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Sulfamoyloxy-Substituted 2-Phenylindoles: Antiestrogen-Based Inhibitors of the Steroid Sulfatase in Human Breast Cancer Cells

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Abstract—Estrone sulfate (E1S) is an endogenous prodrug that delivers estrone and, subsequently, estradiol to the target cells following the hydrolysis by the enzyme estrone sulfatase which is active in various tissues including hormone dependent breast cancer cells. Blockade of this enzyme should reduce the estrogen level in breast cancer cells and prevent hormonal growth stimulation. Sulfamates of a variety of phenolic compounds have been shown to be inhibitors of estrone sulfatase. Our rational is based on findings that these inhibitors can undergo hydrolysis and the pharmacological effects of the free hydroxy compounds contribute to the bioactivity of the sulfamates. A desirable action of the metabolites would be an estrogen antagonism to block stimulatory effects of residual amounts of estrogens. Thus, we synthesized a number of sulfamoyloxy-substituted 2-phenylindoles with side chains at the indole nitrogen that guarantee antiestrogenic activity. All of the new sulfamates were studied for their inhibitory effects on the enzyme estrone sulfatase from human breast cancer cells and their (anti)hormonal activities in stably transfected human MCF-7/2a mammary carcinoma cells. The hormonal profile of the sulfamates was partly reflected by the properties of the corresponding hydroxy precursors. Some of the sulfamoylated antiestrogens strongly inhibited estrone sulfatase activity with IC_{50} values in the submicromolar range. They were devoid of agonist activity and suppressed estrone sulfate-stimulated gene expression mainly by blocking the enzyme. Examples are the disulfamates of the indoles ZK 119, 010 and ZK 164, 015. Their IC_{50}s for sulfatase inhibition were 0.3 and 0.2 μM, respectively, and 50 and 80 nM, respectively, for the inhibition of E1S-stimulated luciferase expression in transfected MCF-7 cells. With some of the new sulfamates an additional direct antiestrogenic effect was noticed which might be due to a partial hydrolysis during incubation and would improve the growth inhibitory effect on estrogen-sensitive breast cancer cells.

Introduction

The majority of breast cancers are hormone-dependent, at least in their early stages, and require estrogens for growth. Blockade of estrogen receptors by antiestrogens and inhibition of estrogen biosynthesis by aromatase inhibitors are therapeutic options which both have proved effective in the treatment of postmenopausal patients although 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.[1] Mammary tumor tissue has been shown to be capable of cleaving this conjugate to liberate free estrone which can subsequently be converted by 17β-HSD to 17β-estradiol.[2,3] It has 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.[4] In mammary tumors the levels of free and conjugated estrogens as well as the sulfatase activity is significantly higher than in normal tissue.[5] These findings have stimulated the search for inhibitors of steroid sulfatase.

The starting point was the natural substrate estrone sulfate which was first modified chemically in the 3-position.[6–8] The most favourable substituent in respect to enzyme inhibition was the sulfamoyloxy group but the inherent estrogenic potency[9] made this derivative (EMATE, Fig. 1) unsuitable for further development as sulfatase inhibitor. Meanwhile a variety of steroidal[10,11] and nonsteroidal sulfamates[12,13] have been synthesized and evaluated as enzyme inhibitors.[14] Some of these agents have shown estrogenic activity[10,15] while others were devoid of a hormonal action.[13] Since these sulfamates can act as both inhibitor and substrate of

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steroid sulfatase the bioactivity of the free phenols is of great importance and has to be considered in the design of sulfatase inhibitors for therapeutic application. For clinical use as drug for the treatment of estrogen-dependent breast cancer it would be desirable to have an inhibitor of estrone sulfatase which can be hydrolyzed to a potent antiestrogen to offer the possibility of a dual mode of action (Fig. 2).

In our first study on non-steroidal inhibitors of the enzyme steroid sulfatase we used the 2-phenylindole system as basic structure because of its inherent antiestrogenic properties, and converted the phenolic hydroxy groups into sulfooxy functions with sodium as the cation.[16] The most active compound in this series was the fluorinated derivative **15** (Fig. 1). The following studies were aimed to increase the antiestrogenic potency of the 2-phenylindole system by introduction of functional side chains.[17–19] Some of these new compounds proved to be pure estrogen antagonists because they lack residual estrogenic activity. Parallel to these studies, other groups optimized the functional group necessary for the interaction with steroid sulfatase, and identified the sulfamoyloxy group as the most potent function.[20] Based on these findings, we also used this element and converted 2-phenyl-3-methylindoles with different side chains at the nitrogen and a variable substitution pattern in the aromatic rings into the sulfamates **8**–**14** (Fig. 1). All sulfamoyl derivatives were tested in whole cell assays for inhibitory activity against steroid sulfatase from human MCF-7 breast cancer cells, for estrogenic and antiestrogenic activity in stably transfected MCF-7/2a mammary carcinoma cells and for their effects on estrone sulfate-stimulated MCF-7/2a cells which mimic the in vivo situation. Both, the sulfamates and the corresponding free hydroxy compounds as possible products of a chemical or enzymatic hydrolysis were also tested for their ability of binding to the estrogen receptor to estimate hormonal effects.

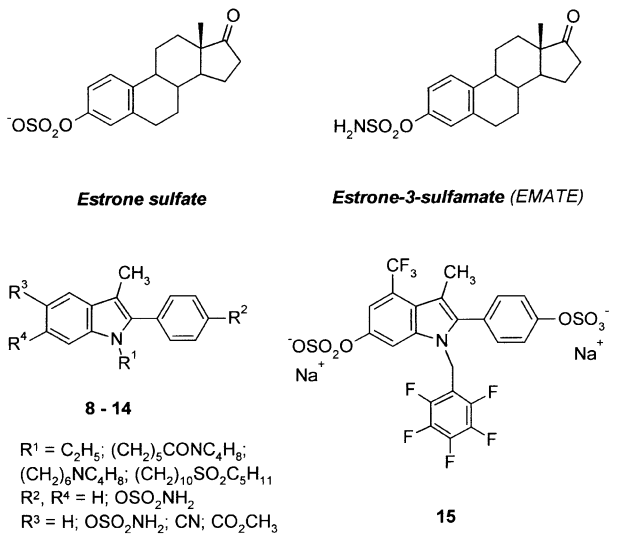


Figure. 1. Chemical structures of estrone sulfate, estrone-3-sulfamate (EMATE) and 2-phenylindole-based inhibitors of steroid sulfatase.

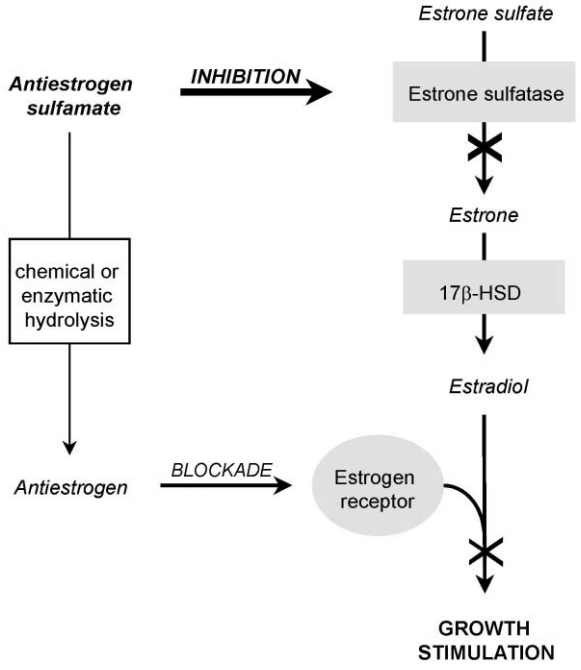
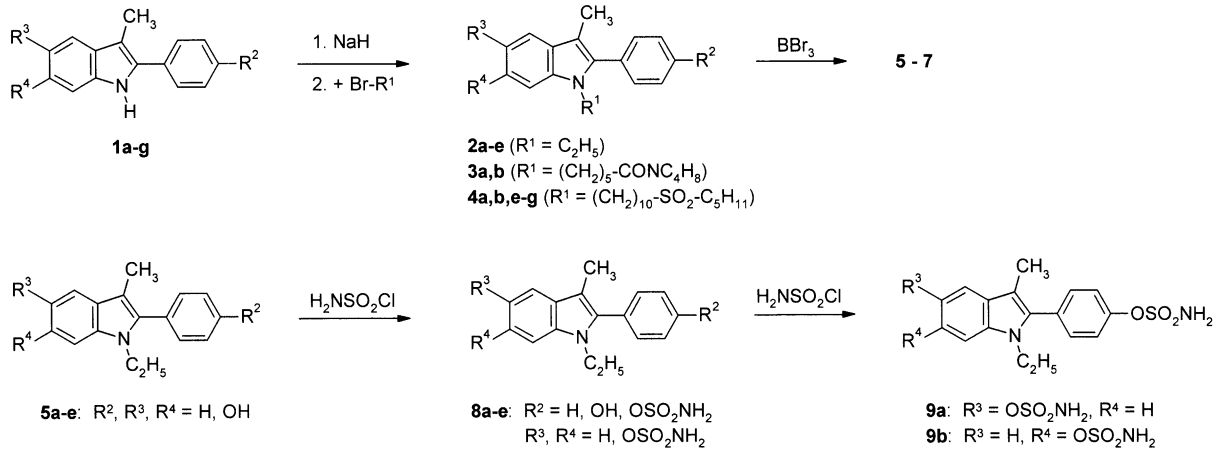


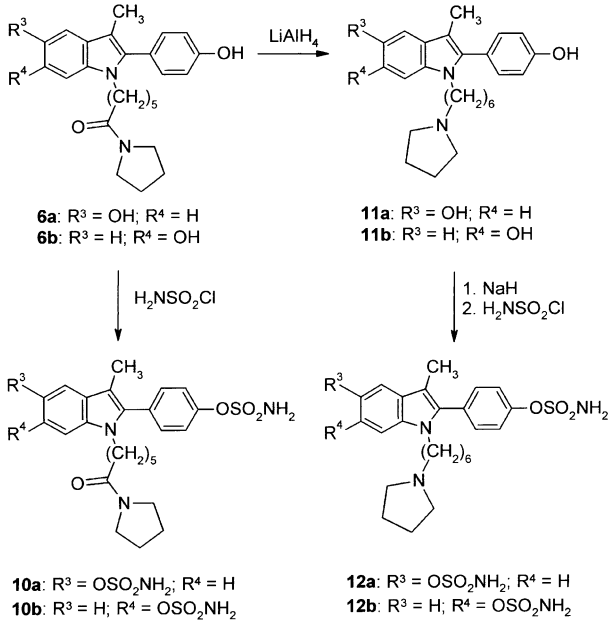
Figure 2. Stimulation of breast cancer growth by estrone sulfate: points of intervention by antiestrogen-based steroid sulfatase inhibitors.

