1 [20, $(M-SO_2NH_2)^+$]; MS *m/z* (FAB−) 450.1 [20, $(M+NBA)^-$], 296.1 [100, $(M-H)^-$]; 216.1 [20, $(M-H-SO_2NH_2)^-$]; Acc. MS (FAB+) 297.0129, $C_{12}H_{11}NO_4S_2$ requires 297.0129. Found C, 48.2; H, 3.89; N, 4.57; $C_{12}H_{11}NO_4S_2$ requires C, 48.47; H, 3.73; N, 4.71.

**5.6.12. 4,4′-Dihydroxydiphenylmethane (17).** To a solution of 4,4′-dihydroxybenzophenone (1.0 g, 4.67 mmol) in ethanol (25 mL) Pd–C (10%, 200 mg) was added and the resulting suspension was subjected to hydrogenation at balloon pressure at room temperature for 6 h. Upon removal of the supported catalyst by filtration, the filtrate was evaporated to give a white residue (1.02 g), which was recrystallised from ethyl acetate/hexane (1:2) to give **17** as white crystals (854 mg, 91%); mp 158–160 °C [lit.[24] 161–162 °C]; TLC (chloroform/acetone, 8:1 and 4:1): $R_f$s 0.28 and 0.63, respectively; $\nu_{max}$ (KBr) 3500–3000 (OH), 1600 $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 3.8 (2H, s, $CH_2$), 6.75 (4H, m, ArH), 7.03 (4H, m, ArH) and 8.1 (2H, br s, ex. with $D_2O$, 2×OH); MS *m/z* (FAB+) 200.1 [100, $(M)^+$]; Acc. MS (FAB+) 200.0842, $C_{13}H_{12}O_2$ requires 200.0915.

**5.6.13. 4,4′-Bis-sulfamoyloxydiphenylmethane (18) and 4-sulfamoyloxy-4′-hydroxydiphenylmethane (19).** Upon sulfamoylation, **17** (825 mg, 4.12 mmol) gave a beige crude product (1.3 g), which was fractionated by flash chromatography (chloroform/acetone, gradient). The fraction at $R_f$ 0.43 (chloroform/acetone, 4:1) gave a white residue (471 mg), which was further purified by recrystallisation from acetone/chloroform (1:2) to give **18** as white crystals (389 mg, 26%); mp 175–177 °C; $\nu_{max}$ (KBr) 3500–3000 ($NH_2$), 1390 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 4.03 (2H, s, $CH_2$), 7.10 (4H, br s, ex. with $D_2O$, 2×$OSO_2NH_2$), 7.25 (4H, d, *J* 8.8 Hz, C2-H, C2′-H, C6-H and C6′-H) and 7.34 (4H, d, *J* 8.8 Hz, C3-H, C3′-H, C5-H and C5′-H); MS *m/z* (FAB+) 512.0 [40, $(M+H+NBA)^+$], 358.0 [90, $(M)^+$], 279.0 [50, $(M+H-SO_2NH_2)^+$]; MS *m/z* (FAB−) 511.1 [40, $(M+NBA)^+$], 357.1 [100, $(M-H)^+$], 278.0 [30, $(M-SO_2NH_2)^+$]. Found C, 43.5; H, 3.89; N, 7.64; $C_{13}H_{14}O_6N_2S_2$ requires C, 43.57; H, 3.94; N, 7.82.

The fraction at $R_f$ 0.52 (chloroform/acetone, 4:1) gave a beige residue (150 mg), which was recrystallised from

acetone/hexane (1:2) to give **19** as white crystals (120 mg, 10%); mp 128–130 °C; TLC (chloroform/acetone, 8:1): $R_f$ 0.27; $\nu_{max}$ (KBr) 3500–3300 ($NH_2$), 3240 (OH) 1390 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 3.90 (2H, s, $CH_2$), 6.77 (2H, d, $J$ 8.8 Hz, C3′-H and C5′-H), 7.07 (4H, d, $J$ 8.1 Hz, 2H ex. with $D_2O$, ArH and $OSO_2NH_2$), 7.21 (2H, d, $J$ 8.8 Hz, ArH), 7.28 (2H, d, $J$ 8.8 Hz, ArH) and 8.18 (1H, br s, ex. with $D_2O$, OH); MS $m/z$ (FAB+) 279.0 [100, $(M)^+$], 200.1 [30, $(M+H-SO_2NH_2)^+$]; MS $m/z$ (FAB−) 432.2 [40, $(M+NBA)^-$], 278.1 [100, $(M-H)^-$]; Acc. MS (FAB+) 279.0584, $C_{13}H_{13}O_4NS$ requires 279.0643. Found C, 56.0; H, 5.16; N, 4.74; $C_{13}H_{13}O_4NS$ requires C, 55.90; H, 4.69; N, 5.01.

