Antiestrogenic Activities of 3,8-Dihydroxy-6,11-dihydrobenzo[*a*]carbazoles with Sulfur-Containing Side Chains

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***Key Words**: Benzocarbazole; phenylindole; antiestrogen; antitumor activity; transfection assay*

Summary

The objective of this study was to explore whether the conversion of the 2-phenylindole system into the tetracyclic benzo[*a*]carbazole changes the endocrine profile when the side chain structure was kept constant. Five different sulfur-containing side chains were linked to the nitrogen of the tetracycle. The biological evaluation revealed that the character of the indole derivatives remained unchanged after the conversion to the respective benzocarbzoles but the potency decreased by one order of magnitude. *In vitro*, all derivatives acted as pure antiestrogens without any agonist activity. They strongly inhibited the growth of estrogen-sensitive MCF-7 breast cancer cells with IC_{50}-values in the nanomolar range. In the mouse uterine weight test, the derivatives with an aliphatic side chain were devoid of estrogenic activity and antagonized the effect of estradiol. The presence of an aromatic ring in the side chain gave rise to significant agonist activity *in* vivo independently of the carrier structure. All data revealed the equivalence of both carrier structures in respect to the endocrine profile but showed a decrease in potency upon the conversion of the 2-phenylindole system into the benzocarbazole structure.

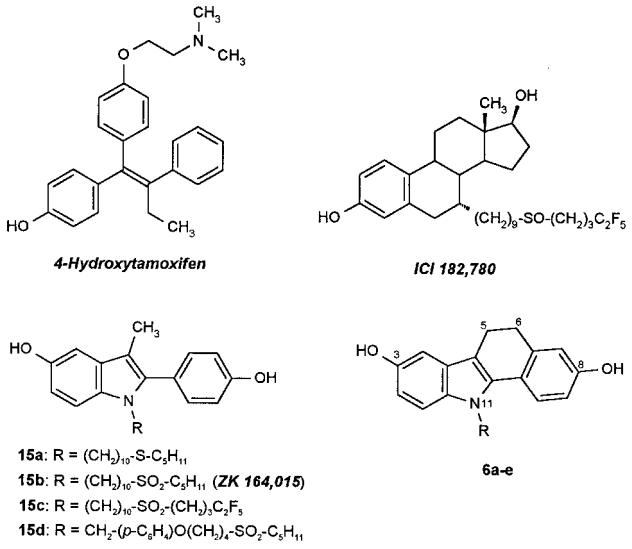

Figure 1. Structures of 3,8-dihydroxy-6,11-dihydrobenzo[*a*]carbazoles (**6**), 5-hydroxy-2-(4-hydroxyphenyl)-3-methylindoles (**15**), and reference compounds.

Introduction

For a long time the treatment of hormone-dependent breast cancer has been the dominant therapeutic application of antiestrogens, mainly tamoxifen. However, the partial agonist activity of tamoxifen has been regarded as a major disadvantage and led to the development of so-called pure antiestrogens that are devoid of any residual estrogenic activity [1–4]. This type of drugs might be a valuable alternative for patients whose tumors had become resistant to tamoxifen but have retained their dependency on estrogens. A number of compounds both steroidal and non-steroidal in structure have been discovered and one particular estradiol derivative, ICI 182,780 (Faslodex) is currently undergoing clinical evaluation [5]. Besides the search for pure antagonists there is a growing interest in antiestrogens with agonist activity in certain tissues such as the bone. These investigations have been stimulated by the discovery of raloxifene which displays a certain degree of tissue specificity and is now in clinical use for the treatment of osteoporosis [6,7].

The development of new estrogen antagonists now follows two avenues: the enhancement of the *in vivo* potency of pure antiestrogens since most of the presently known agents suffer from poor bioavalibility following oral administration and the search for mixed agonists/antagonists with a better tissue selectivity than raloxifene. Several groups including our own have shown that the structure of the side chain in steroidal and non-steroidal antiestrogens is crucial for the degree of antagonism that can be reached [4,8,9]. The main role of the carrier molecule is to guarantee both receptor binding and the correct orientation of the side chain inside the binding pocket. However, it is still unclear how strongly the basic structure influences the endocrine profile if the side chain structure is kept constant. A suitable structure for these investigations is the 2-phenylindole system. In a previous study we have shown that the indole nitrogen can be replaced by sulfur provided the side chain is transferred to carbon 3 and the hydroxy group is shifted from position 5 to 6 without major influence on the endocrine profile [10].