Chemistry

The 2-phenylindole-based sulfamates were prepared as outlined in Schemes 1–3. The starting material for the syntheses was 2-(4-methoxyphenyl)-3-methylindole and 2-phenyl-3-methylindole, respectively, with methoxy or other groups in the 5- or 6-position (**1**).[21] Three different side chains which give rise to different degrees of estrogen antagonism were introduced by a nucleophilic substitution reaction. The ethyl group was used because it is also found in the zindoxifene structure.[22] The side chains with a pyrrolidine ring or sulfone group were used in analogy to the potent antiestrogens ZK 119.010[23] and ZK 164.015[19] Ether cleavage of the derivatives **2**–**4** with boron tribromide gave the compounds **5**–**7** (Scheme 1). Generally, the side chains were introduced without further modification except for the tertiary amines **11a** and **11b** which were obtained by $LiAlH_4$ reduction of the corresponding amides **6a** and **6b** after ether cleavage (Scheme 2). All of the phenolic compounds were converted to the corresponding sulfamates by reacting them with sulfamoyl chloride. When hydroxy groups were present in both aromatic rings often the monosulfamates could be 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. For the conversion of the two pyrrolidinohexyl derivatives (**11a**, **b**) into the sulfamates the procedure had to be modified due to the reduced reactivity of the hydroxy functions. Prior to the reaction with sulfamoyl chloride the phenolic groups had to be converted to the anions by sodium hydride (Scheme 2).



Scheme 1.

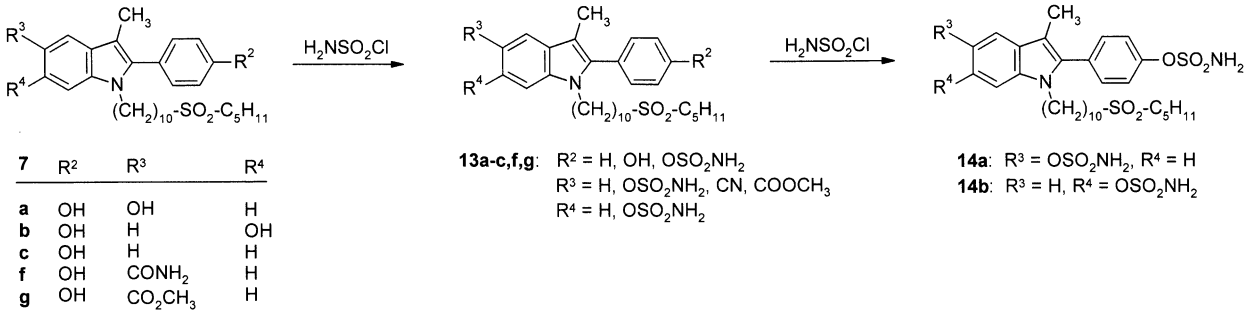


Scheme 2.

Results

Since the compounds were evaluated for biological activity in estrogen receptor (ER) positive breast cancer cells their affinity for this particular receptor was determined as the first step of biological characterization. As in previous studies[18] calf uterine cytosol was used as the receptor source and no attempt was made to distinguish between ERα and ERβ. The binding affinities decreased considerably by the conversion of the phenols into the sulfamates. Generally, the RBA values dropped by one order of magnitude after one hydroxy group had been sulfamoylated and two orders of magnitude when both hydroxy functions had been modified (Table 1).

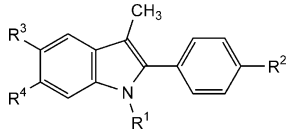
The target enzyme of these investigations is the steroid sulfatase present in various human tissues including breast cancer cells. An appropriate source for this enzyme are humam MCF-7 breast cancer cells which express this enzyme in sufficient quantities[24] and might be more relevant than placental microsomes used by others. The enzyme inhibitory activities were determined in a whole cell assay[25] because intact cells were



7	R2	R3	R4
a	OH	OH	H
b	OH	H	OH
c	OH	H	H
f	OH	$CONH_2$	H
g	OH	CO_2CH_3	H

Scheme 3.

Table 1. Relative binding affinities of 2-phenylindole sulfamates and their parent hydroxy derivatives for the estrogen receptor



Compd	R^1	R^2	R^3	R^4	RBA[a]
5a	C_2H_5	OH	OH	H	14
8a	C_2H_5	OH	OSO_2NH_2	H	0.85
9a	C_2H_5	OSO_2NH_2	OSO_2NH_2	H	0.04
5b	C_2H_5	OH	H	OH	21
8b	C_2H_5	OH	H	OSO_2NH_2	0.57
9b	C_2H_5	OSO_2NH_2	H	OSO_2NH_2	0.05
5c	C_2H_5	OH	H	H	3.4
8c	C_2H_5	OSO_2NH_2	H	H	0.37
5d	C_2H_5	H	H	OH	0.37
8d	C_2H_5	H	H	OSO_2NH_2	<0.001
5e	C_2H_5	H	OH	H	0.47
8e	C_2H_5	H	OSO_2NH_2	H	0.01
6a	$(CH_2)_5CON(CH_2)_4$	OH	OH	H	18.9
10a	$(CH_2)_5CON(CH_2)_4$	OSO_2NH_2	OSO_2NH_2	H	0.18
6b	$(CH_2)_5CON(CH_2)_4$	OH	H	OH	12.2
10b	$(CH_2)_5CON(CH_2)_4$	OSO_2NH_2	H	OSO_2NH_2	0.03
11a	$(CH_2)_6N(CH_2)_4$	OH	OH	H	33
12a	$(CH_2)_6N(CH_2)_4$	OSO_2NH_2	OSO_2NH_2	H	0.64
11b	$(CH_2)_6N(CH_2)_4$	OH	H	OH	8.4
12b	$(CH_2)_6N(CH_2)_4$	OSO_2NH_2	H	OSO_2NH_2	0.25
7a	$(CH_2)_{10}SO_2C_5H_{11}$	OH	OH	H	4.9
13a	$(CH_2)_{10}SO_2C_5H_{11}$	OH	OSO_2NH_2	H	0.45
14a	$(CH_2)_{10}SO_2C_5H_{11}$	OSO_2NH_2	OSO_2NH_2	H	0.07
7b	$(CH_2)_{10}SO_2C_5H_{11}$	OH	H	OH	2.4
13b	$(CH_2)_{10}SO_2C_5H_{11}$	OH	H	OSO_2NH_2	0.12
14b	$(CH_2)_{10}SO_2C_5H_{11}$	OSO_2NH_2	H	OSO_2NH_2	0.01
7c	$(CH_2)_{10}SO_2C_5H_{11}$	OH	H	H	0.19
13c	$(CH_2)_{10}SO_2C_5H_{11}$	OSO_2NH_2	H	H	0.06
7f	$(CH_2)_{10}SO_2C_5H_{11}$	OH	$CONH_2$	H	0.93
13f	$(CH_2)_{10}SO_2C_5H_{11}$	OSO_2NH_2	CN	H	0.03
7g	$(CH_2)_{10}SO_2C_5H_{11}$	OH	$COOCH_3$	H	0.24
13g	$(CH_2)_{10}SO_2C_5H_{11}$	OSO_2NH_2	$COOCH_3$	H	n.d.

[a]Relative binding affinities for the calf uterine estrogen receptor, determined by incubation at 4 °C for 20 h. RBA value for 17β-estradiol = 100.

also used for the following assays. Cells were incubated with [^{3}H]estrone sulfate in the presence of an inhibitor for 20 h. Enzyme activity was estimated by the amount of [^{3}H]estrone that was formed during incubation and extracted with toluene. The extraction yield was quantified by the addition of [^{14}C]-labeled estrone and measurement of both radioactive nuclei. The inhibitory activity of the new sulfamates varied over a wide range (Table 2). Some of the indole-based monosulfamates were completely devoid of activity (**8e**, **13a**, **13b**). The strongest inhibition was observed for the disulfamate **9b** (IC_{50}: 5 nM) with an ethyl side chain and a sulfamoyloxy group in position 6. Its effect was only one order of magnitude weaker than that of the steroidal reference drug estrone-3-sulfamate (EMATE) but higher than other nonsteroidal sulfamates such as the coumarin-based inhibitor COUMATE with an IC_{50} value of 380 nM.[12] High inhibitory activity was also found for **9a**, for the two pyrrolidinocarbonyl derivatives (**10a**, **10b**), and the disulfamate **14b** with a 10-(pentylsulfonyl)decyl side chain with IC_{50} values in the range of other nonsteroidal structures tested in MCF-7 cells.[12] The data for the three monosulfamates **8c**, **8d**, and **8e** without an additional functional group in the other ring revealed that the sulfamoyloxy group in the phenyl ring provides the most favorable conditions for inhibition. The values for **14a**, **13f**, and **13g** indicate the importance of a polar function in the indole moiety (Table 2).

The aim of this study was the identification of nonsteroidal compounds that inhibit the enzyme steroid sulfatase without estrogenic side effects but possibly with antiestrogenic activity. Both actions were determined in stably transfected MCF-7/2a breast cancer cells. These cells express the estrogen receptor like the wild-type cells and carry the luciferase gene under control of an estrogen responsive element (ERE) as the reporter for estrogen-driven gene expression. All of the 2-phenylindole-based sulfamates with an ethyl group at the nitrogen (**8**, **9**) showed moderate to strong estrogenic activity at a concentration of 1 μM (Table 2). The effect of estradiol at 10^{-8} M, however, was not reached. All sulfamates with side chains other than ethyl were devoid of agonist activity in this assay (Table 2). When the results were compared with data for the derivatives lacking one or both the sulfamoyl groups no marked difference was noticed (Fig. 3a and b). As expected from the literature, EMATE proved to be a potent agonist and exceeded the value for estradiol considerably when tested at 1 μM (Table 2).