**5.6.14. (1,3-Adamantanediyl)diphenyl-4,4′-*O*,*O*-bis-sulfamate (20) and 4′-hydroxy-(1,3-adamantanediyl)diphenyl-4-*O*-sulfamate (21).** Upon sulfamoylation, 4,4′-(1,3-adamantanediyl)diphenol (210 mg, 655 μmol) gave a crude product (273 mg), which was fractionated by flash chromatography (chloroform/acetone, gradient). The fraction at $R_f$ 0.25 (chloroform/acetone, 4:1) gave a white residue (159 mg), which was further purified by recrystallisation from acetone/hexane (1:2) to give **20** as white crystals (112 mg, 36%); mp 197–199 °C; $\nu_{max}$ (KBr) 3420–3240 ($NH_2$), 1390 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 1.8–2.25 (14H, m), 7.12 (4H, br s, ex. with $D_2O$, 2×$SO_2NH_2$), 7.24–7.32 (4H, AA′BB′, ArH), 7.48–7.56 (4H, AA′BB′, ArH); MS $m/z$ (FAB+) 478.1 [80, $(M)^+$], 399.2 [30, $(M+H-SO_2NH_2)^+$]; MS m/z (FAB−) 631.2 [30, $(M+NBA)^-$], 477.2 [100, $(M-H)^-$], 398.2 [20, $(M-SO_2NH_2)^-$]; Acc. MS (FAB+) 478.1227, $C_{22}H_{26}N_2O_6S_2$ requires 478.1232. Found C, 55.1; H, 5.63; N, 5.85; $C_{22}H_{26}N_2O_6S_2$ requires C, 55.21; H, 5.48; N, 5.85.

The fraction at $R_f$ 0.39 (chloroform/acetone, 4:1) gave a white residue (38 mg), which was further purified by recrystallisation from ethyl acetate/hexane (1:2) to give **21** as white crystals (20 mg, 8%); mp 140–142 °C; $\nu_{max}$ (KBr) 3500–3000 ($NH_2$ and OH), 1370 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 1.8–2.29 (14H, m), 6.78 (2H, d, $J$ 8.8 Hz, C3′-H and C5′-H), 7.08 (2H, br s, ex. with $D_2O$, $SO_2NH_2$), 7.26 (4H, br d, $J$ 8.79 Hz, ArH), 7.5 (2H, d,$J$ 8.8 Hz, ArH) and 8.15 (1H, br s, ex. with $D_2O$, OH); MS $m/z$ (FAB+) 399.1 [100, $(M)^+$], 320.2 [15, $(M+H-SO_2NH_2)^-$], 306.1 (45), 288.1 (15); MS $m/z$ (FAB−) 552.3 [30, $(M+NBA)^-$], 398.2 [100, $(M-H)^-$]; Acc. MS (FAB+) 400.1533, $C_{22}H_{26}NO_4S$ requires 400.1583.

**5.6.15. 4,4′-(Di-*tert*-butyldimethylsilyloxy)benzophenone (22).** To a solution of 4,4′-dihydroxybenzophenone (5.0 g, 23.3 mmol) in anhydrous DMF (15 mL), *tert*-butyldimethyl-chlorosilane (4.22 g, 28 mmol) and imidazole (4.0 g, 58.8 mmol) were added and the reaction mixture stirred under $N_2$ for 3 h. After dilution with 5% aq $NaHCO_3$ (100 mL) and ethyl acetate (200 mL), the organic layer that separated was washed with water, brine, dried ($MgSO_4$), filtered and evaporated to give **22** as a pale white oil, which solidified on standing (10 g, 97%); TLC (chloroform): $R_f$ 0.69; $\nu_{max}$ (KBr) 1620 (C=O) $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) 0.24 (12H, s, 2×$Si(CH_3)_2$), 1.0 (18H, s, 2×$(CH_3)_3$), 6.9 (4H, AA′BB′, C3-H, C5-H, C3′-H and C5′-H) and 7.73 (4H, AA′BB′, C2-H, C6-H, C2′-H and C6′-H); MS $m/z$ (FAB+) 443.4 [60, $(M+H)^+$], 385.3 [10, $(M-C(CH_3)_3)^+$], 235 (90), 73.0 (100); MS $m/z$ (FAB−) 441.3 [10, $(M-H)^-$], 401.2 (10), 327.2 [100, $(M-H-2\times C(CH_3)_3)^+$], 255.1 (10). Found C, 67.6; H, 8.64; $C_{25}H_{38}O_3Si_2$ requires C, 67.82; H, 8.65.

