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4,4′-Benzophenone-*O,O′*-disulfamate: A Potent Inhibitor of Steroid Sulfatase

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Abstract—We investigated whether the benzophenone moiety can be used as core element of steroid sulfatase (STS) inhibitors. While 4- and 3-benzophenone-*O*-sulfamates inhibit STS with IC_{50} values between 5 and 7 μM irrespective of additional hydroxy and methoxy substituents at the second phenyl ring, benzophenone-*O,O′*-disulfamates show increased activity. With an IC_{50} value of 190 nM the 4,4′-derivative is the first small monocyclic STS inhibitor coming close to the potency of the steroidal standard estrone sulfamate.

Steroid sulfatase (estrone sulfatase, E.C. 3.1.6.2., STS) regulates the local production of estrogens from systemic precursors, such as estrone sulfate and dehydroepiandrosterone sulfate in normal and malignant breast tissues. There is increasing evidence that in breast and endometrial tissues the steroid sulfatase pathway is the major source of estrogens, which support the growth of endocrine-dependent tumours. Inhibitors of STS are therefore considered as potential new therapeutic agents for the treatment of estrogen-dependent cancers.[1]

Both irreversible and reversible inhibitors of human STS have been reported.[2] All potent irreversible inhibitors feature the sulfamate functionality, which is responsible for the irreversible mode of action. While with irreversible inhibitors blocking of enzyme activity in the nanomolar range can be achieved, the few known reversible inhibitors are less potent by several orders of magnitude. The design of potent, reversible inhibitors is hampered by the lack of the 3-D-structure of STS. In contrast to the related arylsulfatases A and B,[3,4] information on structure and catalytic mechanism of STS is not available. Photolabeling of the active site of an enzyme is a way to get insight into the residues involved in catalysis. We, therefore, envisaged the design and synthesis of photolabeling ligands for STS. Incorporation of a benzophenone substituent in photolabeling substrates has proven several advantages over other photophores, but its steric requirement limits wider applicability.[5]

We asked whether for STS the benzophenone moiety itself could be used as core element of a ligand and not only as an appendix to known ligands. To estimate the magnitude of the binding affinity of benzophenone-based ligands we prepared the corresponding sulfamates and tested them for their inhibitory potencies, analogously to the successful search for a new STS substrate.[6] Following this approach, we discovered benzophenone disulfamates with high inhibitory potency against STS.

Chemistry

The test compounds **1a**–**1l** were synthesized by sulfamoylation of the corresponding hydroxybenzophenones using sodium hydride followed by amidochlorosulfonic acid[7] (3-fold excess of both reagents) in dimethylformamide. With 3,3′- and 3,4′-dihydroxybenzophenone as starting material both, the mono- and the disulfamated products were obtained (**1g** + **1j** and **1i** + **1k**, respectively). All test compounds were purified by silica gel chromatography and characterized by ^{1}H NMR, MS (ESI), and, in the case of new compounds, also by elemental analysis.

The non-commercially available phenolic precursors were prepared as outlined in Scheme 1. Addition of in situ generated methoxyphenyllithium salts **2** to TBDMS protected hydroxybenzaldehydes **3** gave benzhydrol

