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Journal of Steroid Biochemistry & Molecular Biology 88 (2004) 409–420
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

2-Phenylindole sulfamates: inhibitors of steroid sulfatase with antiproliferative activity in MCF-7 breast cancer cells

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Accepted 29 December 2003

Abstract

A number of 2-phenylindole sulfamates with lipophilic side chains in 1- or 5-position of the indole were synthesized and evaluated as steroid sulfatase (estrone sulfatase) inhibitors. Most of the new sulfamates inhibited the enzymatic hydrolysis of estrone sulfate in MDA-MB 231 breast cancer cells with IC_{50} values between 2 nM and 1 μM. A favorable position for a long side chain is the nitrogen of a carbamoyl group at C-5 of the indole when the phenyl ring carries the sulfamate function. These derivatives inhibit gene activation in estrogen receptor (ER)-positive MCF-7 breast cancer cells in submicromolar concentrations and reduce cell proliferation with IC_{50} values of ca. 1 μM. All of the potent inhibitors were devoid of estrogenic activity and have the potential for in vivo application as steroid sulfatase inhibitors.

Keywords: Steroid sulfatase; Breast cancer; Phenylindole sulfamate; Enzyme inhibition

1. Introduction

Since estrogens are known to play a dominant role in the promotion of breast cancer several strategies have been developed to reduce the mitogenic effects of estrogens on mammary carcinoma cells. Blockade of the estrogen receptor (ER) by antiestrogens and inhibition of estrogen biosynthesis by aromatase inhibitors are therapeutic options which have proved effective in the treatment of postmenopausal patients though number and duration of remissions are far from being acceptable. Studies on estrogen metabolism in man have identified estrone sulfate as the predominant form of circulating estrogens, and has also been detected in breast cancer tissue as the main estrogen [1,2]. Mammary tumor cells have been shown to be capable of cleaving this conjugate to free estrone which can subsequently be converted to 17β-estradiol [3,4]. The enzyme involved in this reduction is 17β-HSD which exists in several isoforms showing unidirectional reactivity, preferred substrates and cofactors (NAD^+/NADH or $NADP^+$/NADPH), and different tissue distribution [5]. The conversion of estrone to 17β-estradiol is mainly catalyzed by the 17β-HSD type 1 enzyme, but type 7 has also the ability for this reduction [5,6]. Increased levels of type 1 and decreased concentrations of type 2 17β-HSD in breast cancer tissue have been associated with poor prognosis for patients [7,8].

It has also been demonstrated that the steroid sulfatase present in breast cancer cells plays a more important role than the enzyme aromatase in the formation of free estrogens [2,9]. In mammary tumors the levels of free and conjugated estrogens as well as the sulfatase activity is significantly higher than in normal tissue [1,10]. High expression of steroid sulfatase mRNA has been associated with poor prognosis in patients with ER-positive breast cancer [11]. These findings have stimulated the search for inhibitors of steroid sulfatase as a new therapeutic option or as co-medication in endocrine therapy of patients with mammary carcinomas.

In the first studies the natural substrate estrone sulfate was chemically modified in the relevant 3-position [12–14]. The most favourable substituent in respect to enzyme inhibition proved to be the sulfamoyloxy group, but the inherent estrogenic potency [15] made this derivative (**7**, EMATE, Fig. 1) unsuitable for further development as sulfatase inhibitor. Meanwhile a variety of steroidal [16,17] and non-steroidal sulfamates [18,19] have been synthesized and evaluated as enzyme inhibitors [20]. Some recently reported examples comprise 2-methoxyestradiol-bis-sulfamate (8) [21], tricyclic coumarin derivatives, such as compound 9 (667-COUMATE) [22], 2-adamantylthiochromenone sulfamate (10) [23], and the phenyl sulfamates 11 [24] and 12

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doi:10.1016/j.jsbmb.2003.12.012

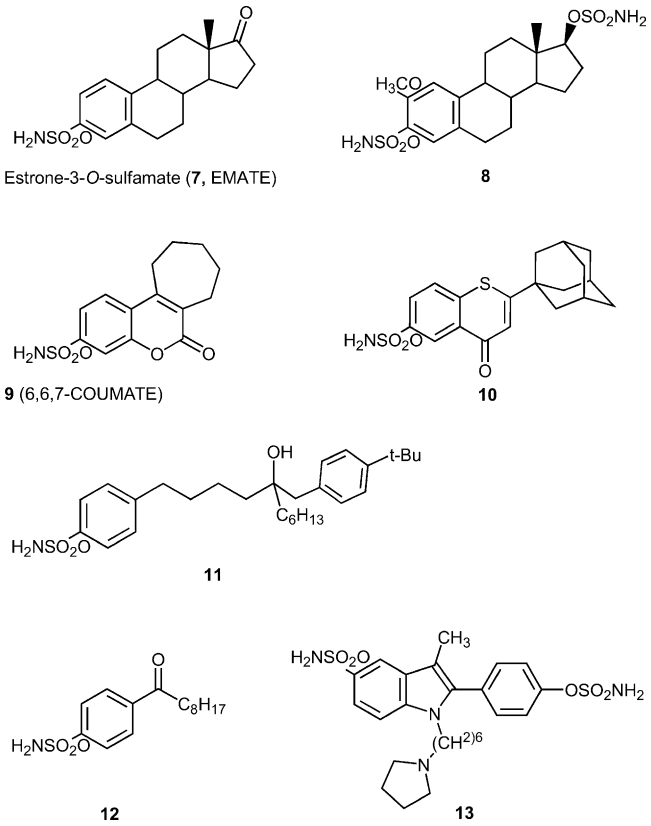


Fig. 1. Chemical structures of steroidal and non-steroidal steroid sulfatase inhibitors.

[25] with lipophilic residues in *para*-position of the aromatic ring. Recently, we have shown that the non-steroidal antiestrogen ZK 119,010 can be converted into the potent steroid sulfatase inhibitor **13** by sulfamoylation of both phenolic functions [26].

Since these sulfamates can act as both inhibitor and substrate of steroid sulfatase the bioactivity of the free phenols is of great importance and has to be considered in the design of new sulfatase inhibitors for therapeutic application. Therefore, we developed the concept of an antiestrogen based inhibitor of the enzyme steroid sulfatase. We could demonstrate that non-steroidal antiestrogens of appropriate structure can be converted to steroid sulfatase inhibitors after the phenolic hydroxy groups had been modified by sulfamoylation. An important finding of this study was that the enzyme inhibitory activity of the 2-phenylindole derivatives increases in parallel to the estrogenic potency of the parent structure [26]. This observation can be rationalized by overlapping structural requirements for binding to the ER and the active site of steroid sulfatase. For further development it is necessary to find structural elements in the 2-phenylindole system that increase enzyme inhibition without giving rise to estrogenic side effects.

A suitable strategy for improving the enzyme inhibitory effects is the introduction of lipophilic side chains into appropriate phenyl sulfamates as it has been demonstrated by other groups [19,27–30]. Since the antiestrogenic potency of steroidal and non-steroidal ligands of the ER benefits from a side chain of 10 or more atoms including a functional group such as sulfoxide [31], sulfone [32] or amide, it was reasonable to link the lipophilic element to the indole nitrogen which generally carries the side chain in indole-based antiestrogens. An alternative would be to modify one of the aromatic rings in the 2-phenylindole system to use it as carrier for the side chain. Both modifications were performed in this study. Free phenolic hydroxy groups were reacted with sulfamoyl chloride to give the respective sulfamates which were tested for enzyme inhibitory activity in human MDA-MB 231 mammary carcinoma cells, for direct (anti)estrogenic effects and inhibition of estrone sulfate action in stably transfected MCF-7/2a breast cancer cells, and for antiproliferative activity in wild type MCF-7 cells stimulated with estrone sulfate.

2. Material and methods

2.1. General methods

Melting points were determined on a Büchi 510 apparatus and are uncorrected. NMR spectra were recorded on a Bruker AC-250 spectrometer with TMS as internal standard and DMSO-d_6 as solvent, and were in accord with the assigned structures. Elemental analyses of crystalline compounds were performed by the Mikroanalytisches Laboratorium, University of Regensburg, and were within 0.4% of the calculated values except where noted. Purity (98–100%) of non-crystalline final products was checked by HPLC (MeCN/buffer mixtures; buffer: 0.5% NEt_3 adjusted to pH 7.0 with H_3PO_4), detection: UV, $\lambda = 250$ nm, or fluorescence, EX 300 nm, EM 360 nm).

2.2. Synthesis of starting material

The starting 1*H*-2-phenylindoles **1** were obtained by the Bischler method as described previously [26,33]. *N*-Alkylation to **2a–e** and ether cleavage with BBr_3 to **3a–e** were performed according to reference [26]. Analytical data of 1-ethyl-2-(4-hydroxyphenyl)-3-methylindol-6-ol (**3a**) and 1-butyl-2-(4-hydroxyphenyl)-indol-6-ol (**3b**) have been reported [33].