**5.6.16. 1-Cyclohexyl-1,1-di-[(4-(*tert*-butyldimethylsilyloxy)-phenyl]methanol (23).** To a solution of **22** (500 mg, 1.13 mmol) in dry ether (50 mL), cyclohexyl magnesium chloride (1.2 mL, 2.4 mmol) was added dropwise with stirring at 0 °C under $N_2$. The reaction mixture after being stirred overnight at room temperature was diluted with dilute HCl and extracted with ether. The combined ethereal extracts were washed with water, brine, dried ($MgSO_4$), filtered and evaporated to give crude **23** as a pale white oil (550 mg, 92%). TLC analysis of this crude has shown a single spot although $^1H$ NMR has indicated the presence of starting material (ca. 10–20%); TLC (chloroform): $R_f$ 0.61; $\nu_{max}$ (film) 3420 (OH), 1600 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) 0.14 (12H, s, 2×$Si(CH_3)_2$), 0.92 (18H, s, 2×$(CH_3)_3$), 0.96–2.32 (11H, m), 4.95 (1H, s, ex. with $D_2O$, OH), 6.7 (4H, d, $J$ 8.5 Hz, C3-H, C5-H, C3′-H and C5′-H) and 7.31 (4H, d, $J$ 8.8 Hz, C2-H, C6-H, C2′-H and C6′-H); MS $m/z$ (FAB+) 525.5 [30, $(M-H)^+$], 443.4 [100, $(M-cyclohexyl)^+$], 427.4 (50), 319 (20), 73.0 (60); MS $m/z$ (FAB−) 679.0 [10, $(M+NBA)^-$], 525.5 [40, $(M-H)^-$], 441.3 [20, $(M-H-cyclohexyl)^-$], 411.4 (100), 327.3 [50, $(M-cyclohexane-Si(CH_3)_2C(CH_3)_3]^-$, 317.3 (40); Acc. MS (FAB+) 526.3214, $C_{31}H_{50}O_3Si_2$ requires 526.3298.

**5.6.17. 1-Cyclohexyl-1,1-di-(4-hydroxyphenyl)methanol (24).** To a solution of **23** (500 mg, 950 μmol) in dry THF (10 mL), *tetra-n*-butylammonium fluoride (5.6 mL of 1 M solution in THF, 5.68 mmol) was added dropwise. The resulting mixture was stirred for about 5–10 min and then diluted with ethyl acetate (100 mL). The organic layer was washed with water (100 mL), dried ($MgSO_4$), filtered and evaporated to give a white solid (280 mg), which was recrystallised from acetone/hexane (1:2) to give **24** as white crystals (260 mg, 92%); mp 115–117 °C; TLC (chloroform and chloroform/acetone, 8:1): $R_f$s 0.21 and 0.52; $\nu_{max}$ (KBr) 3500–3140 (OH), 1600 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) 0.96–2.26 (11H, m), 4.74 (1H, s, ex. with $D_2O$, OH), 6.66 (4H, d, $J$ 8.8 Hz, C3-H, C5-H, C3′-H and C5′-H), 7.21 (4H, d, $J$ 8.8 Hz, C2-H, C6-H, C2′-H and C6′-H) and 9.1 (2H, s, ex. with $D_2O$, 2×OH); MS $m/z$ (FAB+) 298.2 [10, $(M)^+$], 215.1 [100, $M-cyclohexyl]^+$, 205.2 (15); MS $m/z$ (FAB−) 451.3 [40, $(M+NBA)^-$], 297.3 [100, $(M-H)^-$], 279.3 (30), 213.2 [30, $(M-H-cyclohexane)^-$]; Acc. MS (FAB+) 298.1558, $C_{19}H_{22}O_3$ requires 298.1569.