Table 2. Sulfatase inhibition, estrogenic and antiestrogenic activities, and combined hormonal effects of 2-phenylindole sulfamates in wild-type or transfected MCF-7 breast cancer cells

Compd	Inhibition of estrone sulfatase[a] [IC_{50} (μM) or % at 1 μM]	Estrogenic activity[b] (% of E2)	Antiestrogenic activity[c] (% inhibition)	Inhibition of E1 sulfate action[d] IC_{50} (μM) or % at 1 μM
8a	0.5	73	−8	−19%
9a	0.01	73	−7	−7%
8b	0.2	82	−2	−7%
9b	0.005	79	11	−10%
8c	0.2	72	−24	−10%
8d	20%	41	1	5%
8e	0%	15	−2	26%
10a	0.07	0	4	0.20
10b	0.03	18	8	0.40
12a	0.3	0	8	0.05
12b	0.3	0	4	0.07
13a	0%	0	22	0.14
14a	0.2	1	37	0.08
13b	0%	0	24	0.46
14b	0.07	0	16	0.44
13c	5	0	7	0.67
13f	0.15	0	5	0.10
13g	0.27	0	6	0.20
EMATE	0.0003	128	6	−50%

[a]Inhibitory effect on the conversion of [^{3}H]estrone sulfate (2 nM) to [^{3}H]estrone by MCF-7 cells.
[b]Stimulation of luciferase activity by test compounds (1 μM) in stably transfected MCF-7/2a cells; given in% of the value for estradiol (10 nM). Basal activity (15% of E2) was subtracted from all values.
[c]Inhibition of estradiol stimulated luciferase activiy by test compounds (1 μM) in stably transfected MCF-7/2a cells; given in % of the value for estradiol (10 nM). Negative values indicate an additional estrogenic effect.
[d]Inhibition of estrone sulfate stimulated luciferase activity in stably transfected MCF-7/2a cells; given in% of the value for estrone sulfate (100 nM). Negative values indicate an additional estrogenic effect.

Antiestrogenic properties were determined in a similar way except that the MCF-7/2a cells were treated with the 2-phenylindole derivatives (1 μM) in the presence of 10^{-8} M estradiol. All sulfamates with an ethyl group were devoid of antiestrogenic activity as expected from the test on agonist action (Table 2). The disulfamates with pyrrolidinocarbonyl-pentyl or pyrrolidinohexyl as side chains showed no significant antagonism, but three out of the four phenols (**6a**, **11a**, **11b**) acted as antiestrogens (Fig. 4a). This activity has been previously reported for **11a** (ZK 119.010) in mice and rats.[23] The only sulfamates with antiestrogenic properties in this assay are characterized by a (pentylsulfonyl)decyl side chain which is typical for pure antiestrogens (Table 2). The strongest effect was observed for the disulfamate **14a** (37% inhibition at 1 μM). The value for the corresponding monosulfamate **13a** with a free hydroxy group at the phenyl ring was 22% whereas the dihydroxy derivative **7a** completely antagonized the effect of estradiol as described previously (Fig. 4b).[19]

The majority of these compounds was shown to act as steroid sulfatase inhibitors of variable potencies. The overall effect of these agents on gene expression may arise from both the inhibition of this particular enzym and their (anti)hormonal action. After these three different activities had been quantified separately, the combined effect of the sulfamates on the transcriptional activity was estimated in MCF-7/2a cells by using estrone sulfate (0.1 μM) as the agonist instead of estradiol or estrone. All ethyl derivatives with estrogenic activity rather enhanced the effect of estrone sulfate than inhibited it (Table 2). All of the compounds with other side chains strongly inhibited the stimulation by estrone sulfate as measured by the luciferase expression. The IC_{50} values were in the submicromolar range (Table 2). The most potent derivatives were the two pyrrolidinohexyl derivatives **12a** and **12b** (IC_{50}: 50 and 70 nM, respectively) and the indole **14a** with a (pentylsulfonyl)decyl side chain (IC_{50}: 80 nM). The 2-phenylindoles with functionalities other than sulfamoyloxy in the indole part (**13f**, **13g**) displayed similar activities. In those cases (**12a**, **12b**, **14a**) in which the inhibitory effect on estrone sulfate-stimulated luciferase expression in transfected cells exceeded the inhibition of steroid sulfatase measured in wild-type MCF-7 cells a contribution of estrogen antagonism to the overall effect is likely (Table 2).

Discussion

For the interpretation of these results both, enzyme inhibition and hormonal effects on the cellular level have to be considered. Possibly, the enzyme-catalyzed hydrolysis of the sulfamates to the free hydroxy derivatives can contribute to overall activity if the sulfamates function as substrates. All sulfamoyloxy derivatives with inherent estrogenic activity were incapable of interfering with the estrone sulfate mediated stimulation of gene expression although they displayed the strongest inhibitory effects on the enzyme. Obviously, the ability of binding to the active site of the enzyme coincides with the capability of acting as agonist. The inhibitory activities

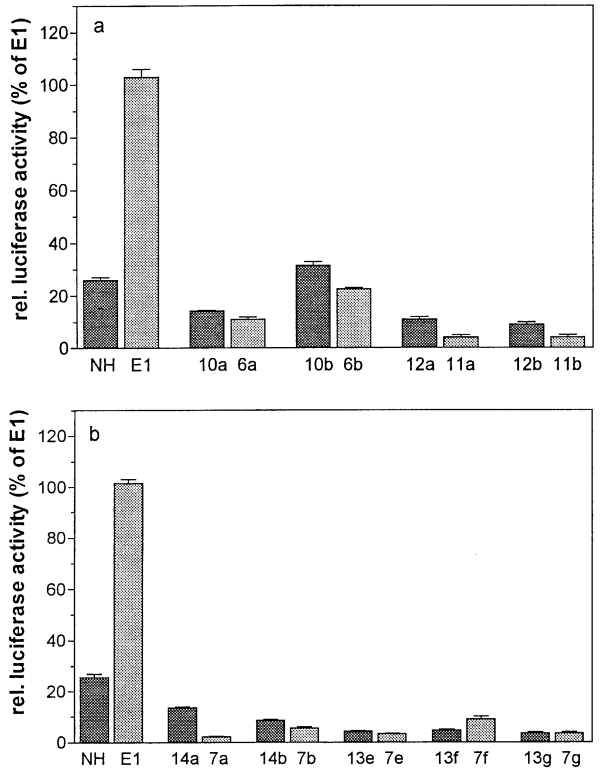


Figure 3. Comparison of the estrogenic activities of 2-phenylindole sulfamates (1 μM) and their corresponding hydroxy derivatives (1 μM) in stably transfected MCF-7/2a breast cancer cells. Panel a: Derivatives with pyrrolidine-containing side chains. Panel b: Derivatives with 10-(pentylsulfonyl)decyl side chains. E1 = estrone (10 nM). Values are means ±SD of three independent experiments.

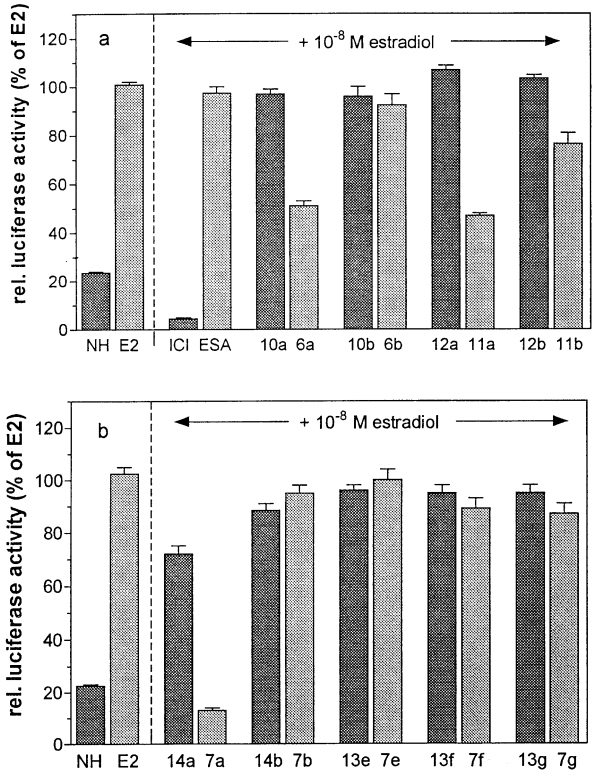


Figure 4. Comparison of direct antiestrogenic effects of 2-phenylindole sulfamates (1 μM) and their corresponding hydroxy derivatives (1 μM) in stably transfected MCF-7/2a breast cancer cells. Panel a: Derivatives with pyrrolidine-containing side chains. Panel b: Derivatives with 10-(pentylsulfonyl)decyl side chains. E2 = 17β-estradiol (10 nM); ICI = fulvestrant (ICI 182, 780; 1 μM); ESA = estrone sulfamate (EMATE; 1 μM). Values are means ±SD of three independent experiments.

of the pyrrolidinocarbonyl derivatives **10a** and **10b** with submicromolar IC_{50} values can be rationalized by the enzyme inhibition. A contribution of the free hydroxy compounds is unlikely for **10b** but cannot be ruled out for **10b** because its hydroxy derivative (**6a**) displayed significant antiestrogenic effects. The activity profile changed when the carbonyl group had been reduced to a methylene group to give **12a** and **12b**. These amino derivatives proved to be the most active compounds in this series in respect to the blockade of estrone sulfate action in transfected MCF-7/2a cells with IC_{50} values of 50 and 70 nM, respectively. The observed increase in overall activity was not paralleled by an enhanced enzym inhibition but appears to be an additive effect of estrogen antagonism due to the formation of the free hydroxy derivatives **11a** and **11b**. This result supports the assumption that these agents can act by a dual mode of action.

In the series of 2-phenylindoles with a (pentylsulfonyl)decyl side chain which is responsible for the strong antiestrogenic character of this type of molecule both, mono- and disulfamoylated products were studied. It was interesting to note that the monosulfamates **13a** and **13b** though devoid of enzyme inhibition strongly decreased the stimulatory effect of estrone sulfate. Obviously, hydrolysis of estrone sulfate to estrone took place but the monosulfamates blocked the action of thus formed estrone and/or estradiol by virtue of their antiestrogenic properties. A much higher activity in estrone sulfate-stimulated MCF-7/2a cells was found for the two monosulfamate **13f** and **13g** though their antiestrogenic effects were hardly significant. Their potency can only be rationalized by a strong inhibition of the steroid sulfatase. The comparison of the IC_{50} values of compounds **14a** and **14b** revealed that the antiestrogenic effect in this particular series has a greater share than the enzyme inhibition because the stronger antagonist **14a** inhibited the action of estrone sulfate better than the isomer **14b** despite its lower inhibitory effect on the enzym (Table 2). The most favorable conditions are provided by strong enzyme inhibition combined with antiestrogenic activity of the free phenols as demonstrated for the bis(sulfamoyloxy)-substituted 2-phenylindole **14a** with an IC_{50} value of 80 nM.