2.2.1. 1-Heptyl-2-(4-hydroxyphenyl)-indol-6-ol (3c)

Colorless viscous oil; 93% yield. ^{1}H NMR δ: 0.79 (t, ^3J = 7.1 Hz, 3H, CH_2–C$\underline{H}_3$), 1.07–1.20 (m, 8H, (C$\underline{H}_2$)$_4$–CH_3), 1.51–1.64 (m, 2H, C$\underline{H}_2$–$(CH_2)_4$), 4.05 (t, ^3J = 7.3 Hz, 2H, C$\underline{H}_2$–$(CH_2)_5$), 6.19 (s, 1H, indole-H^3), 6.63 (dd, ^3J = 8.7 Hz, ^4J = 2.3 Hz, 1H, indole-H^5), 6.82 (d, ^4J = 2.3 Hz, 1H, indole-H^7), 6.87 and 7.29 (AA′BB′, ^3J = 8.5 Hz, 4H, ArH), 7.24 (d, ^3J = 8.7 Hz, 1H, Indol-H^4), 8.68 (s, 1H, –OH), 9.66 (s, 1H, –OH).

*2.2.2. 1-Decyl-2-(4-hydroxyphenyl)-indol-6-ol (**3d**)*

Colorless viscous oil; 92% yield. ^{1}H NMR δ: 0.85 (t, ^3J = 6.9 Hz, 3H, CH_2–C$\underline{H}_3$), 1.09–1.27 (m, 14H, (C$\underline{H}_2$)$_7$–CH_3), 1.49 bis 1.52 (m, 2H, C$\underline{H}_2$–$(CH_2)_7$), 4.02–4.07 (m, 2H, C$\underline{H}_2$–$(CH_2)_8$), 6.22 (s, 1H, indole-H^3), 6.56 (dd, ^3J = 8.4 Hz, ^4J = 2.0 Hz, 1H, indole-H^5), 6.75 (d, ^4J = 2.0 Hz, 1H, indole-H^7), 6.85 and 7.26 (AA′BB′, ^3J = 8.5 Hz, 4H, ArH), 7.27 (d, ^3J = 8.4 Hz, 1H, indole-H^4), 8.98 (s, 1H, –OH), 9.62 (s, 1H, –OH).

*2.2.3. 2-(4-Hydroxyphenyl)-1,3-dimethylindole-5-carbamide (**3e**)*

Yellow foam; 74% yield. ^{1}H NMR δ: 2.22 (s, 3H, C–CH_3), 3.59 (s, 3H, N–CH_3), 6.93 and 7.28 (AA′BB′, ^3J = 8.5 Hz, 4H, ArH), 7.10 (s, br, 1H, CO–NH), 7.44 (d, ^3J = 8.7 Hz, 1H, indole-H^7), 7.73 (dd, ^3J = 8.7 Hz, ^4J = 1.6 Hz, 1H, indole-H^6), 7.88 (s, br, 1H, CO–NH), 8.15 (d, ^4J = 1.6 Hz, 1H, indole-H^4), 9.74 (s, 1H, –OH).

2.3. General procedure for the preparation of the sulfamates

A solution of the hydroxy-2-phenylindole (2.77 mmol) in dry DMF (15 ml) was cooled to 10–15 °C. Sulfamoyl chloride [34], 13.9 mmol per hydroxy group, was added in portions. After the addition, the mixture was stirred for 12 h under N_2. It was hydrolyzed with water (40 ml) followed by extraction with EtOAc. The combined organic layers were washed with water and dried ($MgSO_4$). After evaporation of the solvent the residue was chromatographed over SiO_2 with CH_2Cl_2/EtOAc mixtures as eluent. In the case of dihydroxy derivatives the first fractions contained the monosulfamates **5a–d**, the second the disulfamates **4a–d**.

*2.3.1. 1-Ethyl-6-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)indole (**4a**)*

Viscous oil; 38% yield. ^{1}H NMR δ: 1.21 (t, ^3J = 7.0 Hz, 3H, CH_2–C$\underline{H}_3$), 4.23 (q, ^3J = 7.0 Hz, 2H, C$\underline{H}_2$–CH_3), 6.61 (s, 1H, indole-H^3), 7.11 (dd, ^3J = 8.9 Hz, ^4J = 2.3 Hz, 1H, indole-H^5), 7.43 and 7.64 (AA′BB′, ^3J = 8.7 Hz, 4H, ArH), 7.48 (d, ^4J = 2.3 Hz, 1H, indole-H^7), 7.61 (d, ^3J = 8.9 Hz, 1H, indole-H^4), 7.81 (s, br, 2H, OSO_2NH_2), 8.09 (s, br, 2H, OSO_2NH_2). HPLC (MeCN/buffer 55:45 v/v; UV detection): retention time 4.15 min (99%).

*2.3.2. 4-(1-Ethyl-6-sulfamoyloxyindol-2-yl)phenol (**5a**)*

Viscous oil; 34% yield. ^{1}H NMR δ: 1.18 (t, ^3J = 7.0 Hz, 3H, CH_2–C$\underline{H}_3$), 4.21 (q, ^3J = 7.0 Hz, 2H, C$\underline{H}_2$–CH_3), 6.46 (s, 1H, indole-H^3), 6.90 and 7.34 (AA′BB′, ^3J = 8.3 Hz, 4H, ArH), 7.06 (dd, ^3J = 8.8 Hz, ^4J = 2.3 Hz, 1H, indole-H^5), 7.43 (d, ^4J = 2.3 Hz, 1H, indole-H^7), 7.54 (d, ^3J = 8.8 Hz, 1H, indole-H^4), 7.79 (s, br, 2H, OSO_2NH_2), 9.76 (s, br, 1H, –OH). HPLC (MeCN/buffer 78:22 v/v; UV detection): retention time 2.39 min (99%).

*2.3.3. 1-Butyl-6-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)indole (**4b**)*

Colorless oil; 37% yield. ^{1}H NMR δ: 0.74 (t, ^3J = 7.2 Hz, 3H, CH_2–C$\underline{H}_3$), 1.02–1.20 (m, 2H, C$\underline{H}_2$–CH_3), 1.51–1.63 (m, 2H, C$\underline{H}_2$–CH_2–CH_3), 4.19 (t, ^3J = 7.3 Hz, 2H, C$\underline{H}_2$–$(CH_2)_2$–CH_3), 6.58 (s, 1H, indole-H^3), 7.02 (dd, ^3J = 8.6 Hz, ^4J = 2.0 Hz, 1H, Indol-H^5), 7.43 and 7.64 (AA′BB′, ^3J = 8.7 Hz, 4H, ArH), 7.45 (d, ^4J = 2.0 Hz, 1H, indole-H^7), 7.61 (d, ^3J = 8.6 Hz, 1H, indole-H^4), 7.88 (s, br, 2H, OSO_2NH_2), 8.13 (s, br, 2H, OSO_2NH_2). HPLC (MeCN/buffer 55:45 v/v; UV detection): retention time 5.60 min (99%).

*2.3.4. 4-(1-Butyl-6-sulfamoyloxy-indol-2-yl)-phenol (**5b**)*

Colorless oil; 31% yield. ^{1}H NMR δ: 0.73 (t, ^3J = 7.4 Hz, 3H, CH_2–C$\underline{H}_3$), 1.02–1.11 (m, 2H, C$\underline{H}_2$–CH_3), 1.49–1.61 (m, 2H, C$\underline{H}_2$–CH_2–CH_3), 4.13 (t, ^3J = 7.2 Hz, 2H, –C$\underline{H}_2$–$(CH_2)_2$–CH_3), 6.43 (s, 1H, indole-H^3), 6.90 and 7.33 (AA′BB′, ^3J = 8.5 Hz, 4H, ArH), 6.98 (dd, ^3J = 8.5 Hz, ^4J = 2.0 Hz, 1H, indole-H^5), 7.40 (d, ^4J = 2.0 Hz, 1H, indole-H^7), 7.55 (d, ^3J = 8.5 Hz, 1H, indole-H^4), 7.84 (s, br, 2H, OSO_2NH_2), 9.74 (s, br, 1H, OH). HPLC (MeCN/buffer 78:22 v/v; UV detection): retention time 2.67 min (99%).