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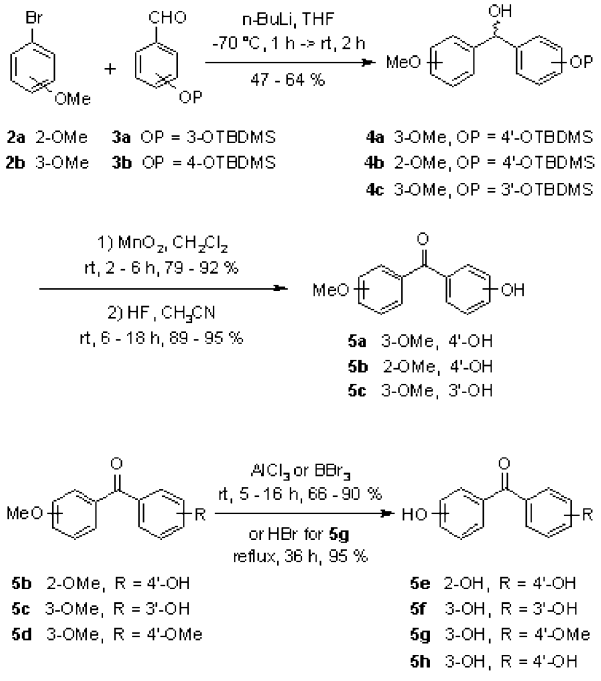
intermediates **4**. Oxidation with manganese dioxide generated the benzophenone core element and subsequent deprotection using HF yielded hydroxybenzophenones **5a**–**c**. Dihydroxybenzophenones **5e** and **5f** were obtained from **5b** and **5c**, respectively, by treatment with Lewis acid (aluminum chloride for **5e**, boron tribromide for **5f**). Similarly, 3,4′-dimethoxybenzophenone[8] (**5d**) was transformed into 3-hydroxy-4′-methoxybenzophenone (**5g**) by treatment with boron tribromide and to 3,4′dihydroxybenzophenone (**5h**) using hydrobromic acid in acetic acid.

Biological Results and Discussion

All known potent STS inhibitors are bi- or polycyclic sulfamates with one exception: Li et al. reported on a series of *N*-acyl tyramine sulfamates,[9] with the best representative showing only about 20-fold less activity than the common standard estrone sulfamate.[10] Evaluation of the structure–activity relationship (SAR) of STS inhibitors suggests that a stretched shape of the molecule is beneficial in all substance classes. However, we were encouraged in investigating benzophenones as



Scheme 1. Synthesis of non-commercially available hydroxybenzophenone precursors **5**.

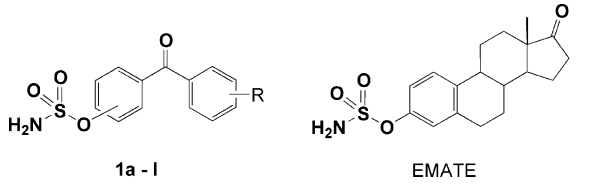


Figure 1. General structure of benzophenone-*O*-sulfamates **1a**–**l** (for definition of substituent R see Table 1) and the structure of the steroidal STS inhibitor estrone sulfamate (EMATE).

core moiety of STS inhibitors by the report that already simple tetralone and indalone sulfamates are able to block STS activity.[1] During the course of our studies, 4-acylphenyl-*O*-sulfamates including 4-benzophenone-*O*-sulfamate (**1a**) itself were reported as inhibitors of STS.[11]

Estrone sulfamate (EMATE, Fig. 1), a well characterized potent STS inhibitor, was used as reference compound in our assay (Table 1). The first compounds we investigated were 4- and 3-benzophenone-*O*-sulfamate (**1a**,[13] **1b**) without any additional substituents. Both regioisomers inhibited STS and were about 100-fold less active than estrone sulfamate. This agrees well with the reported activity of **1a** (126-fold less active than EMATE).[11] The comparable potency of the 3- and the 4-isomer might be explained by the possibility to overlay the sulfamate functionalities and the phenyl rings of the two molecules. All potent STS inhibitors have a stretched shape and published SAR studies circumstantiate that elongation of the (poly)cyclic core elements by side chains results in improved potency. Therefore, we explored the influence of alkoxy and hydroxy substituents in benzophenone-*O*-sulfamates, which could serve as potential attachment sites for side chains to enhance binding to the enzyme. Surprisingly, all these derivatives (selected compounds **1c** to **1i** shown in Table 1) exhibit very similar potency with IC_{50} values in the low micromolar range (about 100 times less potent than EMATE) indicating that electronic and steric effects by these substituents are tolerated at all positions of the second phenyl group. In the course of the synthesis of **1g** we also isolated the 3,3′-disulfamate **1j**. This by-product showed improved activity relative to all benzophenone monosulfamates tested before. This prompted us to synthesise and test the benzophenone disulfamates **1k** and **1l**. Here a clear preference for the

Table 1. Inhibitory potencies (IC_{50}) of benzophenone sulfamates **1a**–**l** against recombinant human steroid sulfatase in comparison to EMATE