**5.6.18. 1-Cyclohexyl-1,1-di-(4-sulfamoyloxyphenyl)methanol (25).** To a solution of **24** (150 mg, 503 μmol) and 2,6-di-*tert*-butyl-4-methylpyridine (930 mg, 4.53 mmol) in dichloromethane (25 mL) was added dropwise with

stirring a freshly concentrated solution of sulfamoyl chloride in toluene (ca. 0.7 M, 3.0 mmol). After 2 h of stirring, the solution was diluted with dichloromethane (100 mL). The organic layer was washed with water, brine, dried ($MgSO_4$), filtered and evaporated to give a residue (450 mg), which was fractionated by flash chromatography (chloroform/acetone, gradient). The white solid that obtained (180 mg) was further purified by recrystallisation from acetone/hexane (1:2) to give **25** as pale white crystals (120 mg, 52%); mp 213–215 °C (dec); TLC (chloroform, and chloroform/acetone, 8:1); $R_f$s 0.12 and 0.31, respectively; $\nu_{max}$ (KBr) 3500–3000 ($NH_2$), 1600, 1380 ($SO_2$) $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) 1.31 (1H, br s), 1.57 (6H, br s), 2.15 (4H, br s), 7.25 (8H, m, ArH), 8.0 (4H, br s, ex. with $D_2O$, $SO_2NH_2$) and 9.68 (1H, br s, ex. with $D_2O$, OH); MS $m/z$ (FAB+) 457.1 [10, $(M+H)^+$], 206.2 (100); MS $m/z$ (FAB−) 608.2 [40, $(M-H+NBA)^-$], 455 [15, $(M-H)^-$], 249.0 (100); Acc. MS (FAB+) 457.1065, $C_{19}H_{25}N_2O_7S_2$ requires 457.1103.

**5.6.19. 3,3′-Dinitrobenzophenone (26).** To a stirred mixture of benzophenone (10 g, 54.88 mmol) in concd sulfuric acid (55 mL) at room temperature was added a mixture of concd nitric acid (6 mL) and concd sulfuric acid (14 mL). The reaction mixture was then slowly heated to 75 °C, and maintained at this temperature for 30 min. After cooling to room temperature, the reaction mixture was poured onto crushed ice. The gummy mass that formed hardened over time and after being grinded into powder was washed with water until the washings were neutral. The cake/powder that collected was air dried and recrystallised from butanone (73 mL). The solid that formed overnight was washed first with butanone and then with ethanol to give crude **26** as a yellow solid (7.9 g, 53%). An analytical sample was further recrystallised from methanol to give **26** as pale yellow crystals; mp 144–146 °C (lit.[25] 148–149 °C); TLC (chloroform/acetone, 8:1): $R_f$ 0.8; $\nu_{max}$ (KBr) 1670 (C=O) $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) 7.78 (2H, t, $J$ 7.9 Hz, C5-H and C5′-H), 8.15 (2H, dt, $J \sim 2$ and 7.7 Hz, ArH), 8.53 (2H, ddd, $J \sim 1$–2 and 8.2 Hz, ArH) and 8.64 (2H, t, $J \sim 2$ Hz, C2-H and C2′-H); MS $m/z$ (FAB+) 273.0 [100, $(M+H)^+$], 259.1 (90), 243.1 (50); MS $m/z$ (FAB−) 425.2 [30, $(M+NBA)^-$], 272.1 [100, $(M)^-$], 257.2 (10); Acc. MS (FAB+) 273.0500, $C_{13}H_9N_2O_5$ requires 273.0512. Found C, 57.1; H, 2.89; N, 10.30; $C_{13}H_8N_2O_5$ requires C, 57.36; H, 2.96; N, 10.29.