*2.3.5. 1-Heptyl-6-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)-indole (**4c**)*

Colorless oil; 34% yield. ^{1}H NMR δ: 0.79 (t, ^3J = 7.1 Hz, 3H, CH_2–C$\underline{H}_3$), 1.09–1.28 (m, 8H, (C$\underline{H}_2$)$_4$–CH_3), 1.53–1.61 (m, 2H, C$\underline{H}_2$–$(CH_2)_4$), 4.22 (t, ^3J = 7.0 Hz, 2H, –C$\underline{H}_2$–$(CH_2)_5$), 6.60 (s, 1H, indole-H^3), 7.10 (dd, ^3J = 8.9 Hz, ^4J = 2.3 Hz, 1H, indole-H^5), 7.43 and 7.64 (AA′BB′, ^3J = 8.6 Hz, 4H, ArH), 7.48 (d, ^4J = 2.3 Hz, 1H, indole-H^7), 7.60 (d, ^3J = 8.9 Hz, 1H, indole-H^4), 7.82 (s, 2H, OSO_2NH_2), 8.13 (s, 2H, OSO_2NH_2). HPLC (MeCN/buffer 55:45 v/v; UV detection): retention time 12.12 min (99%).

*2.3.6. 4-(1-Heptyl-6-sulfamoyloxy-indol-2-yl)-phenol (**5c**)*

Viscous oil; 29% yield. ^{1}H NMR δ: 0.78 (t, ^3J = 7.1 Hz, 3H, CH_2–C$\underline{H}_3$), 1.08–1.25 (m, 8H, (C$\underline{H}_2$)$_4$–CH_3), 1.53–1.59 (m, 2H, C$\underline{H}_2$–$(CH_2)_4$), 4.16 (t, ^3J = 7.3 Hz, 2H, C$\underline{H}_2$–$(CH_2)_5$), 6.45 (s, 1H, indole-H^3), 6.90 and 7.33 (AA′BB′, ^3J = 8.3 Hz, 4H, ArH), 7.05 (dd, ^3J = 8.8 Hz, ^4J = 2.3 Hz, 1H, indole-H^5), 7.43 (d, ^4J = 2.3 Hz, 1H, indole-H^7), 7.53 (d, ^3J = 8.8 Hz, 1H, indole-H^4), 7.79 (s, 2H, OSO_2NH_2), 9.74 (s, 1H, OH). HPLC (MeCN/buffer 78:22 v/v; UV detection): retention time 3.63 min (99%).

*2.3.7. 1-Decyl-6-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)-indole (**4d**)*

Colorless oil; 41% yield. ^{1}H NMR δ: 0.84 (t, ^3J = 6.9 Hz, 3H, CH_2–C$\underline{H}_3$), 1.09–1.20 (m, 14H, (C$\underline{H}_2$)$_7$–CH_3), 1.57 (m, 2H, –C$\underline{H}_2$–$(CH_2)_7$), 4.18 (t, ^3J = 7.3 Hz, 2H, C$\underline{H}_2$–$(CH_2)_8$), 6.58 (s, 1H, indole-H^3), 7.02 (dd, ^3J = 8.5 Hz, ^4J = 2.0 Hz, 1H, indole-H^5), 7.42 and 7.63

(AA′BB′, ^3J = 8.7 Hz, 4H, ArH), 7.44 (d, ^4J = 2.0 Hz, 1H, indole-H^7), 7.60 (d, ^3J = 8.5 Hz, 1H, indole-H^4), 7.91 (s, 2H, OSO_2NH_2), 7.92 (s, 2H, OSO_2NH_2). HPLC (MeCN/buffer 55:45 v/v; UV detection): retention time 31.02 min (99%).

*2.3.8. 4-(1-Decyl-6-sulfamoyloxy-indol-2-yl)-phenol (**5d**)*

Reddish foam; 27% yield. ^{1}H NMR δ: 0.85 (t, ^3J = 6.9 Hz, 3H, CH_2–C$\underline{H}_3$), 1.09–1.26 (m, 14H, (C$\underline{H}_2$)$_7$–CH_3), 1.55 (m, 2H, –C$\underline{H}_2$–$(CH_2)_7$), 4.12 (t, ^3J = 7.3 Hz, 2H, C$\underline{H}_2$–$(CH_2)_8$), 6.42 (s, 1H, indole-H^3), 6.89 and 7.32 (AA′BB′, ^3J = 8.5 Hz, 4H, ArH), 6.98 (dd, ^3J = 8.5 Hz, ^4J = 2.0 Hz, 1H, indole-H^5), 7.39 (d, ^4J = 2.0 Hz, 1H, indole-H^7), 7.54 (d, ^3J = 8.5 Hz, 1H, indole-H^4), 7.84 (s, 2H, OSO_2NH_2), 9.73 (s, 1H, OH). HPLC (MeCN/buffer 78:22 v/v; UV detection): retention time 5.93 min (98%).

*2.3.9. 1,3-Dimethyl-2-(4-sulfamoyloxyphenyl)indole-5-carbamide (**4e**)*

Viscous oil; 23% yield. ^{1}H NMR δ: 2.23 (s, 3H, C–CH_3), 3.64 (s, 3H, N–CH_3), 7.14 (s, br, 1H, CO–NH), 7.45 and 7.59 (AA′BB′, ^3J = 8.7 Hz, 4H, ArH), 7.49 (d, ^3J = 8.7 Hz, 1H, indole-H^7), 7.78 (dd, ^3J = 8.7 Hz, ^4J = 1.6 Hz, 1H, indole-H^6), 7.90 (s, br, 1H, CO–NH), 8.12 (s, br, 2H, OSO_2NH_2), 8.20 (d, ^4J = 1.6 Hz, 1H, indole-H^4). IR (CH_2Cl_2): 1670 (s, C=O) cm^{-1}. HPLC (MeCN/buffer 78:22 v/v; UV detection): retention time 2.16 min (99%).

*2.3.10. 1,3-Dimethyl-2-(4-sulfamoyloxyphenyl)indole-5-carbonitrile (**4f**)*

Second product of the sulfamoylation of **3e**. Viscous oil; 27% yield. ^{1}H NMR δ: 2.25 (s, 3H, C–CH_3), 3.67 (s, 3H, N–CH_3), 7.46 and 7.60 (AA′BB′, ^3J = 8.6 Hz, 4H, ArH), 7.55 (dd, ^3J = 8.5 Hz, ^4J = 1.5 Hz, 1H, indole-H^6), 7.68 (d, ^3J = 8.5 Hz, 1H, indole-H^7), 8.14 (d, ^4J = 1.5 Hz, 1H, indole-H^4), 8.14 (s, br, 2H, –OSO_2NH_2). IR (CH_2Cl_2): 2222 (s, –CN) cm^{-1}. HPLC (MeCN/buffer 78:22 v/v; UV detection): retention time 2.84 min (94%).

*2.4. Preparation of the N-alkylamides **6a–c***

Under N_2, a solution of the 2-(4-methoxyphenyl)-1,3-dimethylindole-5-carbamide (**2e**) (1.47 g, 5 mmol) in dry DMF (25 ml) was added slowly with stirring to an ice-cold suspension of NaH (177 mg, 7.4 mmol) in dry DMF. Stirring was continued until the gas evolution ceased. Then, the respective alkyl bromide (4.47 mmol) in dry DMF (20 ml) was added dropwise with cooling in an ice bath. After the addition, the ice bath was removed and stirring continued for 2 h at RT. The excess of NaH was destroyed carefully by dropwise addition of water, followed by the addition of water (50 ml) and EtOAc (50 ml). The organic layer was separated, and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with water and dried ($MgSO_4$). The solvent was removed in vacuo and the residue purified by chromatography (SiO_2) with CH_2Cl_2/EtOAc mixtures as the eluent.

*2.4.1. 2-(4-Methoxyphenyl)-1,3-dimethyl-N-octylindole-5-carbamide (**6a**)*

Colorless crystals; yield 91%; m.p. 90–91 °C. ^{1}H NMR δ: 0.86 (t, ^3J = 6.9 Hz, 3H, –CH_2–C$\underline{H}_3$), 1.24 bis 1.34 (m, 10H, –(C$\underline{H}_2$)$_5$–CH_3), 1.52 bis 1.55 (m, 2H, N–CH_2–C$\underline{H}_2$–), 2.23 (s, 3H, C–CH_3), 3.27–3.32 (m, 2H, N–C$\underline{H}_2$–CH_2–), 3.60 (s, 3H, –N–CH_3), 3.84 (s, 3H, –OCH_3), 7.13 and 7.39 (AA′BB′, ^3J = 8.8 Hz, 4H, ArH), 7.40 (d, ^3J = 8.7 Hz, 1H, indole-H^7), 7.71 (dd, ^3J = 8.7 Hz, ^4J = 1.6 Hz, 1H, indole-H^6), 8.12 (d, ^4J = 1.6 Hz, 1H, indole-H^4), 8.33 (t, ^3J = 5.6 Hz, 1H, NH–CO). IR 1629 (s, C=O) cm^{-1}. Anal. ($C_{26}H_{34}N_2O_2$) C, H (calcd. 8.43; found 9.03%), N.