In this study we used the tetracyclic 5,6-dihydrobenzo[*a*]-carbazole system as the carrier molecule because this modification allowed us to keep the positions of the side chain and the phenolic hydroxy groups constant. Four different sulfur-containing side chains were used for the comparison between the 2-phenylindole and the benzo[*a*]carbazole system.

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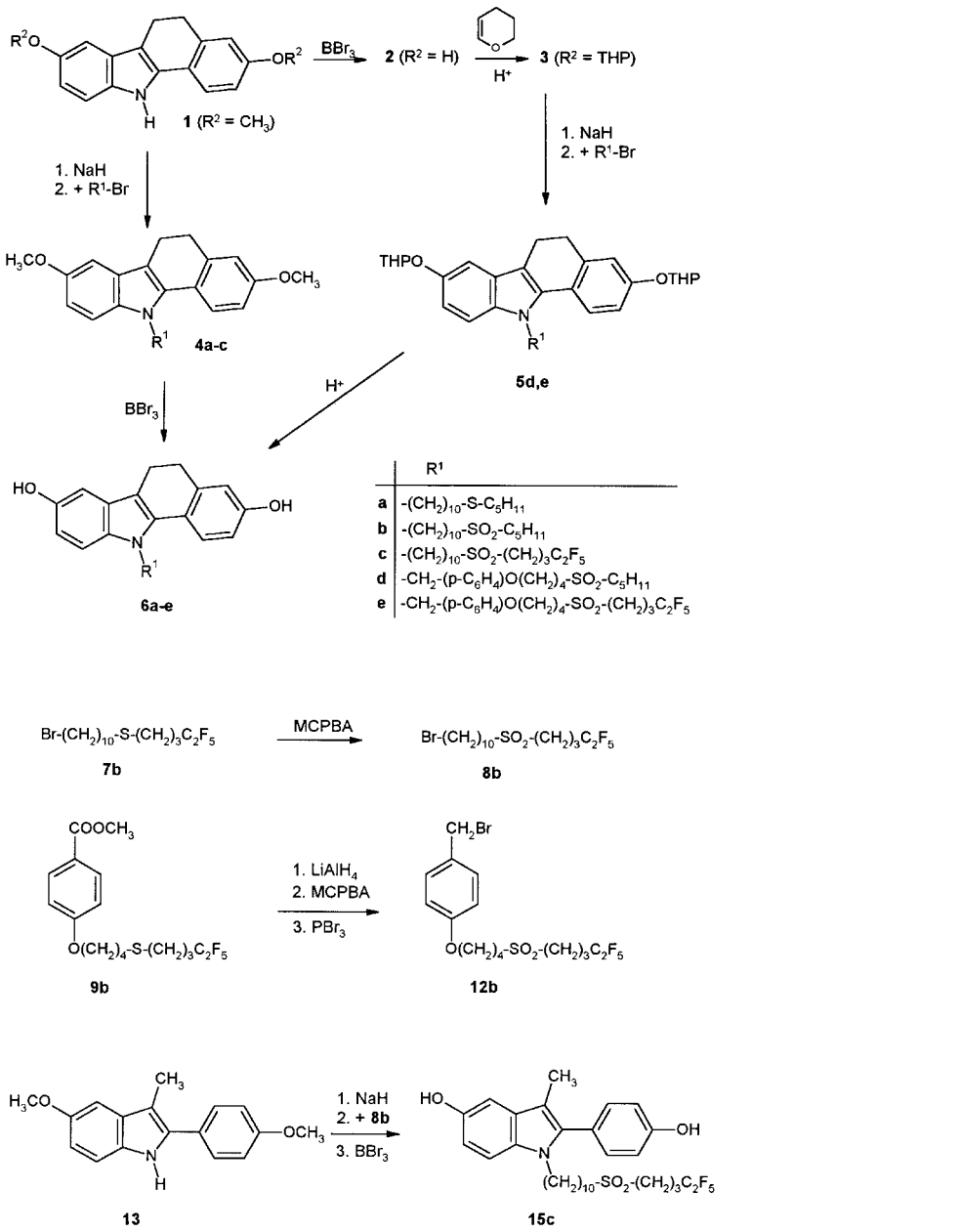