**5.6.20. 3,3′-Dihydroxybenzophenone (27).** A mixture of **26** (5.0 g, 18.37 mmol), tin chloride (25 g, 131.9 mmol) and concentrated hydrochloric acid (35 mL) was stirred at 70 °C for 6 h. The dark yellow crystalline benzophenone-3,3′-diammonium chlorostannate that separated was collected by filtration. After re-suspending the solid collected in concentrated hydrochloric acid (35 mL) at 0 °C, this cold suspension was added dropwise to a cold solution (at 0 °C) of sodium nitrite (2.56 g in 10 mL water). After the addition was completed, the suspension was stirred at 0 °C for 1 h and then quickly filtered by means of a cold glass funnel. The resulting yellow precipitate (tetrazonium salt) was added (cold, 0–5 °C) in small portions to boiling 1 N sulfuric acid (100 mL). The resulting red solution was heated with charcoal for decolourisation and the suspension was filtered hot. On cooling the filtrate, the yellow precipitate that formed was collected by filtration, washed with water and air dried to give a yellow powder (1.92 g, 49%); which was recrystallised from acetone/hexane (1:2) to give **27** as yellow crystals (1.3 g, 33%); mp 161–163 °C (lit.[25] 163–164 °C); TLC (chloroform/acetone, 8:1): $R_f$ 0.63; $\nu_{max}$ (KBr) 3500–3000 (OH), 1630 (C=O) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 7.12 (2H, m, C4-H and C4′-H), 7.23 (4H, m, C6-H, C6′-H, C2-H and C2′-H), 7.38 (2H, t, $J$ 8.1 Hz, C5-H and C5′-H) and 8.74 (2H, br s, ex. with $D_2O$, 2×OH); MS $m/z$ (FAB+) 215.1 [100, $(M+H)^+$], 199.1 (10), 185.1 (15); MS $m/z$ (FAB−) 367.2 [40, $(M+NBA)^+$], 213.1 [50, $(M-H)^-$], 139.1 (5); Acc. MS (FAB+) 215.0715, $C_{13}H_{11}O_3$ requires 215.0708.

**5.6.21. Benzophenone-3,3′-*O*,*O*-bis-sulfamate (28) and 3′-hydroxybenzophenone-3-*O*-sulfamate (29).** Upon sulfamoylation, **27** (1.0 g, 4.67 mmol) gave a dark yellow residue (1.42 g), which was fractionated by flash chromatography (chloroform/acetone, gradient). The band at $R_f$ 0.21 (chloroform/acetone, 4:1) gave a yellow residue (567 mg), which was further purified by recrystallisation from acetone/hexane (1:2) to give **28** as yellow crystals (310 g, 18%); mp 141–143 °C; TLC (chloroform/acetone, 4:1): $R_f$ 0.42; $\nu_{max}$ (KBr) 3360–3580 ($NH_2$), 1650 (C=O), 1380 ($SO_2$); $^1H$ NMR (270 MHz, acetone-$d_6$) 7.29 (4H, br s, ex. with $D_2O$, 2×$SO_2NH_2$) 7.65 (4H, m, ArH) and 7.77 (4H, m, ArH); MS $m/z$ (FAB+) 373.0 [100, $(M+H)^+$], 211.1 [20, $(M-H-2\times SO_2NH_2)^+$]; MS $m/z$ (FAB−) 371.1 [100, $(M-H)^-$]; Acc. MS (FAB+) 373.0165, $C_{13}H_{13}N_2O_7S_2$ requires 373.0164. Found C, 42.2; H, 3.23; N, 7.21; $C_{13}H_{12}N_2O_7S_2$ requires C, 41.93; H, 3.25; N, 7.52.

The fraction at $R_f$ 0.56 (chloroform/acetone, 2:1) gave a yellow residue (215 mg), which was further purified by recrystallisation from ethyl acetate/hexane (1:2) to give **29** as yellow crystals (163 mg, 12%); mp 190–192 °C; TLC (chloroform/acetone, 4:1): $R_f$ 0.31; $\nu_{max}$ (KBr) 3220–3000 ($NH_2$ and OH), 1640 (C=O), 1400 ($SO_2$) $cm^{-1}$; $^1H$ NMR (270 MHz, acetone-$d_6$) 6.64 (2H, br s, ex. with $D_2O$, $OSO_2NH_2$), 7.17 (1H, t, $J$ 7.5 Hz, C5′-H), 7.47 (2H, m, C2′-H and C4′-H), 7.58 (2H, dd, $J$ 8.1 Hz, C4-H and C5-H), 7.79 (1H, d, $J$ 7.3 Hz, C6′-H), 8.0 (1H, d, $J$ 8.8 Hz, C6-H), 8.11 (1H, s, C2-H) and 8.87 (1H, br s, ex. with $D_2O$, OH); MS $m/z$ (FAB+) 447.1 [10, $(M+H+NBA)^+$], 294.0 [100, $(M+H)^+$], 214.1 [10, $(M+H-SO_2NH_2)^+$]; MS $m/z$ (FAB−) 446.1 [20, $(M+NBA)^-$], 292.0 [100, $(M-H)^-$], 213.0 [30, $(M-SO_2-NH_2)^-$]; Acc. MS (FAB+) 294.0411, $C_{13}H_{11}NO_5S$ requires 294.0436.

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