*2.4.2. N-Decyl-2-(4-methoxyphenyl)-1,3-dimethylindole-5-carbamide (**6b**)*

Colorless crystals; yield 82%; m.p. 98–99 °C. ^{1}H NMR δ: 0.85 (t, ^3J = 6.3 Hz, 3H, –CH_2–C$\underline{H}_3$), 1.18–1.25 (m, 14H, –(C$\underline{H}_2$)$_7$–CH_3), 1.52–1.55 (m, 2H, N–CH_2–C$\underline{H}_2$–), 2.23 (s, 3H, C–CH_3), 3.24–3.32 (m, 2H, N–C$\underline{H}_2$–CH_2–), 3.60 (s, 3H, N–CH_3), 3.84 (s, 3H, –OCH_3), 7.10 and 7.40 (AA′BB′, ^3J = 8.8 Hz, 4H, ArH), 7.45 (d, ^3J = 8.7 Hz, 1H, indole-H^7), 7.71 (dd, ^3J = 8.7 Hz, ^4J = 1.6 Hz, 1H, indole-H^6), 8.12 (d, ^4J = 1.6 Hz, 1H, indole-H^4), 8.33 (t, ^3J = 5.5 Hz, 1H, NH–CO); IR 1630 (s, C=O) cm^{-1}. Anal. ($C_{28}H_{38}N_2O_2$) C, H, N.

*2.4.3. N-Dodecyl-2-(4-methoxyphenyl)-1,3-dimethylindole-5-carbamide (**6c**)*

Colorless crystals; 92% yield; m.p. 99–102 °C. ^{1}H NMR δ: 0.85 (t, ^{3}J = 6.3 Hz, 3H, –CH_2–C$\underline{H}_3$), 1.15–1.29 (m, 18H, –(C$\underline{H}_2$)$_9$–CH_3), 1.52–1.57 (m, 2H, N–CH_2–C$\underline{H}_2$–), 2.23 (s, 3H, C–CH_3), 3.24–3.32 (m, 2H, N–C$\underline{H}_2$–CH_2–), 3.60 (s, 3H, N–CH_3), 3.84 (s, 3H, –OCH_3), 7.11 and 7.41 (AA′BB′, ^3J = 8.8 Hz, 4H, ArH), 7.45 (d, ^3J = 8.7 Hz, 1H, indole-H^7), 7.71 (dd, ^3J = 8.7 Hz, ^4J = 1.6 Hz, 1H, indole-H^6), 8.11 (d, ^4J = 1.6 Hz, 1H, indole-H^4), 8.32 (t, ^3J = 5.6 Hz, 1H, NH–CO); IR 1630 (s, (C=O) cm^{-1}). Anal. ($C_{30}H_{42}N_2O_2$) C, H, N.

2.5. Cleavage of the methoxy groups with $AlCl_3$/EtSH

To a solution of **6** (0.5 mmol) in dry CH_2Cl_2 (10 ml) $AlCl_3$ (200 mg, 1.5 mmol) was added with stirring. Stirring was continued for 5 min followed by the addition of ethanethiol (155 mg, 2.5 mmol). After stirring at 50 °C under N_2 for 1 h, the mixture was poored into ice water. The aqueous phase was separated and extracted twice with EtOAc. The combined organic layers were washed with sat. $NaHCO_3$ solution and water, and dried ($MgSO_4$). After the evaporation of the solvent, the residue was purified by chromatography (SiO_2) with CH_2Cl_2/EtOAc mixtures as eluent.

*2.5.1. 2-(4-Hydroxyphenyl)-1,3-dimethyl-N-octylindole-5-carbamide (**3g**)*

Yellow foam; 96% yield. ^{1}H NMR δ: 0.86 (t, ^3J = 7.1 Hz, 3H, CH_2–C$\underline{H}_3$), 1.10–1.29 (m, 10H, –(C$\underline{H}_2$)$_5$–CH_3), 1.52–1.57 (m, 2H, N–CH_2–C$\underline{H}_2$–), 2.22 (s, 3H, C–CH_3), 3.26–3.31 (m, 2H, N–C$\underline{H}_2$–CH_2–), 3.59 (s, 3H, N–CH_3), 6.92 and 7.27 (AA′BB′, ^3J = 8.5 Hz, 4H, ArH), 7.43 (d, ^3J = 8.5 Hz, 1H, indole-H^7), 7.70 (dd, ^3J = 8.5 Hz, ^4J = 1.5 Hz, 1H, indole-H^6), 8.09 (d, ^4J = 1.5 Hz, 1H, indole-H^4), 8.31 (t, ^3J = 5.6 Hz, 1H, NH–CO), 9.73 (s, 1H, OH).

*2.5.2. N-Decyl-2-(4-hydroxyphenyl)-1,3-dimethylindole-5-carbamide (**3h**)*

Viscous oil; 73% yield. ^{1}H NMR δ: 0.85 (t, ^3J = 7.0 Hz, 3H, CH_2–C$\underline{H}_3$), 1.19–1.31 (m, 14H, –(C$\underline{H}_2$)$_7$–CH_3), 1.53–1.56 (m, 2H, N–CH_2–C$\underline{H}_2$), 2.22 (s, 3H, C–CH_3), 3.25–3.30 (m, 2H, N–C$\underline{H}_2$–CH_2), 3.59 (s, 3H, N–CH_3), 6.93 and 7.27 (AA′BB′, ^3J = 8.8 Hz, 4H, ArH), 7.43 (d, ^3J = 8.4 Hz, 1H, indole-H^7), 7.69 (dd, ^3J = 8.4 Hz, ^4J = 1.7 Hz, 1H, indole-H^6), 8.09 (d, ^4J = 1.7 Hz, 1H, indole-H^4), 8.33 (t, ^3J = 5.6 Hz, 1H, NH–CO), 9.77 (s, 1H, OH).

*2.5.3. N-Dodecyl-2-(4-hydroxyphenyl)-1,3-dimethylindole-5-carbamide (**3i**)*

Yellow foam; 89% yield. ^{1}H NMR δ: 0.85 (t, ^3J = 6.3 Hz, 3H, CH_2–C$\underline{H}_3$), 1.18–1.29 (m, 18H, (C$\underline{H}_2$)$_9$–CH_3), 1.52–1.55 (m, 2H, N–CH_2–C$\underline{H}_2$), 2.22 (s, 3H, C–CH_3), 3.25–3.32 (m, 2H, N–C$\underline{H}_2$–CH_2), 3.59 (s, 3H, N–CH_3), 6.93 and 7.27 (AA′BB′, ^3J = 8.6 Hz, 4H, ArH), 7.44 (d, ^3J = 8.7 Hz, 1H, indole-H^7), 7.70 (dd, ^3J = 8.7 Hz, ^4J = 1.5 Hz, 1H, indole-H^6), 8.09 (d, ^4J = 1.5 Hz, 1H, indole-H^4), 8.30 (t, ^3J = 5.6 Hz, 1H, NH–CO), 9.74 (s, br, 1H, OH).

2.6. General procedure for the preparation of the sulfamates by means of base

NaH (5 mmol; 60% suspension in paraffin oil) in dry DMF (10 ml) was cooled to 5 °C followed by the addition of the respective phenol (5 mmol) dissolved in DMF (10 ml) over 5 min. Stirring was continued at RT for 1 h. With cooling in an ice-bath sulfamoyl chloride (50 mmol) was added in portions, and the mixture was stirred at RT overnight. At 5 °C, half-concentrated $NaHCO_3$ solution (70 ml) was added, and the mixture extracted three times with EtOAc (70 ml). The combined organic layers were dried ($MgSO_4$), and the solvent removed in vacuo. The residue was purified by chromatography (SiO_2; CH_2Cl_2/EtOAc 5:1).

*2.6.1. 1,3-Dimethyl-N-octyl-2-(4-sulfamoyloxyphenyl)indole-5-carbamide (**4g**)*

Yellow viscous oil; 51% yield. ^{1}H NMR δ: 0.86 (t, ^3J = 7.0 Hz, 3H, –CH_2–C$\underline{H}_3$), 1.17–1.30 (m, 10H, –(C$\underline{H}_2$)$_5$–CH_3), 1.52–1.58 (m, 2H, N–CH_2–C$\underline{H}_2$–), 2.27 (s, 3H, C–CH_3), 3.26–3.30 (m, 2H, N–C$\underline{H}_2$–CH_2–), 3.64 (s, 3H, N–CH_3), 7.45 and 7.59 (AA′BB′, ^3J = 8.7 Hz, 4H, ArH), 7.49 (d, ^3J = 8.7 Hz, 1H, indole-H^7), 7.74 (dd, ^3J = 8.7 Hz, ^4J = 1.6 Hz, 1H, indole-H^6), 8.12 (s, br, 2H, –OSO_2NH_2), 8.15 (d, ^4J = 1.6 Hz, 1H, indole-H^4), 8.34 (t, ^3J = 5.6 Hz, 1H, NH–CO); IR 1636 (s, C=O) cm^{-1}. HPLC (MeCN/buffer 89:11 v/v; fluorimetric detection): retention time 3.31 min (100%).