Conclusion

The results of this study cleary showed that the 2-phenylindole system is appropriate for the synthesis of new steroid sulfatase inhibitors after one or two phenolic hydroxy groups have been converted to sulfamates. Sufficient potency can be reached when the indole moiety carries a polar substituent besides a sulfamoyloxy group in the phenyl ring. Though the 1-ethyl derivatives possess the highest inhibitory activity they are inappropriate for further development due to their inherent estrogenic

activity. Obviously, sulfamates derived from agonists fit much better into the active site of the enzyme than antiestrogen based sulfamates because they lack the functional side chain. Systematic variation of this side chain, however, made it possible to keep the decrease in enzyme inhibition to a minimum without changing the hormonal profile of the parent compounds. All antiestrogen-derived sulfamates strongly inhibited estrone sulfate-stimulated gene expression in stably transfected MCF-7/2a breast cancer cells mainly by virtue of their enzyme inhibiting activity. One particular compound (**14a**), however, seems to block the estrogen receptor as an antiestrogen after sulfatase catalyzed hydrolysis. A careful analysis of the data for the two most active compounds **12a** and **12b** revealed that they abrogate the effect of estrone sulfate on gene activation by a dual mode of action: enzyme inhibition and receptor blockade. These findings can be taken as proof of principle and provide the basis for further investigations.

Experimental

Melting points were determined on a Büchi 510 apparatus and are uncorrected. NMR spectra were obtained on a Bruker AC-250 spectrometer with TMS as internal standard and were in accord with the assigned structures. Purity of all compounds was checked by tlc. Elemental analyses were performed by the Mikroanalytisches Laboratorium, University of Regensburg. High-resolution mass spectra were recorded on a MAT 95 (Varian). The syntheses of 1-ethyl-5-hydroxy-2-(4-hydroxyphenyl)-3-methylindole (**5a**),[21] 1-ethyl-6-hydroxy-2-(4-hydroxyphenyl)-3-methylindole (**5b**),[21] 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[5-(pyrrolidinocarbonyl)pentyl]indole (**6a**),[18] 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl] indole (**7a**),[19] and 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[6-(pyrrolidino)hexyl]indole (ZK119010, **11a**)[17] have been described previously.

5-Carbamoyl-2-(4-methoxyphenyl)-3-methylindole (1f). A solution of 4-aminobenzamide (51.54 g, 0.38 mol) in *N*,*N*-dimethylaniline (75 mL) was heated to 170 °C followed by the slow addition of 0.18 mol of α-bromo-4-methoxypropiophenone (43.74 g, 0.18 mol) in xylene (240 mL). After heating for another 3 h under reflux, the mixture was cooled to rt and poored into 2 N HCl (600 mL). The organic layer was separated and the aqueous extracted three times with EtOAc. The combined organic layers were washed with 2 N HCl and dried ($MgSO_4$). After the evaporation of the solvent, the residue was crystallized with a small volume of warm EtOH to give a greenish solid (22% yield), mp 245–247 °C. ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H, $-CH_3$); 3.82 (s, 3H, $-OCH_3$); 7.09, 7.61 (AA′BB′, ^3J = 8.8 Hz, 4H, ArH); 7.10 (s, br, 1H, –CO–NH); 7.33 (dd, ^3J = 8.4 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.67 (dd, ^3J = 8.4 Hz, ^4J = 1.6 Hz, 1H, indole-H^6); 7.86 (s, br, 1H, –CO–NH); 8.15 (dd, ^4J = 1.6 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 11.30 (s, 1H, –N–H). Anal. ($C_{17}H_{16}N_2O_2$): C, 72.83; H, 5.76; N, 9.99. Found: C, 72.50; H, 5.71; N, 9.56.

5-Methoxycarbonyl-2-(4-methoxyphenyl)-3-methylindole (1g). Prepared from methyl 4-aminobenzoate by a method similar to that described for **1f**. The crude product was purified by chromatography and recrystallized (EtOH) to give colorless crystals (20% yield), mp 161–162 °C. ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H, $-CH_3$), 3.83 (s, 3H, $-OCH_3$); 3.86 (s, 3H, $-OCH_3$); 7.10, 7.61 (AA′BB′, ^3J = 8.8 Hz, 4H, ArH); 7.40 (dd, ^3J = 8.5 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.74 (dd, ^3J = 8.5 Hz, ^4J = 1.6 Hz, 1H, indole-H^6); 8.20 (dd, ^4J = 1.6 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 11.51 (s, 1H, –N–H). Anal. ($C_{18}H_{17}NO_3$): C, 73.19; H, 5.81; N, 4.74. Found: C, 72.63; H, 5.74; N, 4.61.

1-Ethyl-5-methoxy-3-methyl-2-phenylindole (2c). Under N_2, a solution of 5-methoxy-3-methyl-2-phenylindole (**1c**, 1.19 g, 4.47 mmol) in dry DMF (25 mL) was added slowly with stirring to an ice-cold suspension of NaH (158 mg, 6.6 mmol) in dry DMF. Stirring was continued until the gas evolution ceased. Then, ethyl bromide (0.49 g, 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 residue purified by chromatography (SiO_2) with CH_2Cl_2/EtOAc mixtures as the eluent. Recrystallization with EtOH yielded colorless crystals (59% yield), mp 96–97 °C. ^{1}H NMR ($CDCl_3$) δ 1.19 (t, ^3J = 7.2 Hz, 3H, $C\underline{H}_3$–CH_2–); 2.21 (s, 3H, $-CH_3$); 3.90 (s, 3H, $-OCH_3$); 4.02 (q, ^3J = 7.2 Hz, 2H, CH_3–$C\underline{H}_2$–); 6.90 (dd, ^3J = 8.8 Hz, ^4J = 2.4 Hz, 1H, indole-H^6); 7.05 (dd, ^4J = 2.4 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 7.26 (dd, ^3J = 8.8 Hz, ^5J = 0.5 Hz, 1H, indole-H^7), 7.38–7.53 (m, 5H, phenyl-H). Anal. ($C_{18}H_{19}NO$): C, 81.46; H, 7.23; N, 5.28. Found: C, 81.14; H, 6.90; N, 5.00.

1-Ethyl-6-methoxy-3-methyl-2-phenylindole (2d). Prepared from 6-methoxy-3-methyl-2-phenylindole (**1d**) by a method similar to that described for **2c**. Colorless crystals (75% yield), mp 62–63 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.19 (t, ^3J = 7.2 Hz, 3H, $C\underline{H}_3$–CH_2–); 2.21 (s, 3H, $-CH_3$); 3.90 (s, 3H, $-OCH_3$); 4.02 (q, ^3J = 7.2 Hz, 2H, CH_3–$C\underline{H}_2$–); 6.81 (dd, ^3J = 7.8 Hz, ^4J = 2.2 Hz, 1H, indole-H^5); 6.83 (dd, ^4J = 2.2 Hz, ^5J = 0.5 Hz, 1H, indole-H^7), 7.36–7.51 (m, 6H, phenyl-H, indole-H^4). Anal. ($C_{18}H_{19}NO$): C, 81.46; H, 7.23; N, 5.28. Found: C, 81.42; H, 7.33; N, 5.21.

1-Ethyl-2-(4-methoxyphenyl)-3-methylindole (2e). Prepared from 2-(4-methoxyphenyl)-3-methylindole (**1e**) by a method similar to that described for **2c**. Colorless needles (89% yield), mp 133 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.20 (t, ^3J = 7.1 Hz, 3H, $C\underline{H}_3$–CH_2–); 2.22 (s, 3H, $-CH_3$); 3.88 (s, 3H, $-OCH_3$); 4.05 (q, ^3J = 7.1 Hz, 2H, CH_3–$C\underline{H}_2$–); 7.01, 7.31 (AA′BB′, ^3J = 8.8 Hz, 4H, phenyl-H); 7.04–7.60 (m, 4H, indole-H). Anal. ($C_{18}H_{19}NO$): C, 81.46; H, 7.23; N, 5.28. Found: C, 81.17; H, 7.50; N, 5.12.

6-Methoxy-2-(4-methoxyphenyl)-3-methyl-1-(5-pyrrolidinocarbonylpentyl)indole (3b). Prepared from 6-methoxy-2-(4-methoxyphenyl)-3-methylindole (**1b**)[21] and 6-bromohexanoyl-pyrrolidine by a method similar to that described for **2c**. Yellow oil (85% yield). ^{1}H NMR ($CDCl_3$) δ 1.13–1.20 (m, 2H, –$(CH_2)_2$–C$\underline{H}_2$–); 1.49–1.62 (m, 4H, –CH_2–C$\underline{H}_2$–CH_2–, –CH_2–C$\underline{H}_2$–), 1.85–1.94 (m, 4H, –N(CH_2–C$\underline{H}_2$–)$_2$), 2.12 (t, ^3J = 7.5 Hz, 2H, –CO-CH_2–), 2.18 (s, 3H, –CH_3), 3.30–3.42 (m, 4H, –N–(CH_2–)$_2$), 3.87 (s, 3H, –OCH_3), 3.89 (s, 3H, –OCH_3), 3.95 (t, ^3J = 7.5 Hz, 2H, indole-N–CH_2–), 6.80 (dd, ^3J = 9.1 Hz, ^4J = 2.2 Hz, 1H, indole-H^5), 6.81 (dd, ^4J = 2.2 Hz, ^5J = 0.5 Hz, 1H, indole-H^7), 6.99, 7.27 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H), 7.44 (dd, ^3J = 9.1 Hz, ^5J = 0.5 Hz, 1H, indole-H^4).