Compd	Position of		STS, IC_{50}[a] (μM)
	$-OSO_2NH_2$	R	
EMATE			0.056
1a	4	H	5.1
1b	3	H	5.7
1c	4	3-OMe	5.2
1d	4	2-OMe	4.8
1e	4	2-OH	4.6
1f	3	3-OMe	7.1
1g	3	3-OH	6.9
1h	3	4-OMe	6.9
1i	3	4-OH	5.0
1j	3	$3-OSO_2NH_2$	3.2
1k	3	$4-OSO_2NH_2$	0.78
1l	4	$4-OSO_2NH_2$	0.19

[a]All compounds were tested using a fluorimetric assay.[12] The substrate (0.5 mM of 4-methylumbelliferyl sulfate) was incubated with STS (1.5 nM) in the presence of graded concentrations of inhibitors at pH 7.5 and 37° C for 60 min. Then, 0.2 M NaOH was added and fluorescence intensity ($\lambda_{ex} = 355$ nm, $\lambda_{em} = 460$ nm) was measured. The inhibitory activities of the test compounds (IC_{50}) were calculated using non-linear regression (software Grafit).

4-isomer was observed. Already the 3,4′-disulfamate **1k** showed additionally increased potency and the 4,4′-disulfamate **1l** was the best compound out of the series being only 3.4-fold less active than EMATE. This finding proved for the first time that highly efficient STS inhibitory activity can be also achieved with a very small aryl sulfamate lacking both a steroidal B-ring mimicry and a side chain mimicking the steroidal D-ring and beyond (see for instance elongated 17-estradiol analogues[14]). It still has to be elucidated how the second sulfamate moiety contributes to the substantial increase in activity.

In summary, we have discovered 4,4′-benzophenone-*O,O′*-disulfamate (**1l**) as potent inhibitor of human STS. Moreover, the results from this study indicate that the benzophenone moiety might be suitable as a core structure for the design of photolabeling ligands for STS.

References and Notes

1. Woo, L. W. L.; Howarth, N. M.; Purohit, A.; Jejaz, H. A. M.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **1998**, *41*, 1068.
2. Poirier, D.; Ciobanu, L. C.; Maltais, R. *Exp. Opin. Ther. Pat.* **1999**, *9*, 1083.
3. Lukatela, G.; Krauss, N.; Theis, K.; Selmer, T.; Gieselman, V.; Figura, K.; Saenger, W. *Biochemistry* **1998**, *37*, 3654.
4. Bond, C. S.; Clements, P. R.; Ashby, S. J.; Collyer, C. A.; Harrop, S. J.; Hopwood, J. J.; Guss, J. M. *Structure* **1997**, *5*, 177.
5. Dormán, G.; Prestwich, G. D. *Biochemistry* **1994**, *33*, 5661.
6. Bilban, M.; Billich, A.; Auer, M.; Nussbaumer, P. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 967.
7. Appel, R.; Berger, G. *Chem. Ber.* **1958**, *91*, 1339.
8. Blicke, F. F.; Weinkauff, O. J. *J. Org. Chem.* **1932**, *54*, 1446.
9. Li, P. K.; Milano, S.; Kluth, L.; Rhodes, M. E. *J. Steroid Biochem. Mol. Biol.* **1996**, *59*, 41.
10. Purohit, A.; Williams, G. J.; Howarth, N. M.; Potter, B. V. L.; Reed, M. J. *Biochemistry* **1995**, *34*, 11508.
11. Ahmed, S.; James, K.; Owen, C. P.; Patel, C. K.; Patel, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 841.
12. Roy, A. B. *Methods Enzymol.* **1987**, *143*, 207.
13. **1a** was a known compound but for other use (Lo, Y. S.; Walsh, D. A.; Uwaydah, I. M. US Patent 5025031 A, 1991; *Chem. Abstr.* **1991**, *115*, 255820) and was not tested as steroid sulfatase inhibitor until very recently.[11]
14. Ciobanu, L. C.; Boivin, R. P.; Luu-The, V.; Labrie, F.; Poirier, D. *J. Med. Chem.* **1999**, *42*, 2280.