*2.6.2. N-Decyl-1,3-dimethyl-2-(4-sulfamoyloxyphenyl)indole-5-carbamide (**4h**)*

Colorless viscous oil; 56% yield. ^{1}H NMR δ: 0.85 (t, ^3J = 7.1 Hz, 3H, –CH_2–C$\underline{H}_3$), 1.25–1.31 (m, 14H, –(C$\underline{H}_2$)$_7$–CH_3), 1.52–1.56 (m, 2H, N–CH_2–C$\underline{H}_2$–), 2.27 (s, 3H, C–CH_3), 3.25–3.31 (m, 2H, N–C$\underline{H}_2$–CH_2–), 3.64 (s, 3H, N–CH_3), 7.45 and 7.59 (AA′BB′, ^3J = 8.7 Hz, 4H, ArH), 7.49 (d, ^3J = 8.6 Hz, 1H, indole-H^7), 7.74 (dd, ^3J = 8.6 Hz, ^4J = 1.6 Hz, 1H, indole-H^6), 8.13 (s, br, 2H, –OSO_2NH_2), 8.15 (d, ^4J = 1.6 Hz, 1H, indole-H^4), 8.35 (t, ^3J = 5.8 Hz, 1H, NH–CO); IR 1631 (s, C=O) cm^{-1}. HPLC (MeCN/buffer 89:11 v/v; fluorimetric detection): retention time 4.43 min (99%).

*2.6.3. N-Dodecyl-1,3-dimethyl-2-(4-sulfamoyloxyphenyl)indole-5-carbamide (**4i**)*

Yellow viscous oil; 53% yield. ^{1}H NMR δ: 0.85 (t, ^3J = 6.8 Hz, 3H, –CH_2–C$\underline{H}_3$), 1.18–1.29 (m, 18H, –(C$\underline{H}_2$)$_9$–CH_3), 1.52–1.55 (m, 2H, N–CH_2–C$\underline{H}_2$–), 2.27 (s, 3H, C-CH_3), 3.25–3.31 (m, 2H, N–C$\underline{H}_2$–CH_2–), 3.64 (s, 3H, N-CH_3), 7.45 and 7.58 (AA′BB′, ^3J = 8.8 Hz, 4H, ArH), 7.49 (d, ^3J = 8.7 Hz, 1H, indole-H^7), 7.74 (dd, ^3J = 8.7 Hz, ^4J = 1.7 Hz, 1H, indole-H^6), 8.12 (s, br, 2H, –OSO_2NH_2), 8.15 (d, ^4J = 1.7 Hz, 1H, indole-H^4), 8.34 (t, ^3J = 5.7 Hz, 1H, NH–CO); IR 1629 (s, C=O). HPLC (MeCN/buffer 89:11 v/v; fluorimetric detection): retention time 6.62 min (100%).

2.7. Materials and reagents for bioassays

[^{3}H] 17β-Estradiol, [^{3}H] estrone sulfate (ammonium salt), and [^{14}C] estrone were purchased from NEN (Dreieich, Germany); all other biochemicals were obtained from Sigma (Munich, Germany). Hormone-sensitive human MCF-7 breast cancer cells and hormone-independent human MDA-MB 231 breast cancer cells were obtained from the American Type Culture Collection (ATCC). MCF-7/2a cells with the reporter construct integrated in the genome had been cloned in authors' laboratory [35].

2.8. Steroid sulfatase assay

The previously described assay [26] was used with one major alteration: MCF-7 cells were replaced by the hormone-independent MDA-MB 231 cell line. Cells were grown in 250 ml flasks in phenol red-containing McCoy's medium supplemented with 5% FCS at 37 °C in a humidified atmosphere containing 5% CO_2. Shortly before

confluence they were harvested by addition of trypsin/EDTA solution and suspended in 11 ml of fresh medium. One ml-aliquots of the cell suspension were transferred to 25 ml flasks followed by the addition of 9 ml of medium containing 10% FCS. After 3–4 days of incubation the cells have reached 80% confluence and can be used for this assay. After removal of the medium, cells were washed with PBS (2.5 ml) followed by the addition of medium without FCS (2.5 ml), 20 μl of a 0.25 μM [^{3}H] estrone sulfate solution (520,000 dpm), leading to a final concentration of 2 nM, and 25 μl of inhibitor dissolved in DMF. After an incubation period of 20 h at standard conditions cells were cooled to 4 °C for 15 min before 1 ml of the supernatatant was transferred into a test tube. After the addition of [^{14}C] estrone (7500 dpm) the mixture was vortexed with toluene (5 ml). For an efficient separation of the two phases, the mixture was centrifuged at 1000 × *g* for 10 min. Two ml of the toluene layer and 3 ml of scintillation liquid were used for the separate determination of both nuclei. Values for maximum conversion to [^{3}H] estrone (controls) were obtained with 25 μl DMF without inhibitor. Background radioactivity was determined in the absence of cells.

Since all values were standardized for the protein content of each flask the remaining medium was removed from the flask, the cells were washed with PBS (2.5 ml) and lysed by the addition of lysis buffer (600 μl). The protein content was quantified according to the method of Bradford [36].

2.9. Reversibility of sulfatase Inhibition

Cells were treated as described above (2.8) with one exception. The inhibitor in a standard concentration of 1 μM was added without [^{3}H] estrone sulfate. After incubation for 2 h, medium was removed, the cells were washed twice with 2.5 ml PBS, 2.5 ml of FCS-free medium was added together with 20 μl of a 0.25 μM [^{3}H] estrone sulfate solution. The following steps were identical with those decribed above.

2.10. Estrogen receptor binding assay

Relative binding affinities (RBA) were determined as described previously [32]. The 500 μl incubation mixture comprised 5 nM [^{3}H] 17β-estradiol (added in 100 μl Tris-buffer (0.01 M, pH 7.5), supplemented with EDTA (0.01 M) and NaN_3 (0.003 M)), 10^{-9}–10^{-5} M competing ligand (in 100 μl buffer), 100 μl of calf uterine cytosol, and buffer. The mixture was incubated for 18 h at 4 °C, after which 0.5 ml of dextran-coated charcoal (DCC) slurry (0.8% charcoal Norit A and 0.008% dextran in buffer) was added to the tubes, and the contents were mixed. The tubes were incubated for 90 min at 4 °C and then centrifuged at 700 × *g* for 10 min to pellet the charcoal. An aliquot (100 μl) of the supernatant was removed and radioactivity was determined by liquid scintillation. Non-specific binding was calculated using 5 μM 17β-estradiol as competing ligand. RBA was calculated as the ratio of the molar concentrations of estradiol and test compound required to decrease the amount of bound radioactivity by 50%, multiplied by 100.

2.11. Estimation of estrogenic activity in stably transfected MCF-7/2a cells

One week before the start of the experiment, cells were cultivated with medium supplemented with 10% dextran/charcoal-treated fetal calf serum (ct-FCS). Since the MCF-7/2a cells carry the gene for neomycin resistance cells were grown in the presence of 0.35 mg neomycin (Geneticine®)/ml. Untreated cells were seeded into six-well plates 24 h prior to the addition of test compounds, reference drugs or combinations of estrone sulfate (100 nM) with an inhibitor in various concentrations. The incubation period was 50 h. Before harvesting, cells were washed twice with PBS. Cell lysis buffer (200 μl, pH 7.8) containing 5 mM TRIS-phosphate, 0.4 mM dithioerythritol, 0.4 mM 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetate, 10% glycerol, and 1% Triton X-100 was added to each well. After 20 min at room temperature cells were collected, cleared by centrifugation and stored at −20 °C.

Luciferase activity was assayed using the Promega kit according to manufactor's protocol. The luminescence of 30 μl samples and Promega assay solution (100 ml) was measured in a luminometer Lumat LB 9501 (Berthold, Wildbad, Germany). Luminescence (in relative light units, RLU) was integrated over 10 s. The background was approximately 250 RLU/10s. All measurements were corrected for the protein content of the samples quantified according to Bradford [36] using bovine serum albumin as standard. Unless stated otherwise, average values and the deviations of three independent measurements are shown. IC_{50} values were calculated from the dose–response curves.