6-Methoxy-2-(4-methoxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]indole (4b). Prepared from (**1b**) and 1-bromo-10-(pentylsulfonyl)decane[19] by a method similar to that described for **2c**. Colorless crystals (53% yield), mp 61–63 °C (Et_2O). ^{1}H NMR ($CDCl_3$) δ 0.92 (t, ^3J = 7.0 Hz, 3H, C$\underline{H}_3$–CH_2–); 1.13–1.45 (m, 16H, –CH_2–); 1.54–1.90 (m, 6H, –N–CH_2–C$\underline{H}_2$–, SO_2(CH_2–C$\underline{H}_2$–)$_2$); 2.19 (s, 3H, –CH_3); 2.89–2.96 (m, 4H, SO_2(CH_2–)$_2$); 3.88 (s, 3H, –OCH_3); 3.90 (s, 3H, –OCH_3); 3.93 (t, ^3J = 7.5 Hz, 2H, –N–CH_2–); 6.78–6.83 (m, 2H, indole-H^{5+7}); 7.00, 7.28 (AA′BB′, ^3J = 8.8 Hz, 4H, phenyl-H); 7.45 (dd, ^3J = 8.9 Hz, ^5J = 0.5 Hz, 1H, indole-H^4). Anal. ($C_{32}H_{47}NO_4S$): C, 70, 92; H, 8.76; N, 2.59. Found: C, 70.78; H, 8.67; N, 2.63.

2-(4-Methoxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl] indole (4c). Prepared from **1e** and 1-bromo-10-(pentylsulfonyl)decane[19] by a method similar to that described for **2c**. Colorless solid (64% yield), mp 65 °C. ^{1}H NMR ($CDCl_3$) δ 0.92 (t, ^3J = 7.0 Hz, 3H, C$\underline{H}_3$–CH_2–), 1.13–1.49 (m, 16H, –CH_2–); 1.56–1.90 (m, 6H, N–CH_2–C$\underline{H}_2$–, SO_2(CH_2–C$\underline{H}_2$–)$_2$); 2.22 (s, 3H, –CH_3); 2.89–2.96 (m, 4H, SO_2(C$\underline{H}_2$–)$_2$), 3.89 (s, 3H, –OCH_3); 3.99 (t, ^3J = 7.5 Hz, 2H, N–CH_2–); 7.02, 7.30 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 7.09–7.34 (m, 3H, indole-H$^{5,6+7}$); 7.56–7.59 (m, 1H, indole-H^4). Anal. ($C_{31}H_{45}NO_3S$): C, 72.74; H, 8.88; N, 2.74. Found: C, 72.46; H, 8.86; N, 2.73.

5-Carbamoyl-2-(4-methoxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]indole (4f). Prepared from **1f** and 1-bromo-10-(pentylsulfonyl)decane[19] by a method similar to that described for **2c**. Colorless crystals (80% yield), mp 107–109 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 0.87 (t, ^3J = 7.1 Hz, 3H, C$\underline{H}_3$–CH_2–); 1.03–1.44 (m, 18H, –CH_2); 1.57–1.72 (m, 4H, $\underline{S}O_2$(CH_2–C$\underline{H}_2$–)$_2$); 2.18 (s, 3H, –CH_3); 3.00–3.07 (m, 4H, SO_2(CH_2–)$_2$); 3.84 (s, 3H, –OCH_3); 4.06 (t, ^3J = 7.1 Hz, 2H, N−CH_2−); 7.10, 7.36 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 7.10 (s, br, 1H, –CO–NH–); 7.47 (dd, ^3J = 8.6 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.73 (dd, ^3J = 8.6 Hz, ^4J = 1.5 Hz, 1H, indole-H^6); 7.88 (s, br, 1H, –CO–NH–); 8.16 (dd, ^4J = 1.5 Hz, ^5J = 0.5 Hz, 1H, indole-H^4). Anal. ($C_{32}H_{46}N_2O_4S$): C, 69.26; H, 8.37; N, 5.05. Found: C, 69.06; H, 8.30; N, 4.96.

5-Methoxycarbonyl-2-(4-methoxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]indole (4g). Prepared from **1g** and 1-bromo-10-(pentylsulfonyl)decane[19] by a method similar to that described for **2c**. Light yellow resin (76% yield). ^{1}H NMR ($CDCl_3$) δ 0.92 (t, ^3J = 7.0 Hz, 3H, C$\underline{H}_3$–CH_2–); 1.13–1.49 (m, 16H, –CH_2); 1.57–1.89 (m, 6H, N–CH_2–C$\underline{H}_2$–, SO_2(CH_2–C$\underline{H}_2$–)$_2$); 2.24 (s, 3H, –CH_3); 2.89–2.96 (m, 4H, SO_2(CH_2–)$_2$); 3.89 (s, 3H, –OCH_3); 3.95 (s, 3H, –$COOCH_3$); 4.01 (t, ^3J = 7.5 Hz, 2H, N–CH_2–); 7.02, 7.30 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 7.31 (dd, ^3J = 8.7 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.92 (dd, ^3J = 8.7 Hz, ^4J = 1.6 Hz, 1H, indole-H^6); 8.34 (dd, ^4J = 1.6 Hz, ^5J = 0.5 Hz, 1H, indole-H^4).

1-Ethyl-2-(4-hydroxyphenyl)-3-methylindole (5c). Under N_2 a solution of **2c** (143 mg, 0.54 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise with stirring to a solution of BBr_3 (0.54 g, 2.16 mmol) in dry CH_2Cl_2 (10 mL) that had been cooled to −20 °C. After the addition the cooling bath was removed and stirring continued for 3 h. With cooling satd $NaHCO_3$ solution (60 mL) was added slowly with stirring followed by the addition of EtOAc (100 mL). The aqueous phase was separated and extracted twice with EtOAc. The combined organic layers were washed with satd $NaHCO_3$ 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 (10:1) as the eluent. Crystallization from CH_2Cl_2 gave colorless crystals (52% yield), mp 122–124 °C (CH_2Cl_2/*n*-hexane 2:1). ^{1}H NMR (DMSO-d_6) δ 1.08 (t, ^3J = 7.1 Hz, 3H, C$\underline{H}_3$–CH_2–); 2.13 (s, 3H, –CH_3); 4.04 (q, ^3J = 7.1 Hz, 2H, CH_3–C$\underline{H}_2$–); 6.93, 7.23 (AA′BB′, ^3J = 8.6 Hz, 4H, phenyl-H); 7.01–7.51 (m, 4H, indole-H), 9.70 (s, br, 1H, –OH). Anal. ($C_{17}H_{17}NO$): C, 81.23; H, 6.83; N, 5.57. Found: C, 81.07; H, 6.82; N, 5.58.

1-Ethyl-6-hydroxy-3-methyl-2-phenylindole (5d). Prepared from **2d** by the method described for **5c**. Colorless crystals (61% yield), mp 149–150 °C (CH_2Cl_2). ^{1}H NMR (DMSO-d_6) δ 1.05 (t, ^3J = 7.1 Hz, 3H, CH_3–C$\underline{H}_2$–); 2.11 (s, 3H, –CH_3); 3.94 (q, ^3J = 7.1 Hz, 2H, CH_3–C$\underline{H}_2$–); 6.60 (dd, ^3J = 8.4 Hz, ^4J = 2.1 Hz, 1H, indole-H^5); 6.76 (dd, ^4J = 2.1 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.30 (dd, ^3J = 8.4 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 7.36–7.57 (m, 5H, phenyl-H); 9.03 (s, 1H, –OH). Anal. ($C_{17}H_{17}NO$): C, 81.23; H, 6.83; N, 5.57. Found: C, 81.57; H, 6.79; N, 5.68.

1-Ethyl-5-hydroxy-3-methyl-2-phenylindole (5e). Prepared from **2e** by the method described for **5c**. Colorless crystals (65% yield), mp 146–147 °C. ^{1}H NMR (DMSO-d_6) δ1.04 (t, ^3J = 7.4 Hz, 3H, C$\underline{H}_3$–CH_2–); 2.08 (s, 3H, –CH_3); 3.99 (q, ^3J = 7.4 Hz, 2H, CH_3–C$\underline{H}_2$–); 6.69 (dd, ^3J = 8.6 Hz, ^4J = 2.3 Hz, 1H, indole-H^6); 6.82 (dd, ^4J = 2.3 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 7.26 (dd, ^3J = 8.6 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.36–7.56 (m, 5H, phenyl-H); 8.72 (s, 1H, –OH). Anal. ($C_{17}H_{17}NO$): C, 81.23; H, 6.83; N, 5.57. Found: C, 81.19; H, 6.89; N, 5.56.

6-Hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-(5-pyrrolidinocarbonylpentyl)indole (6b). Prepared from **3b** by the method described for **5c**. Grey powder (64% yield), mp 182–183 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 1.03–1.09 (m, 2H, N–(CH_2)$_2$–C$\underline{H}_2$–); 1.31–1.50 (m, 4H, N–CH_2–C$\underline{H}_2$–, –CO–CH_2–C$\underline{H}_2$–); 1.70–1.86 (m, 4H, –N(CH_2–

$C\underline{H}_2–)_2$); 2.03–2.08 (m, 2H, –CO–CH_2–); 2.07 (s, 3H, –$C\underline{H}_3$); 3.20–3.32 (m, 4H, –N–$(CH_2–)_2$), 3.87 (t, $^3J=7.2$ Hz, 2H, indole-N–CH_2–); 6.56 (dd, $^3J=8.4$ Hz, $^4J=2.0$ Hz, 1H, indole-H^5); 6.71 (d, $^4J=2.0$ Hz, 1H, indole-H^7); 6.87, 7.17 (AA′BB′, $^3J=8.5$ Hz, 4H, phenyl-H); 7.25 (d, $^3J=8.4$ Hz, 1H, indole-H^4); 8.93 (s, 1H, indole-OH); 9.60 (s, 1H, phenyl-OH). Anal. ($C_{25}H_{30}N_2O_3$): C, 73.85H, 7.45; N, 6.89. Found: C, 72.45; H, 7.49; N, 6.57.