Scheme 1

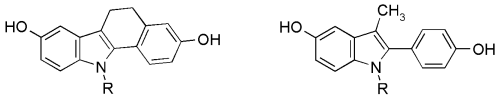
Chemistry

The route to the various benzo[*a*]carbazoles is outlined in Scheme 1. The tetracycle was prepared by the Fischer method as described previously [11]. The side chains were synthesized separately and carried a terminal bromo substituent to allow the nucleophilic substitution by the benzocarbazole anion generated with sodium hydride. The oxygen functions in the heterocycle were protected as methoxy groups (**1**) or as tetrahydropyranyl ethers (**3**) if a labile ether linkage was present in the side chain. In the last step of synthesis the protecting groups were removed to yield the free phenols. Under the reaction conditions applied no oxidation of the dihydro products to the fully aromatic tetracycle was observed. Three of the four 2-phenylindole derivatives that were used for comparison have been synthesized previously. The fourth example (**15c**) was prepared by a method similar to that of the corresponding benzocarbazole.

Results and Discussion

All new derivatives were first tested for their ability to bind to the estrogen receptor. As in previous studies [12], we used the calf uterine cytosol as a convenient source of estrogen receptors. The RBA values of the new compounds ranged from 0.1 to 4.2 (Table 1). The rather low values for the sulfides **6a** and **15a** can be rationalized by the low polarity of this functional group which is only a weak hydrogen bridge acceptor. No significant differences between the benzocarbazoles and the indole derivatives were noticed.

Table 1. Binding affinities for the estrogen receptor, antiestrogenic activities, and antiproliferative properties of 5,6-dihydrobenzo[*a*]carbazoles **6** and corresponding 2-phenylindoles **15**.



6 **15**

Compd	R	RBA[a]	Antiestrogenic activity[b] IC_{50} (nM)	Cytostatic activity[c] IC_{50} (nM)
6a	$-(CH_2)_{10}-S-C_5H_{11}$	0.10	(3%)[d]	18
15a	$-(CH_2)_{10}-S-C_5H_{11}$	0.13	n.d.[e]	3.4
6b	$-(CH_2)_{10}-SO_2-C_5H_{11}$	2.3	860	18
15b	$-(CH_2)_{10}-SO_2-C_5H_{11}$	3.0	200	2.2
6c	$-(CH_2)_{10}-SO_2-(CH_2)_3C_2F_5$	1.5	710	6.7
15c	$-(CH_2)_{10}-SO_2-(CH_2)_3C_2F_5$	2.3	200	0.6
6d	$-CH_2-(p-C_6H_4)O(CH_2)_4-SO_2-C_5H_{11}$	4.2	550	44
15d	$-CH_2-(p-C_6H_4)O(CH_2)_4-SO_2-C_5H_{11}$	4.1	80	1.7
6e	$CH_2C_6H_4-O(CH_2)_4-SO_2-(CH_2)_3C_2F_5$	3.8	310	4.9
OH-Tam		6.8	60	83
ICI 182,780		6.2	40	0.22

[a] Relative binding affinities for the calf uterine estrogen receptor; value for 17*ß*-estradiol = 100. [b]Inhibition of luciferase activity in estrogen receptor-positive MCF-7/2a breast cancer cells stably transfected with the EREwtc luc reporter plasmid and stimulated by 17ß-estradiol (10^{-8} M). Mean of three independent experiments. [c]Inhibition of the growth of estrogen-sensitive human MCF-7 breast cancer cells. Mean of two independent experiments. [d]Inhibition at 1000 nM. [e]Not determined.