2.12. Determination of antiproliferative activity in MCF-7 cells

MCF-7 cells were grown in EMEM, supplemented with sodium pyruvate (110 mg/ml), gentamycin sulfate (50 mg/l), $NaHCO_3$ (2.2 g/l) and 10% FCS (Gibco). The serum was sterilized through a 0.20 μM filter (Sartorius, Göttingen, FRG) and stored at −20 °C. Cells were grown in a humidified incubator in 5% CO_2 at 37 °C and harvested with 0.05% trypsin/0.02% EDTA in 0.15 M NaCl. At the start of the experiment, the cell suspension was transferred to 96-well microplates (100 μl per well). After growing them for 3 days in a humidified incubator with 5% CO_2 at 37 °C, medium was replaced by one containing the test compound, 100 nM estrone sulfate, and 10% ct-FCS. Control wells (16 per plate) contained 0.1% of DMF that was used for the preparation of the stock solution. The initial cell density was determined by addition of glutaric dialdehyde (1% in PBS; 100 μl per well). After incubation for 4–7 days, medium was removed and 100 μl of glutaric aldehyde in PBS (1%) were added for fixation. After 15 min, the solution of aldehyde was

decanted. Cells were stained by treating them for 25 min with 100 μl of an aqueous solution of crystal violet (0.02%). After decanting, cells were washed several times with water to remove adherent dye. After addition of 100 μl of ethanol (70%), plates were gently shaken for 2 h. Optical density of each well was measured in a microplate autoreader EL 309 (Bio-tek) at 578 nm.

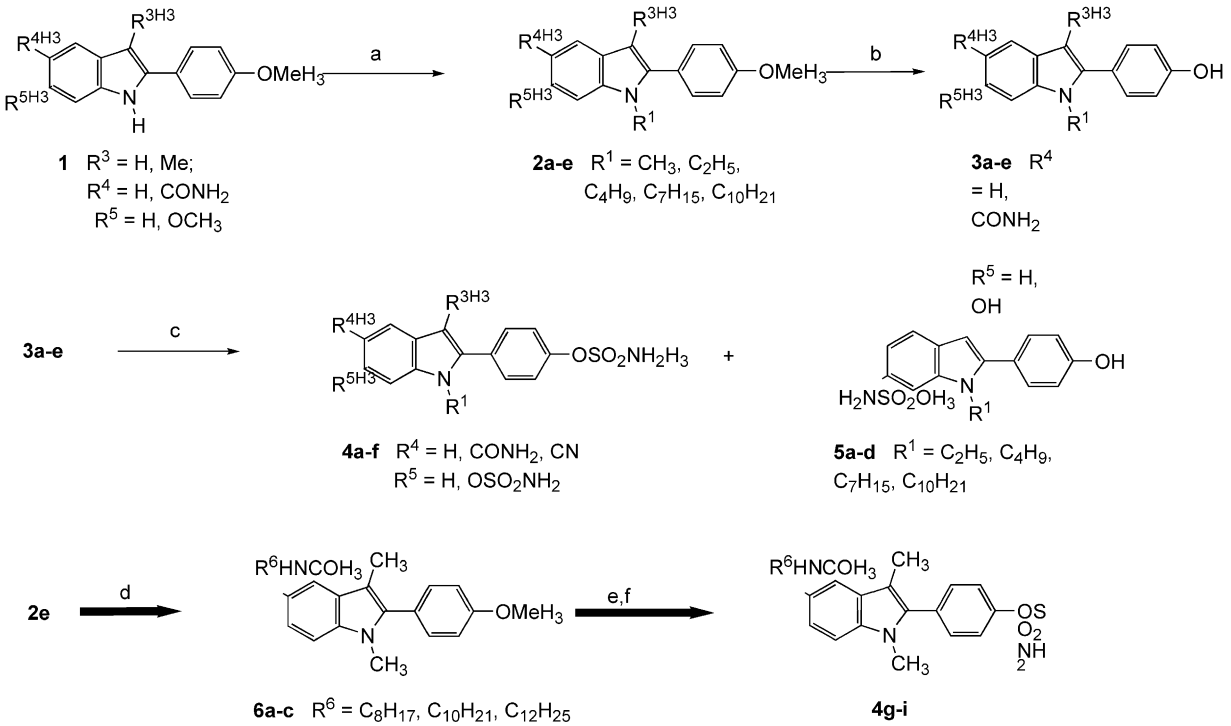
3. Results

3.1. Synthesis of non-steroidal inhibitors

The 2-phenylindole-based sulfamates were prepared as outlined in Scheme 1. The 2-(4-methoxyphenyl)indoles **1a–e** with various substituents in the indole moiety were obtained by the Bischler method [33]. The side chains in 1-position were introduced by deprotonation of the 1H-indole with sodium hydride followed by alkylation with the respective alkyl bromides to give the 1-substituted 2-(4-methoxyphenyl)indoles **2a–e**. Ether cleavage of these derivatives with boron tribromide yielded compounds **3a–e** with one or two free hydroxy groups which reacted with sulfamoyl chloride to give the corresponding sulfamates **4a–e** (Scheme 1). When hydroxy groups were present in both aromatic rings the monosulfamates **5a–d** were isolated as byproducts. In all these cases, the phenolic group in the indole part reacted first and it was not possible to obtain monosulfamoylated products with the sulfamate function in the phenyl ring. In further studies a route to the isomeric monosulfamates should be elaborated. The sulfamoylation reaction of the carbamoyl derivative **3e** gave predominantly the corresponding nitrile **4g** as the result of a water elimination. This sulfamoyl chloride catalyzed dehydration proceeded smoothly at ambient temperature and only small quantities of the sulfamoylated amide **4e** could be isolated. For the synthesis of the *N*-alkylcarbamoyl derivatives **6a–c** the amide **2e** was deprotonated with sodium hydride and reacted with the respective alkyl bromide. The methoxy group in these derivatives was cleaved with ethanethiol in the presence of $AlCl_3$ to yield the phenols **3g–i**. The sulfamoylation of **3g–i** to **4g–i** required the deprotonation of the hydroxy groups with sodium hydride prior to the reaction with sulfamoyl chloride.

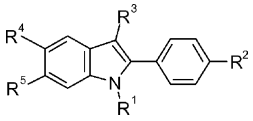
3.2. Inhibition of steroid sulfatase

The target enzyme of these investigations is the steroid sulfatase present in various human tissues including breast cancer cells. An appropriate source are human breast cancer cells which express this enzyme in sufficient quantities [37,38] and might be more relevant for the intended application than placental microsomes used by others [39]. Unlike in our previous studies [26] we used ER negative MDA-MB



Scheme 1. Synthetic routes for the preparation of sulfamoyloxy-substituted 2-phenylindoles **4** and **5**: (a) 1.5 equivalent NaH, DMF, 0 °C, 1 equivalent R^1Br, RT, 2 h; (b) 4 equivalent BBr_3, CH_2Cl_2, −20 °C, 3 h; (c) 5 equivalent H_2NSO_2Cl, DMF, RT, 12 h; (d) 1.5 equivalent NaH, DMF, 0 °C, 1 equivalent R^6Br, RT, 2 h; (e) 1.5 equivalent $AlCl_3$, CH_2Cl_2, 2.5 equivalent EtSH, 50 °C, 1 h; (f) NaH, DMF, 5 °C, 5 min, RT, 1 h, 10 equivalent H_2NSO_2Cl, 5 °C → RT, 12 h.

Table 1
Inhibition of estrone sulfatase by 1-alkyl-2-phenylindole sulfamates



Compound	R^1	R^2	R^3	R^4	R^5	Inhibition of sulfatase[a] IC_{50} (μM) (% at 1 μM)	Inhibition of E1 sulfate action[b] IC_{50} (μM) (% at 1 μM)
4a	C_2H_5	OSO_2NH_2	H	H	OSO_2NH_2	0.002	>1 (29%)
5a	C_2H_5	OH	H	H	OSO_2NH_2	0.72	>1 (17%)
4b	C_4H_9	OSO_2NH_2	H	H	OSO_2NH_2	0.004	0.17
5b	C_4H_9	OH	H	H	OSO_2NH_2	>1 (23%)	>1 (34%)
4c	C_7H_{15}	OSO_2NH_2	H	H	OSO_2NH_2	0.35	0.37
5c	C_7H_{15}	OH	H	H	OSO_2NH_2	>1 (28%)	0.8
4d	$C_{10}H_{21}$	OSO_2NH_2	H	H	OSO_2NH_2	>1 (33%)	1.0
5d	$C_{10}H_{21}$	OH	H	H	OSO_2NH_2	>1 (6%)	0.9
4e	CH_3	OSO_2NH_2	CH_3	$CONH_2$	H	0.21	0.31
4f	CH_3	OSO_2NH_2	CH_3	CN	H	0.066	0.12
3g	CH_3	OH	CH_3	$CONHC_8H_{17}$	H	>1 (10%)	>1 (1%)
4g	CH_3	OSO_2NH_2	CH_3	$CONHC_8H_{17}$	H	0.097	0.11
3h	CH_3	OH	CH_3	$CONHC_{10}H_{21}$	H	>1 (12%)	>1 (25%)
4h	CH_3	OSO_2NH_2	CH_3	$CONHC_{10}H_{21}$	H	0.106	0.02
3i	CH_3	OH	CH_3	$CONHC_{12}H_{25}$	H	>1 (7%)	>1 (4%)
4i	CH_3	OSO_2NH_2	CH_3	$CONHC_{12}H_{25}$	H	0.039	0.09
EMATE[c]						0.0006	−(−50%)[d]

[a] Inhibitory effect on the conversion of [^{3}H] estrone sulfate (2 nM) to [^{3}H] estrone by MDA-MB 231 cells; mean of triplicates.
[b] Inhibition of luciferase activity, stimulated by 100 nM estrone sulfate, in stably transfected MCF-7/2a cells; mean values of two independent experiments with six replicates; S.D. are <10%.
[c] Estrone-3-*O*-sulfamate.
[d] Negative % values indicate an additional estrogenic effect.