6-Hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]indole (7b). Prepared from **4b** by the method described for **5c**. Colorless resin (91% yield). ^{1}H NMR ($CDCl_3$) δ 0.93 (t, $^3J=7.0$ Hz, 3H, $C\underline{H}_3$–CH_2–); 1.06–1.57 (m, 16H, –CH_2–); 1.68–1.97 (m, 6H, N–CH_2–$C\underline{H}_2$–, $SO_2(CH_2–C\underline{H}_2–)_2$); 2.17 (s, 3H, –$CH_3$); 2.95–3.02 (m, 4H, $SO_2(C\underline{H}_2–)_2$); 3.90 (t, $^3J=7.4$ Hz, 2H, –N–CH_2–), 5.01 (s, br, 1H, indole-OH); 6.13 (s, br, 1H, phenyl-OH); 6.69 (dd, $^3J=8.5$ Hz, $^4J=2.2$ Hz, 1H, indole-H^5); 6.78 (dd, $^4J=2.2$ Hz, $^5J=0.5$ Hz, 1H, indole-H^7); 6.95, 7.21 (AA′BB′, $^3J=8.6$ Hz, 4H, phenyl-H); 7.39 (dd, $^3J=8.5$ Hz, $^5J=0.5$ Hz, 1H, indole-H^4).

2-(4-Hydroxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]indole (7c). Prepared from **4e** by the method described for **5c**. Colorless solid (80% yield), mp 68–69 °C (CH_2Cl_2/*n*-hexane 1:4). ^{1}H NMR ($CDCl_3$) δ 0.93 (t, $^3J=7.0$ Hz, 3H, $C\underline{H}_3$–CH_2–); 1.07–1.48 (m, 16H, –CH_2–); 1.56–1.91 (m, 6H, N–CH_2–$C\underline{H}_2$–, $SO_2(CH_2–C\underline{H}_2–)_2$); 2.22 (s, 3H, –$CH_3$); 2.94–3.02 (m, 4H, $SO_2(C\underline{H}_2–)_2$); 4.01 (t, $^3J=7.4$ Hz, 2H, N–CH_2–); 6.12 (s, 1H, –OH); 6.96, 7.24 (AA′BB′, $^3J=8.6$ Hz, 4H, phenyl-H); 7.09–7.25 (m, 2H, indole-H), 7.31–7.34 (m, 1H, indole-H); 7.56–7.59 (m, 1H, indole-H). Anal. ($C_{30}H_{43}NO_3S$): C, 72.38; H, 8.72; N, 2.81. Found: C, 71.98; H, 8.89; N, 2.82.

5-Carbamoyl-2-(4-hydroxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]indole (7f). Prepared from **4f** by the method described for **5c**. Light yellow resin (86% yield). ^{1}H NMR (DMSO-d_6) δ 0.87 (t, $^3J=7.0$ Hz, 3H, $C\underline{H}_3$–CH_2–); 1.05–1.44 (m, 18H, –CH_2–); 1.54–1.72 (m, 4H, $SO_2(CH_2–C\underline{H}_2–)_2$); 2.17 (s, 3H, –$CH_3$); 3.00–3.07 (m, 4H, $SO_2(C\underline{H}_2–)_2$); 4.05 (t, $^3J=7.2$ Hz, 2H, N–CH_2–); 6.92, 7.23 (AA′BB′, $^3J=8.5$ Hz, 4H, phenyl-H); 7.10 (s, br, 1H, –CO–NH–); 7.45 (dd, $^3J=8.6$ Hz, $^5J=0.5$ Hz, 1H, indole-H^7); 7.72 (dd, $^3J=8.6$ Hz, $^4J=1.4$ Hz, 1H, indole-H^6); 7.87 (s, br, 1H, –CO–NH–); 8.14 (dd, $^4J=1.4$ Hz, $^5J=0.5$ Hz, 1H, indole-H^4); 9.72 (s, br, 1H, –OH).

2-(4-Hydroxyphenyl)-5-methoxycarbonyl-3-methyl-1-[10-(pentylsulfonyl)decyl]indole (7g). Due to the sensitivity of the ester function the cleavage reaction had to be modified: To a solution of **4g** (285 mg, 0.5 mmol) in dry CH_2Cl_2 (10 mL) $AlCl_3$ (200 mg, 1.5 mmol) was added with stirring. After the addition stirring was continued for 5 min followed by the addition of ethanethiol (155 mg, 2.5 mmol). After stirring for 3 h at rt under N_2, the mixture was pored into ice-water. The conditions for working-up and purification were similar to those described for **5c**. Slightly red resin (46% yield). ^{1}H NMR (DMSO-d_6) δ 0.87 (t, $^3J=7.0$ Hz, 3H, $C\underline{H}_3$–CH_2–); 1.05–1.45 (m, 16H, –CH_2–); 1.57–1.72 (m, 6H, N–CH_2–$C\underline{H}_2$–, $SO_2(CH_2–C\underline{H}_2–)_2$); 2.17 (s, 3H, –$CH_3$); 2.99–3.07 (m, 4H, $SO_2(CH_2–)_2$); 3.86 (s, 3H, –$COOCH_3$); 3.99–4.09 (m, 2H, N–CH_2–): 6.92, 7.23 (AA′BB′, $^3J=8.5$ Hz, 4H, phenyl-H); 7.53 (dd, $^3J=8.7$ Hz, $^5J=0.5$ Hz, 1H, indole-H^7); 7.77 (dd, $^3J=8.7$ Hz, $^4J=1.6$ Hz, 1H, indole-H^6); 8.18 (dd, $^4J=1.6$ Hz, $^5J=0.5$ Hz, 1H, indole-H^4); 9.70 (s, br, 1H, –OH); MS (EI, 70 eV) *m*/*z* 556.3 (MH^+); HRMS calcd for $C_{32}H_{45}NO_5S$ (M^+) 555.30181, found 555.30179.

6-Hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-(6-pyrrolidinohexyl)indole (11b). A solution of **6b** (700 mg, 1.72 mmol) in dry THF (60 mL) was added slowly to a refluxing suspension of $LiAlH_4$ (176 mg, 4.64 mmol) in dry THF (50 mL). Heating was continued for 1 h followed by stirring overnight at rt. For hydrolysis water (75 mL) and satd $NaHCO_3$ solution (25 mL) were added. The mixture was extracted three times with EtOAc. The combined organic layers were washed with saline and water, and dried ($MgSO_4$). After evaporation of the solvents the crude product was purified by chromatography (SiO_2) with EtOAc/MeOH (1:10) as eluent. Brownish resin (81% yield). ^{1}H NMR (DMSO-d_6) δ 1.06–1.08 (m, 4H, –CH_2–$(C\underline{H}_2–)_2$–CH_2–); 1.21–1.32 (m, 2H, N–CH_2–$C\underline{H}_2$–); 1.42–1.47 (m, 2H, indole-N–CH_2–$C\underline{H}_2$–); 1.60–1.68 (m, 4H, –$N(CH_2–C\underline{H}_2–)_2$); 2.07 (s, 3H, –$CH_3$); 2.22 (t, $^3J=7.2$ Hz, 2H, –N–CH_2–); 2.29–2.34 (m, 4H, –$N(CH_2–)_2$); 3.86 (t, $^3J=7.2$ Hz, 2H, indole-N–CH_2–); 6.56 (dd, $^3J=8.4$ Hz, $^4J=1.9$ Hz, 1H, indole-H^5); 6.70 (dd, $^4J=1.9$ Hz, $^5J=0.5$ Hz, 1H, indole-H^7); 6.86, 7.13 (AA′BB′, $^3J=8.5$ Hz, 4H, phenyl-H); 7.24 (dd, $^3J=8.4$ Hz, $^5J=0.5$ Hz, 1H, indole-H^4); 8.50–10.50 (s, br, 2H;–OH). Anal. ($C_{25}H_{32}N_2O_2$): C, 76.48; H, 8.22; N, 7.13. Found: C, 74.34; H, 8.32; N, 6.73.

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 choride, [26] 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 the dihydroxy derivatives it was sometimes possible to isolate one of the monosulfamates from the first fraction as a by-product.

1-Ethyl-2-(4-hydroxyphenyl)-3-methyl-5-sulfamoyloxyindole (8a). Beige resin (13% yield). ^{1}H NMR (DMSO-d_6) δ 1.07 (t, $^3J=7.1$ Hz, 3H, $C\underline{H}_3$–CH_2–); 2.12 (s, 3H, –CH_3); 4.04 (q, $^3J=7.1$ Hz, 2H, $C\underline{H}_3$–CH_2–); 7.06 (dd, $^3J=8.8$ Hz, $^4J=2.3$ Hz, 1H, indole-H^6); 6.92, 7.24 (AA′BB′, $^3J=8.5$ Hz, 4H, phenyl-H); 7.40 (dd, $^4J=2.3$ Hz, $^5J=0.5$ Hz, 1H, indole-H^4); 7.48 (dd, $^3J=8.8$ Hz, $^5J=0.5$ Hz, 1H, indole-H^7); 7.78 (s, br, 2H, –SO_2NH_2); 9.74 (s, br, 1H, phenyl-OH). MS (EI, 70 eV) *m*/*z* 347.2 (MH^+); HRMS calcd for $C_{17}H_{19}N_2O_4S$ (MH^+) 347.10652, found 347.10650.

1-Ethyl-3-methyl-5-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)indole (9a). Grey solid (21% yield), mp 193–195 °C (dec.). ^{1}H NMR (DMSO-d_6) δ 1.10 (t, ^3J = 6.8 Hz, 3H, C$\underline{H}_3$–CH$_2$–); 2.16 (s, 3H, –CH$_3$); 4.10 (q, ^3J = 6.8 Hz, 2H, CH$_3$–C$\underline{H}_2$–); 7.11 (dd, ^3J = 8.9 Hz, ^4J = 2.3 Hz, 1H, indole-H^6); 7.45 (dd, ^4J = 2.3 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 7.46, 7.53 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 7.55 (dd, ^3J = 8.9 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.79 (s, br, 2H, indole-OSO$_2$NH$_2$); 8.14 (s, br, 2H, phenyl-OSO$_2$NH$_2$). Anal. (C$_{17}$H$_{19}$N$_3$O$_6$S$_2$): C, 47.98; H, 4.51; N, 9.88. Found: C, 48.09; H, 4.83; N, 9.58.

1-Ethyl-2-(4-hydroxyphenyl)-3-methyl-6-sulfamoyloxyindole (8b). Brown resin (15% yield). ^{1}H NMR (DMSO-d_6) δ 1.09 (t, ^3J = 7.1 Hz, 3H, C$\underline{H}_3$–CH$_2$–); 2.13 (s, 3H, –CH$_3$); 4.02 (q, ^3J = 7.1 Hz, 2H, CH$_3$–C$\underline{H}_2$–); 6.98 (dd, ^3J = 8.6 Hz, ^4J = 2.0 Hz, 1H, indole-H^5); 6.92, 7.22 (AA′BB′, ^3J = 8.5 Hz, 4H, phenyl-H); 7.36 (dd, ^4J = 2.0 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.52 (dd, ^3J = 8.6 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 7.81 (s, 2H, –SO$_2$NH$_2$); 9.73 (s, 1H, phenyl-OH).