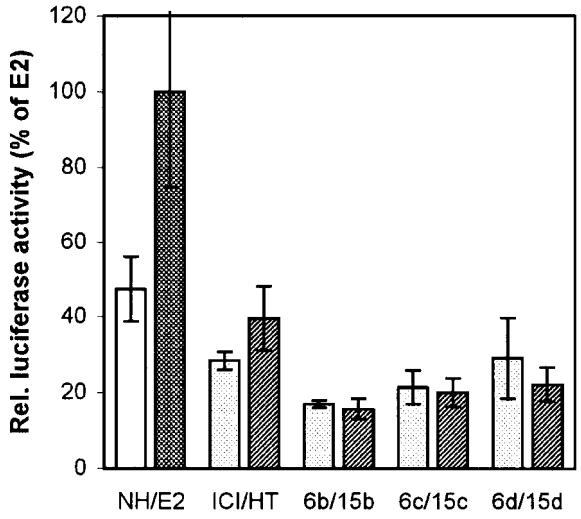


Figure 2. Luciferase expression in HeLa cells cotransfected with the reporter plasmid EREwtc luc and the estrogen receptor expression vector HEG0 and treated with 17*ß*-estradiol (E2, 10 nM), ICI 182,780 (ICI, 1 µM), 4-hydroxytamoxifen (HT, 1 µM), benzo[*a*]carbazoles **6b–d** (1 µM), and 2-phenylindole analogues **15b–d** (1 µM). Control wells (NH) contained only the vehicle (1% EtOH).Values are means of three independent experiments ± SD.

Since the carbazole derivatives of this study bind to the estrogen receptor endocrine activity could be expected. An *in vitro* model that had proved to be appropriate for the detection of agonist activity are estrogen receptor negative HeLa cells cotransfected with the luciferase reporter plasmid EREwtc luc and the expression vector HEG0 for the human wild-type estrogen receptor [4]. Figure 2 shows the comparison of the benzocarbazoles **6b–d** with the corresponding 2-phenylindoles **15b–d** and the values for the reference compounds ICI 182,780 and 4-hydroxytamoxifen which can be considered a partial antagonist. The main problem of this assay is the rather high luciferase activity found in the control cells despite the use of steroid-depleted medium. The reason is probably the presence of various peptides such as growth factors in the serum which are known to activate the estrogen receptor in the absence of the natural ligand [13–15]. This effect can partly be abolished by the addition of a potent antiestrogen such as ICI 182,780. Benzocarbazoles **6b–d** and the corresponding 2-phenylindoles (**15b–d**) were devoid of agonist activity at the standard concentration of 10 µM and lead to stronger reduction of basal luciferase activity than ICI 182,780 did.

For the estimation of antiestrogenic activity estrogen receptor-positive MCF-7/2a breast cancer cells stably transfected with the reporter construct were used [14]. Originally, this line was also used for the *in vitro* determination of estrogenic activity but it proved to be less sensitive for the detection of

Table 2. Estrogenic and antiestrogenic activity of 5,6-dihydrobenzo[*a*]carbazoles **6b–d** and 2-phenylindole **15d** in the mouse uterine weight test.

		Uterotrophic test:		Antiuterotrophic test:	
Compd	dose[a](mg/kg)	rel. uterus weight[b]	agonism (%)	rel. uterus weight[b,c]	antagonism (%)
control	–	20.9 ± 3.5	0		
E2	0.01	64.6 ± 7.3	100		
6b	2.5	20.3 ± 2.2	–1	64.2 ± 14.9	1
	12.5	20.7 ± 3.2	0	39.5 ± 10.9	57[d]
	62.5	14.5 ± 4.0	–15[e]	38.4 ± 10.4	60[d]
6c	2.5	27.3 ± 6.1	15[f]	40.8 ± 3.4	54[d]
	12.5	22.7 ± 5.2	4	43.4 ± 7.7	49[d]
	62.5	20.7 ± 2.2	0	35.9 ± 3.5	66[d]
control	–	19.5 ± 2.7	0		
E2	0.01	56.7 ± 6.2	100		
6d	0.5	26.8 ± 2.8	20[e]	56.6 ± 6.3	0
	2.5	23.8 ± 3.8	12[f]	51.8 ± 3.3	13
	12.5	23.9 ± 4.9	12[f]	52.9 ± 5.0	10
	62.5	27.5 ± 5.2	22[e]	52.3 ± 5.3	12
control	–	14.5 ± 2.1	0		
E2	0.01	47.8 ± 4.6	100		
15d	0.25	17.9 ± 2.5	10[f]	43.0 ± 3.1	14
	1.2	20.4 ± 1.7	18[e]	36.9 ± 6.2	33[d]
	6.0	20.0 ± 2.9	17[e]	28.5 ± 3.4	58[d]
	30	26.7 ± 5.6	37[e]	29.6 ± 5.8	55[d]