231 cells instead of ER-positive MCF-7 cells to avoid hormonal interference with cell proliferation [40]. Inhibition of enzyme activity was quantified by adding [^{3}H] estrone sulfate to the cells and measuring the conversion to free [^{3}H] estrone. The inhibitory activity of the new sulfamates varied over a wide range (Table 1). The data clearly showed that a sulfamoyloxy group has to be located in the phenyl ring, since all the IC_{50} values of the 6-sulfamoyloxyindoles with a free hydroxy group in the phenyl ring exceeded 1 μM except for derivative **5a** (IC_{50} 0.72 μM). In the 1-alkylindole series the IC_{50} value increased with the length of the alkyl chain. This result was unexpected since the corresponding disulfamate with a 10-(pentylsulfonyl)decyl side chain showed a value of 0.07 μM [26], obviously due to the presence of the polar sulfonyl function. In the same study, we demonstrated that the additional sulfamoyloxy group in the indole moiety can be replaced by other polar residues such as ester, amide or nitrile functions. This result was confirmed by the amide **4e** and the nitrile **4f** with IC_{50} values of 210 and 66 nM, respectively (Table 1). Since the introduction of a long alkyl side chain into the 1-position decreased the inhibitory effect, we decided to link the lipophilic residues to the amide group in **4e**. The enzyme inhibition increased upon this modification. The most potent inhibitor (**4i**) with a dodecyl group displayed a IC_{50} value of 39 nM, which is only exceeded by the disulfamates **4a** and **4b**.

These data suggest that the lipophilic residue has to be located in a position opposite of the sulfamate function for strong inhibition. In previous studies [33] we showed that the 2-phenylindole system mimics the steroid structure and the phenyl ring can play the role of the A-ring. Others have demonstrated that lipophilic substituents in the 17α-position of 3-sulfamoylestradiol such as benzyl or *tert*-butyl increase the sulfatase inhibitory activity considerably [17]. Non-steroidal inhibitors of steroid sulfatase also benefit from lipophilic groups as it has been shown for COUMATE [22]. The positive effect of the lipophilic residue can be rationalized by the association of the steroid sulfatase with the membrane of the endoplasmic reticulum [41,42] and the possibility of achoring the inhibitor in the membrane.

The inhibition of steroid sulfatase by potent inhibitors is irreversible (Table 2). This observation is in agreement with those reported for other sulfamates [43]. The inactivation of the enzyme lasted for several days (data not shown). The partial reversibility of derivatives **5a** and **4c** can be attributed to their incomplete blockade of the enzyme at the concentration used in this assay.

3.3. Estrogenic activity in MCF-7/2a cells

The aim of this study was the identification of non-steroidal compounds that inhibit the enzyme steroid sulfatase without estrogenic side effects, but possibly with antiestrogenic

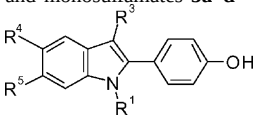
Table 2
Reversibility of enzyme inhibition after preincubation with inhibitor

Compound	Inhibition of steroid sulfatase[a] IC_{50} (nM)	Sulfatase activity after preincubation[b] (%)
4a	2	0
5a	720	66
4b	4	0
4c	350	52
4f	66	0
4I	39	0
EMATE	0.6	0

[a] Determined by incubation of MDA-MB 231 cells with [^{3}H] estrone sulfate and inhibitor for 20 h.
[b] Determined after preincubation of MDA-MB 231 cells with inhibitor (1 μM) for 2 h and subsequent removal of inhibitor.

activity. Both actions were determined in stably transfected MCF-7/2a breast cancer cells [35]. These cells express the ER as the wild-type cells and carry the luciferase gene under the control of an estrogen response element (ERE) as the reporter for estrogen-driven gene expression. Significant estrogenic activity was observed for the disulfamate **4a** and its dihydroxy precursor **3a** at a concentration of 1 μM (Fig. 2). The estrogenic effect of **3a** was not unexpected since 2-phenylindol-6-ols with short alkyl groups are known to act as estrogens in vivo [33]. The hormonal activity of **4a** can be rationalized by hydrolysis of the sulfamoyloxy groups because only the hydroxy derivatives bind to ER (Table 3). As expected from the literature, EMATE proved to be a potent agonist and exceeded the value for estradiol considerably when tested at 1 μM (Fig. 2). Interestingly, the two homologues with a heptyl and a decyl chain showed

Table 3
Relative binding affinities of 1-alkyl-2-(4-hydroxyphenyl)indolols **3a–e** and monosulfamates **5a–d**



Compound	R^1	R^3	R^4	R^5	RBA[a]
E2[b]					100
3a	C_2H_5	H	H	OH	7.1
5a	C_2H_5	H	H	OSO_2NH_2	0.08
3b	C_4H_9	H	H	OH	5.7
5b	C_4H_9	H	H	OSO_2NH_2	0.15
3c	C_7H_{15}	H	H	OH	2.0
5c	C_7H_{15}	H	H	OSO_2NH_2	0.04
3d	$C_{10}H_{21}$	H	H	OH	0.95
5d	$C_{10}H_{21}$	H	H	OSO_2NH_2	0.11
3e	CH_3	CH_3	$CONH_2$	H	0.02

[a] Relative binding affinities for the calf uterine estrogen receptor, determined by incubation at 4 °C for 20 h.
[b] 17β-Estradiol.

antiestrogenic activity in this assay when given in combination with 1 nM estradiol (data not shown).

3.4. Inhibition of estrone sulfate-stimulated gene expression in MCF-7/2a cells

All compounds with the hydroxy groups converted to sulfamate were shown to act as steroid sulfatase inhibitors of variable potencies. The overall effect of these agents on gene expression of estrogen-sensitive cells may arise from both

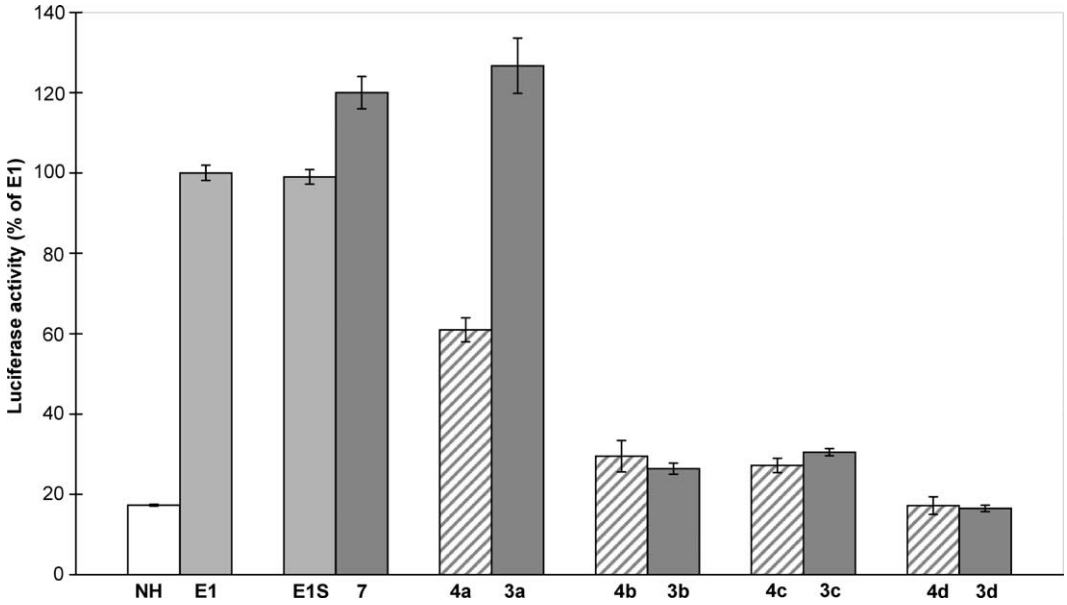


Fig. 2. Estrogenic activities of the 2-phenylindole disulfamates **4a–d** in stably transfected MCF-7/2a breast cancer cells in comparison to the corresponding dihydroxy derivatives **3a–d** and the reference drugs estrone (E1), estrone sulfate (E1S), and estrone-3-*O*-sulfamate (**7**). Concentrations of estrone and estrone sulfate were 1 nM, all other concentrations were 1 μM. Control cells (NH) received only the vehicle. Values are means ± S.D. of three independent experiments.