1-Ethyl-3-methyl-6-sulfamoyloxy-2(4-sulfamoyloxyphenyl) indole (9b). Colorless solid (37% yield), mp 186–187 °C (dec.; MeOH). ^{1}H NMR (DMSO-d_6) δ 1.12 (t, ^3J = 7.0 Hz, 3H, C$\underline{H}_3$–CH$_2$–); 2.18 (s, 3H, –CH$_3$); 4.05 (q, ^3J = 7.0 Hz, 2H, CH$_3$–C$\underline{H}_2$–); 7.02 (dd, ^3J = 8.5 Hz, ^4J = 1.7 Hz, 1H, indole-H^5); 7.40 (dd, ^4J = 1.7 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.45, 7.53 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 7.58 (dd, ^3J = 8.5 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 7.87 (s, br, 2H, indole-OSO$_2$NH$_2$); 8.10 (s, br, 2H, phenyl-OSO$_2$NH$_2$). Anal. (C$_{17}$H$_{19}$N$_3$O$_6$S$_2$): C, 47.98; H, 4.51; N, 9.88. Found: C, 48.39; H, 4.70; N, 9.75.

1-Ethyl-3-methyl-2-(4-sulfamoyloxyphenyl)indole (8c). Colorless crystals (58% yield), mp 173–175 °C (MeOH). ^{1}H NMR (DMSO-d_6) δ 1.10 (t, ^3J = 7.1 Hz, 3H, C$\underline{H}_3$–CH$_2$–); 2.18 (s, 3H, –CH$_3$); 4.09 (q, ^3J = 7.1 Hz, 2H, CH$_3$–C$\underline{H}_2$–); 7.05–7.22 (m, 2H, indole-H); 7.42–7.56 (m, 2H, indole-H); 7.44, 7.54 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 8.13 (s, 2H, –SO$_2$NH$_2$). Anal. (C$_{17}$H$_{18}$N$_2$O$_3$S): C, 61.79; H, 5.50; N, 8.48. Found: C, 61.65; H, 5.67; N, 8.28.

1-Ethyl-3-methyl-2-phenyl-6-sulfamoyloxyindole (8d). Colorless crystals (47% yield), mp 163–165 °C (dec.; MeOH). ^{1}H NMR (DMSO-d_6) δ 1.09 (t, ^3J = 7.1 Hz, 3H, C$\underline{H}_3$–CH$_2$–); 2.16 (s, 3H, –CH$_3$); 4.05 (q, ^3J = 7.1 Hz, 2H, CH$_3$–C$\underline{H}_2$–); 7.02 (dd, ^3J = 8.5 Hz, ^4J = 2.0 Hz, 1H, indole-H^5); 7.41 (dd, ^4J = 2.0 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.57 (dd, ^3J = 8.5 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 7.38–7.60 (m, 5H, phenyl-H); 7.84 (s, 1H, –SO$_2$NH$_2$). Anal. (C$_{17}$H$_{18}$N$_2$O$_3$S): C, 61.79; H, 5.50; N, 8.48. Found: C, 62.33; H, 5.67; N, 8.78.

1-Ethyl-3-methyl-2-phenyl-5-sulfamoyloxyindole (8e). Colorless resin (81% yield). ^{1}H NMR (DMSO-d_6) δ 1.07 (t, ^3J = 7.1 Hz, 3H, C$\underline{H}_3$–CH$_2$–); 2.15 (s, 3H, –CH$_3$); 4.08 (q, ^3J = 7.1 Hz, 2H, CH$_3$—C$\underline{H}_2$–); 7.10 (dd, ^3J = 8.8 Hz, ^4J = 2.3 Hz, 1H, indole-H^6); 7.44 (dd, ^4J = 2.3 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 7.54 (dd, ^3J = 8.8 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.39–7.60 (m, 5H, phenyl-H); 7.78 (s, 2H, –SO$_2$NH$_2$).

3-Methyl-1-(5-pyrrolidinocarbonylpentyl)-5-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)indole (10a). Colorless resin (66% yield). ^{1}H NMR (DMSO-d_6) δ 1.01–1.10 (m, 2H, N–(CH$_2$)$_2$–C$\underline{H}_2$–); 1.24–1.36 (m, 2H, –CO–CH$_2$–C$\underline{H}_2$–); 1.41–1.50 (m, 2H, indole-N–CH$_2$–C$\underline{H}_2$–); 1.70–1.88 (m, 4H, –N(CH$_2$–C$\underline{H}_2$–)$_2$); 2.04 (t, ^3J = 7.3 Hz, 2H, –CO–CH$_2$–); 2.16 (s, 3H, –CH$_3$); 3.19–3.31 (m, 4H, –N–(CH$_2$–)$_2$); 4.08 (t, ^3J = 7.0 Hz, 2H, indole–N–CH$_2$–); 7.10 (dd, ^3J = 8.8 Hz, ^4J = 2.2 Hz, 1H, indole-H^6); 7.44 (dd, ^4J = 2.4 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 7.44, 7.53 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 7.54 (dd, ^3J = 8.8 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.80 (s, 2H, indole-OSO$_2$NH$_2$); 8.14 (s, 2H, phenyl-OSO$_2$NH$_2$); MS (EI, 70 eV) *m*/*z* 565.2 (MH$^+$); HRMS calcd for C$_{25}$H$_{33}$N$_4$O$_7$S$_2$ (MH$^+$) 565.17901, found 565.18031.

3-Methyl-1-(5-pyrrolidinocarbonylpentyl)-6-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)indole (10b). Colorless resin (74% yield). ^{1}H NMR (DMSO-d_6) δ 1.03–1.08 (m, 2H, –N–(CH$_2$)$_2$–C$\underline{H}_2$–); 1.24–1.37 (m, 2H, –CO–CH$_2$–C$\underline{H}_2$–); 1.44–1.52 (m, 2H, indole-N–CH$_2$–C$\underline{H}_2$–); 1.70–1.88 (m, 4H, –N(CH$_2$–C$\underline{H}_2$–)$_2$); 2.05 (t, ^3J = 7.3 Hz, 2H, –CO–CH$_2$–); 2.18 (s, 3H, –CH$_3$); 3.19–3.31 (m, 4H, –N–(CH$_2$–)$_2$); 4.04 (t, ^3J = 7.1 Hz, 2H, indole-N–CH$_2$–); 7.02 (dd, ^3J = 8.6 Hz, ^4J = 2.0 Hz, 1H, indole-H^5); 7.40 (dd, ^4J = 2.0 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.44, 7.52 (AA′BB′, ^3J = 8.7 Hz, 4H, phenyl-H); 7.57 (dd, ^3J = 8.6 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); 7.84 (s, 2H, indole–OSO$_2$NH$_2$); 8.12 (s, 2H, phenyl-OSO$_2$NH$_2$).

2-(4-Hydroxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]-5-sulfamoyloxyindole (13a). Colorless resin (27% yield). ^{1}H NMR (CDCl$_3$) δ 0.93 (t, ^3J = 7.0 Hz, 3H, C$\underline{H}_3$–CH$_2$–); 1.05–1.48 (m, 16H, –CH$_2$–); 1.50–1.93 (m, 6H, N–CH$_2$–C$\underline{H}_2$–, SO$_2$(CH$_2$–C$\underline{H}_2$–)$_2$); 2.18 (s, 3H, –CH$_3$); 2.95–3.03 (m, 4H, SO$_2$(CH$_2$–)$_2$): 4.00 (t, ^3J = 7.3 Hz, 2H, N–CH$_2$–); 4.97 (s, br, 2H, –SO$_2$NH$_2$); 6.26 (s, 1H, phenyl-OH); 6.94, 7.19 (AA′BB′, ^3J = 8.6 Hz, 4H, phenyl-H); 7.17 (dd, ^3J = 8.8 Hz, ^4J = 2.4 Hz, 1H, indole-H^6); 7.30 (dd, ^3J = 8.8 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.51 (dd, ^4J = 2.4 Hz, ^5J = 0.5 Hz, 1H, indole-H^4).

3-Methyl-1-[10-(pentylsulfonyl)decyl]-5-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)indole (14a). Colorless resin (71% yield). ^{1}H NMR (CDCl$_3$) δ (t, ^3J = 7.0 Hz, 3H, C$\underline{H}_3$–CH$_2$–); 1.04–1.47 (m, 16H, –CH$_2$–); 1.49–1.90 (m, 6H, N–CH$_2$–C$\underline{H}_2$–, SO$_2$(CH$_2$–C$\underline{H}_2$–)$_2$); 2.19 (s, 3H, –CH$_3$); 2.96–3.03 (m, 4H, SO$_2$(C$\underline{H}_2$–)$_2$); 3.98 (t, ^3J = 7.4 Hz, 2H, N–CH$_2$–); 5.08 (s, br, 2H, indole-OSO$_2$NH$_2$); 5.51 (s, 2H, phenyl-OSO$_2$NH$_2$); 7.20 (dd, ^3J = 8.8 Hz, ^4J = 2.3 Hz, 1H, indole-H^6); 7.31 (dd, ^3J = 8.8 Hz, ^5J = 0.5 Hz, 1H, indole-H^7); 7.36, 7.44 (AA′BB′, ^3J = 8.8 Hz, 4H, phenyl-H); 7.52 (dd, ^4J = 2.3 Hz, ^5J = 0.5 Hz, 1H, indole-H^4); MS (EI, 70 eV) *m*/*z* 672.3 (MH$^+$; 100%).

2-(4-Hydroxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]-6-sulfamoyloxyindole (13b). Colorless resin (18% yield). ^{1}H NMR (CDCl$_3$) δ 0.93 (t, ^3J = 7.0 Hz, 3H, C$\underline{H}_3$–CH$_2$–); 1.05–1.51 (m, 16H, –CH$_2$–); 1.54–1.92 (m, 6H, N–CH$_2$–CH$_2$–, SO$_2$(CH$_2$–CH$_2$–)$_2$); 2.19 (s, 3H, –CH$_3$); 2.96–3.03 (m, 4H, SO$_2$(CH$_2$–)$_2$); 3.97 (t, ^3J = 7.4 Hz, 2H, N–CH$_2$–); 5.03 (s, 2H, –SO$_2$NH$_2$); 7.08 (dd,

$^3J = 8.5$ Hz, $^4J = 2.1$ Hz, 1H, indole-H^5), 6.94, 7.18 (AA′BB′, $^3J = 8.6$ Hz, 4H, phenyl-H); 7.32 (dd, $^4J = 2.1$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^7); 7.54 (dd, $^3J = 8.6$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^4); MS (EI, 70 eV) m/z 593.3 (MH^+); HRMS calcd for $C_{30}H_{45}N_2O_6S_2$ (MH^+) 593.27186, found 593.27105.