[a] Dose per kg body weight, administered at three consecutive days s.c. [b] Uterus dry weight (mg)/body weight (g) × 100, determined 24 h after the last injection; mean of 6 animals ± SD. [c] Simultaneous administration of 0.01 mg estradiol per kg body weight. [d] Significant difference vs estradiol-treated animals ($p < 0.01$). [e] Significant difference vs control animals ($p < 0.01$). [f] Significant difference vs control animals ($p < 0.05$).

residual estrogenic activities in antiestrogens than the transiently transfected HeLa cells described above. All of the compounds studied reduced the luciferase activity of MCF-7/2a cells stimulated with 10 nM estradiol down to the control level (Table 1). The IC_{50} values of the benzocarbazoles were three to four times higher than those for the corresponding indoles. The most potent derivative **15d** displayed activity similar to that of the two reference compounds.

The main goal of our studies has been the identification of agents that might be useful for treatment of hormone-dependent breast cancer. Therefore, we investigated the activity of the new benzocarbazoles in estrogen receptor-positive human MCF-7 breast cancer cells. All of the compounds strongly inhibited the growth of these cells. The antitumor effect was dependent on both the side chain structure and the carrier (Table 1). The highest activity was observed with the indole **15c** and the steroidal antiestrogen ICI 182,780 with IC_{50} values of 0.6 and 0.22 nM, respectively. This agreement is mainly due to the structural similarity of the side chains and reflects the biological equivalence of the carrier molecules. The comparison of the fluoro-substituted derivatives with their non-halogenated analogues (**6b/6c; 15b/15c; 6d/6e**) revealed a positive effect of the fluorination of the terminal carbon atoms which was thought to improve primarily the pharmacokinetics of these agents.

Since the antiproliferative effects of these compounds could possibly be the result of a cytotoxic action all derivatives were also tested in hormone independent human MDA-MB 231 mammary tumor cells [16]. The lack of activity at concentrations below 1 μM (data not shown) makes a receptor mediated action in MCF-7 cells likely.

Based on the data from the transcription assays we submitted three benzo[*a*]carbazoles (**6b–d**) and the indole **15d** to the mouse uterine weight test. Both benzocarbazoles with aliphatic side chains appeared to be pure antagonists although complete suppression of estrogen-stimulated uterine growth could not be achieved with the highest doses applied (Table 2). Despite the strong antagonism observed in the transfection assays the indole derivative **15d** behaved as a partial antagonist in mice whereas the corresponding benzocarbazole (**6d**) was inactive. Possibly, these derivatives can undergo metabolic reactions in the side chain structure such as oxidative cleavage of the side chain at the ether function that would eliminate the sulfonylalkyl element from the molecule.

The data of this and some other studies clearly showed that the carrier molecule does not change the endocrine profile but influences the potency of these agents. The benzocarbazole derivatives were consistently less active than the corresponding 2-phenylindoles though the chemical structure had only been altered slightly. The ethane bridge across carbon-3 and the *ortho* position in the phenyl ring reduces the dihedral angle of the two aromatic systems from 60°[17] to about 30°. The reason for the differences in activity which can be up to one order of magnitude remains unclear because the binding affinities for the estrogen receptor are not significantly different. Because of the structural similarity it is unlikely that the relative differences in binding will increase when a human estrogen receptor preparation is used instead of calf uterine cytosol. Obviously, other effects such as the penetration of cellular membranes play an important role. This might also explain the positive effect of the fluorination of the terminal carbon atoms in these antiestrogens.

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Experimental Section

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Elemental analyses of crystalline compounds were performed by the Mikroanalytisches Laboratorium, University of Regensburg, and were within ±0.40 % of the calculated values except where noted. Purity was checked by tlc (SiO_2) and HPLC (Lichrosphere 5m, RP-18; MeCN/H_2O (70 : 30) as eluent, detection by UV (250 nm) and fluorescence (ex. 300 nm; em. 370 nm)), respectively. NMR spectra were obtained on a Bruker AC-250 spectrometer with TMS as internal standard. The syntheses of 3,8-dimethoxy-6,11-dihydrobenzo[*a*]carbazole (**1**)[11], 5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**13**)[18], 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl- 1-[10-(pentylthio)decyl]indole (**15a**)[4], 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]indole (**15b**)[4], and 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[4-[4-(pentylsulfonyl)butoxy]benzyl]indole (**15d**)[9] have been described previously. The preparation of the ω-bromo-substituted side chains **7a** and **8a**[4], and **12a**[9] has been reported.