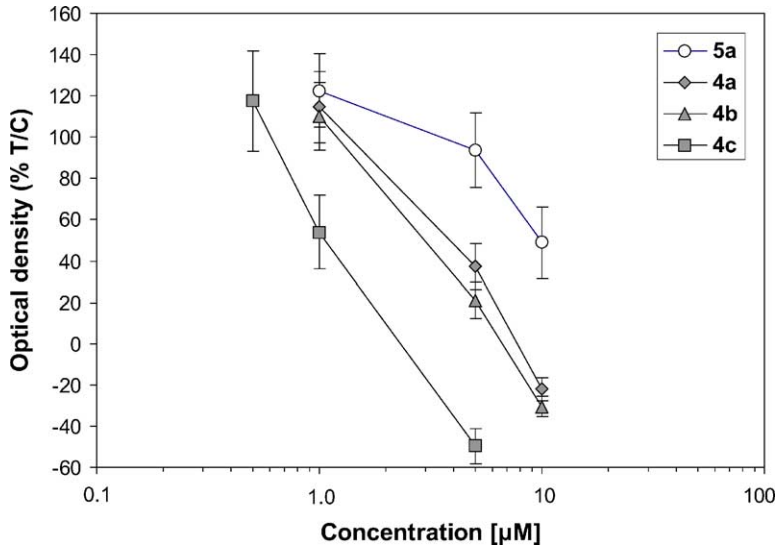


Fig. 3. Antiproliferative effects of disulfamates **4a–c** and of the monosulfamate **5a** on estrogen-sensitive MCF-7 breast cancer cells stimulated with 100 nM estrone sulfate. T/C values are given as ratio of treated cells and control cells × 100 after incubation and crystal violet staining, and are means ± S.D. of 16–24 replicates.

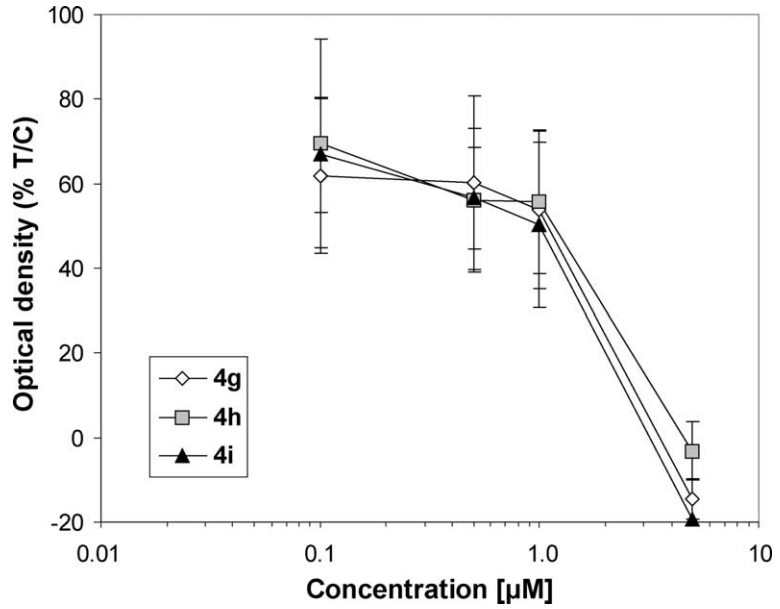


Fig. 4. Antiproliferative effects of carbamoyl sulfamates **4g–i** on estrogen-sensitive MCF-7 breast cancer cells stimulated with 100 nM estrone sulfate. T/C values are given as ratio of treated cells and control cells × 100 after incubation and crystal violet staining, and are means ± S.D. of 16–24 replicates.

enzyme inhibition and (anti)hormonal action. After these different activities had been quantified separately, the combined effects of the sulfamates on transcriptional activity was estimated in MCF-7/2a cells by using estrone sulfate (100 nM) as the agonist instead of estradiol or estrone. Generally there was a good agreement betwen both inhibitory effects (Table 1). An exception was the indole **4a**, the most potent sulfatase inhibitor in this series, which did hardly interfere with estrone sulfate in this assay. This can be rationalized by the estrogenic activity of the free hydroxy form and is in agreement with the effect of EMATE (Table 1 and Fig. 2).

3.5. Inhibition of estrone sulfate-stimulated proliferation of MCF-7 cells

Our interest in sulfatase inhibitors is mainly focussed on their potential use as therapeutic drugs for the treatment of hormone-dependent breast cancer. Therefore, most of the sulfamates were tested for antiproliferative activity

in MCF-7 breast cancer cells stimulated with estrone sulfate at 100 nM, a concentration also used by others [44]. All of the tested indoles inhibited the growth of these cells with IC_{50} values in the range from 1 to 10 μM (Figs. 3 and 4). The most active derivatives displayed values close to 1 μM which is one order of magnitude higher than the IC_{50} values for gene activation. An exception is compound **4c** which showed similar activity in both assays. This result can be explained by the moderate antiestrogenic properties of the corresponding hydroxy derivative (data not shown). ER-independent cytostatic effects which have been observed for 2-methoxyestrone-3-*O*-sulfamate [45] can be ruled out since the growth of hormone-independent MDA-MB 231 is not inhibited at 1 μM (data not shown).

4. Discussion

The array of assays used for the evaluation of new non-steroidal sulfatase inhibitors was designed to study the effects of the substances in systems of increasing complexity. The whole cell assay for measuring sulfatase in ER-negative MDA-MB 231 cells is not influenced by hormonal activities which become apparent in transfected MCF-7/2a cells whose gene expression is stimulated by estrone sulfate after its hydrolysis to estrone and subsequent reduction to estradiol. When inhibitors are included in this assay they can act by different mechanisms: Either by virtue of enzyme inhibition or as ER ligand with agonist or antagonist properties after chemical or enzymatic conversion. This complex mode of action can only be expected for those sulfamates which derive from the dihydroxy derivatives **3a–d** which were shown to bind to the ER and act as agonists when the alkyl chain is short. Enzyme inhibition and estrogenic activity are opposing effects as demonstrated by both, compound **4a** and EMATE [44], whereas antiestrogenic activity would increase the effect of the sulfatase inhibitor on gene expression [26]. When endocrine activity of the parent compounds is lacking, IC_{50} values for enzyme inhibition and suppression of luciferase activity are comparable as shown for derivatives **4g–i**.

For therapeutic application antiproliferative activity in target cells is important. ER-positive, estrogen-sensitive MCF-7 breast cancer cells have proved to be an appropriate in vitro model [44] and can also used as in vivo model when transplanted into nude mice [46]. Four of the 2-phenylindole sulfamates in this series show appreciable IC_{50} values of about 1 μM, but they are lower than those reported for some other non-steroidal compounds [44]. In the biological evaluation of aromatic sulfamates the chemical and enzymatic stability of the sulfamate function has to be considered. From our previous studies on antiestrogen-based steroid sulfatase inhibitors we know that the sulfamates are at least partially converted to the free hydroxy derivatives [26]. This effect might become dominant when the conjugates are given to man or animals.

In a preliminary study with one of the 2-phenylindole sulfamates, we found that the sulfamate is hydrolyzed to the free hydroxy derivative within a few days when kept under tissue culture conditions. In the presence of MCF-7 cells, the phenolic metabolite is converted to its sulfate by sulfotransferases present in breast cancer cells [38]. The formation of conjugates with sulfuric acid is obviously faster than their hydrolysis by steroid sulfatase. Maybe some of the effects attributed to the sulfamates are in fact due to the formation of the analoguous sulfates, which have been shown to be both inhibitors and substrates of steroid sulfatases [47]. The facile hydrolysis and the equilibrium between the hydroxy form and the sulfates add to the biological complexity of sulfamates whose free hydroxy forms and/or sulfates possess also hormonal activity. For further development of sulfamates as potentially useful inhibitors of steroid sulfatase in the therapy of hormone-dependent breast cancer it might be necessary to analyze the biological profiles of both the sulfamate and its metabolites.

Acknowledgements

The authors wish to thank the Deutsche Forschungsgemeinschaft for financial support.

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