3-Methyl-1-[10-(pentylsulfonyl)decyl]-6-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)indole (14b). Colorless resin (62% yield). ^{1}H NMR ($CDCl_3$) δ 0.91 (t, $^3J = 7.0$ Hz, 3H, C$\underline{H}_3$–CH_2–); 1.03–1.88 (m, 22H, –CH_2–); 2.18 (s, 3H, –$\overline{C}H_3$); 2.95–3.01 (m, 4H, $SO_2(CH_2–)_2$); 3.94 (t, $^3J = 7.3$ Hz, 2H, N–CH_2–); 5.28 (s, 2H, indole-OSO_2NH_2); 5.59 (s, 2H, phenyl-OSO_2NH_2); 7.09 (dd, $^3J = 8.6$ Hz, $^4J = 2.0$ Hz, 1H, indole-H^5); 7.32 (dd, $^4J = 2.0$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^7); 7.34, 7.42 (AA′BB′, $^3J = 8.8$ Hz, 4H, phenyl-H); 7.53 (dd, $^3J = 8.6$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^4).

3-Methyl-1-[10-(pentylsulfonyl)decyl]-2-(4-sulfamoyloxyphenyl)indole (13c). Yellow resin (77% yield). ^{1}H NMR ($CDCl_3$) δ 0.93 (t, $^3J = 7.0$ Hz, 3H, C$\underline{H}_3$–CH_2–); 1.06–1.47 (m, 16H, –CH_2–); 1.54–1.91 (m, 6H, N–CH_2–C$\underline{H}_2$–, $SO_2(CH_2–C\underline{H}_2–)_2$); 2.24 (s, 3H, –$CH_3$); 2.96–3.02 (m, 4H, $SO_2(C\overline{H}_2–)_2$), 4.01 (t, $^3J = 7.4$ Hz, 2H, N–CH_2–); 5.48 (s, 2H, –SO_2NH_2); 7.11–7.61 (m, 8H, indole-H, phenyl-H).

5-Cyano-3-methyl-1-[10-(pentylsulfonyl)decyl]-2-(4-sulfamoyloxyphenyl)indole (13f). Yellow resin (67% yield). ^{1}H NMR (DMSO-d_6) δ 0.87 (t, $^3J = 7.0$ Hz, 3H, C$\underline{H}_3$–CH_2–); 1.04–1.44 (m, 18H, –CH_2–); 1.54–1.72 (m, 4H, $SO_2(CH_2–C\underline{H}_2–)_2$); 2.20 (s, 3H, –$CH_3$); 2.99–3.06 (m, 4H, $SO_2(C\overline{H}_2–)_2$); 4.13 (t, $^3J = 7.0$ Hz, 2H, N–CH_2–); 7.46, 7.56 (AA′BB′, $^3J = 8.7$ Hz, 4H, phenyl-H); 7.53 (dd, $^3J = 8.6$ Hz, $^4J = 1.5$ Hz, 1H, indole-H^6); 7.71 (dd, $^3J = 8.6$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^7); 8.12 (dd, $^4J = 1.5$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^4); 8.16 (s, br, 2H, –SO_2NH_2).

5-Methoxycarbonyl-3-methyl-1-[10-(pentylsulfonyl)decyl]-2-(4-sulfamoyloxyphenyl)indole (13g). Colorless resin (64% yield). ^{1}H NMR (DMSO-d_6) δ 0.87 (t, $^3J = 7.0$ Hz, 3H, C$\underline{H}_3$–CH_2–), 1.05–1.46 (m, 16H, –CH_2–); 1.56–1.72 (m, 6H, N–CH_2–C$\underline{H}_2$–, $SO_2(CH_2–C\underline{H}_2–)_2$); 2.22 (s, 3H, –$CH_3$), 2.99–3.06 (m, 4H, $SO_2(C\overline{H}_2–)_2$); 3.87 (s, 3H, –OCH_3); 4.11 (t, $^3J = 7.0$ Hz, 2H, N–CH_2–); 7.45, 7.55 (AA′BB′, $^3J = 8.7$ Hz, 4H, phenyl-H); 7.59 (dd, $^3J = 8.7$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^7); 7.81 (dd, $^3J = 8.7$ Hz, $^4J = 1.7$ Hz, 1H, indole-H^6); 8.15 (s, 2H, –SO_2NH_2); 8.23 (dd, $^4J = 1.7$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^4).

3-Methyl-1-(6-pyrrolidinohexyl)-5-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)indole (12a). Under N_2 a solution of **11a** (196 mg, 0.5 mmol) in dry DMF (5 mL) was added slowly at 0 °C to a stirred suspension of NaH (50 mg, 2 mmol) in dry DMF (5 mL). After stirring for 1.5 h, sulfamoyl chloride (575 mg, 5 mmol) was added in portions at 0 °C. After removal of the cooling bath, the mixture was stirred overnight and then poured into a sat. $NaHCO_3$ solution (40 mL). The aqueous mixture was extracted twice with EtOAc. The combined organic layers were washed with water and dried ($MgSO_4$). After evaporation of the solvent, the crude product was purified by chromatography over SiO_2 with EtOAc/MeOH (1:10) as eluent to afford a colorless resin (64% yield). ^{1}H NMR (DMSO-d_6) δ 1.04–1.06 (m, 4H, –CH_2–); 1.22–1.24 (m, 2H, –N–CH_2–C$\underline{H}_2$–); 1.44–1.46 (m, 2H, indole-N–CH_2–C$\underline{H}_2$–); 1.57–1.69 (m, 4H, –N(CH_2–C$\underline{H}_2$–)$_2$); 2.16 (s, 3H, –CH_3); 2.21–2.24 (m, 2H, –N–C$\underline{H}_2$–); 2.31–2.33 (m, 4H, –N(CH_2–)$_2$); 4.07 (t, $^3J = 7.0$ Hz, 2H, indole-N–CH_2–); 7.09 (dd, $^3J = 8.8$ Hz, $^4J = 2.3$ Hz, 1H, indole-H^6); 7.49 (dd, $^4J = 2.3$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^4); 7.41, 7.45 (AA′BB′, $^3J = 8.8$ Hz, 4H, phenyl-H); 7.52 (dd, $^3J = 8.8$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^7); MS (FAB) m/z 551.3 (MH^+); HRMS calcd for $C_{25}H_{35}N_4O_6S_2$ (MH^+) 551.19975, found 551.20073.

Methyl-1-(6-pyrrolidinohexyl)-6-sulfamoyloxy-2-(4-sulfamoyloxyphenyl)indole (12b). Prepared from **11b** by the method described for **12a**. Colorless resin (56% yield). ^{1}H NMR (DMSO-d_6) δ 1.05–1.06 (m, 4H, –CH_2–); 1.23–1.49 (m, 2H, –N–CH_2–C$\underline{H}_2$–); 1.50–1.63 (m, 2H, indole-N–CH_2–C$\underline{H}_2$–); 1.76–1.82 (m, 4H, –N(CH_2–C$\underline{H}_2$–)$_2$); 2.18 (s, 3H, –CH_3); 2.13–2.22 (m, 2H, –N–C$\underline{H}_2$–); 2.32–2.41 (m, 4H, –N(CH_2–)$_2$); 4.04 (t, $^3J = 6.9$ Hz, 2H, indole-N–CH_2–); 7.01 (dd, $^3J = 8.5$ Hz, $^4J = 1.9$ Hz, 1H, indole-H^5); 7.38 (dd, $^4J = 1.9$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^7); 7.42, 7.48 (AA′BB′, $^3J = 8.9$ Hz, 4H, phenyl-H); 7.56 (dd, $^3J = 8.5$ Hz, $^5J = 0.5$ Hz, 1H, indole-H^4). Anal. ($C_{25}H_{34}N_4O_6S_2$): C, 51.17; H, 6.52; N, 9.54. Found: C, 51.28; H, 6.02; N, 9.00.

Materials and reagents for bioassays

[^{3}H]17β-Estradiol, [^{3}H]estrone sulfate (ammonium salt), and [^{14}C]estrone were purchased from New England Nuclear (Dreieich, Germany); all other biochemicals were obtained from Sigma (Munich, Germany). ICI 182, 780 was generously provided by Dr. M.R. Schneider, Berlin (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.[27]

Estrogen receptor binding assay

For the determination of relative binding affinities (RBA), the previously described procedure was applied with modifications.[21] 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 700g for 10 min to pellet the charcoal. An aliquot (100 μL) of the supernatant was removed and radioactivity was determined by liquid scintillation spectrometry after addition of 3 mL of Quickszint 212 (Zinsser). Nonspecific binding was calculated using 5

μM 17β-estradiol as competing ligand. Radioactivity was plotted as a function of the log concentration of competing ligand in the assay. 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.

Steroid sulfatase assay[28]

MCF-7 human breast cancer cells were grown in 75 mL-flasks in MEM Eagles's medium supplemented with $NaHCO_3$ (2.2 g/L), sodium pyruvate (110 mg/L), gentamycin (50 mg/L), and 10% 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 transfered 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 supernatant 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.[29]

Estimation of estrogenic and antiestrogenic activity in stably transfected MCF-7/2a cells

Approximately 1 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 estradiol (10 nM) or 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 with a rubber policeman, 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. With samples from *Photinus pyralis* (Boehringer, Mannheim, Germany) a linear correlation between luminescence (RLU/10s) and the amount of luciferase was established in the range from 0.0001 pg to 30 pg enzyme with 3000 RLU referring to 10 fg luciferase. Measurements were corrected for the protein content of the samples quantified according to Bradford[29] 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. Estrone (E1; 10 nM) and 17β-estradiol (E2; 10 nM) were used as reference estrogens, estrone sulfate (100 nM) for estimation of combined sulfatase inhibiting and antiestrogenic effects.

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