*6,11-Dihydro-3,8-dihydroxy-5H-benzo[a]carbazole (**2**)*

Under N_2, a solution of 3,8-dimethoxy-6,11-dihydrobenzo[*a*]carbazole (**1**, 14.2 mmol) in 90 ml of dry CH_2Cl_2 was added slowly to a solution of 8.0 ml (85 mmol) of BBr_3 in 175 ml of dry CH_2Cl_2 at a temperature of –10 °C. After addition, stirring was continued for 1 h at room temperature followed by heating for 5 h at 40–50 °C. With cooling 250 ml of a saturated solution of $NaHCO_3$ was added in portions. After addition of 300 ml of EtOAc the mixture was stirred vigorously for 30 min. The layers were separated and the aqueous layer extracted three times with EtOAc. After washing with water and drying ($MgSO_4$), the solvent was removed *in vacuo* and the residue purified by chromatography (SiO_2; EtOAc/CH_2Cl_2, 3 : 1) to give **2** as a beige amorphous powder, mp. 249–251 °C (dec.), yield 64%.– Anal. ($C_{16}H_{13}NO_2$) C; calcd. 76.47, found 75.13; H, N.– ^{1}H NMR ($CDCl_3$) δ 2.97 (m; 4H; Ar-$(CH_2)_2$-Ar); 3.82 (s; 3H; -OCH_3); 3.87 (s; 3H; -OCH_3); 6.75 (dd; ^3J = 8.4 Hz, ^4J = 2.6 Hz; 1H; Ar-H^9); 6.80 (dd; ^3J = 8.9 Hz, ^4J = 2.5 Hz; 1H; Ar-H^2); 6.84 (d; ^4J = 2.5 Hz; 1H; Ar-H^4); 6.97 (d; ^4J = 2.6 Hz; 1H; Ar-H^7); 7.20 (d; ^3J = 8.4 Hz; 1H; Ar-H^{10}); 7.23 (d; ^3J = 8.9 Hz; 1H; Ar-H^1); 8.01 (s; 1H; N-H).

*6,11-Dihydro-3,8-bis(tetrahydropyran-2-yl-oxy)-5H-benzo[a]carbazole (**3**)*

3,8-Dihydroxy-6,11-dihydrobenzo[*a*]carbazole (**2**, 10.1 mmol) was dissolved in 30 ml of EtOAc followed by addition of 8 ml (87.5 mmol) of 3,4-dihydro-2*H*-pyran. Under N_2, 1.5 ml of EtOAc, saturated with dry HCl gas, was added dropwise. After stirring for 3.5 h at room temperature 100 ml of saturated $NaHCO_3$ solution was added and the mixture extracted four times with EtOAc. The combined organic layers were washed with $NaHCO_3$ solution and water, and dried over $MgSO_4$. After evaporation of the solvent *in vacuo*, the residue was dissolved in hot CCl_4. On cooling to –18 °C, an amorphous light yellow solid preciptated, mp 174–175 °C (EtOH/hexane 1:5), yield 45%. Anal. ($C_{26}H_{29}NO_4$).– ^{1}H NMR ($CDCl_3$) δ 1.57–2.17 (m; 12H; -$(CH_2)_3$-); 2.87–3.05 (m; 4H; Ar-$(CH_2)_2$-Ar); 3.52–3.74 (m; 2H; -CH_2-O-); 3.89–4.11 (m; 2H; -CH_2O-); 5.41–5.46 (m; 2H; -O(CH-)O-); 6.89–6.94 (m; 2H; Ar-H^{9+2}); 6.96 (d; ^4J = 2.5 Hz; 1H; Ar-H^4); 6.99 (d; ^4J = 2.3 Hz; 1H; Ar-H^7); 7.21 (d; ^3J = 8.9 Hz; 1H; Ar-H^{10}); 7.23 (d; ^3J = 8.3 Hz; 1H; Ar-H^1); 8.03 (s; 1H; N-H).

General Procedure for the N-Alkylation

Under N_2, a solution of **1** or **3** (4.3 mmol) in 15 ml of dry DMF was added to a stirred suspension of 6.1 mmol of NaH in 5 ml of dry DMF at 0 °C. Stirring was continued until the gas evolution ceased (30 min). At 0 °C, the respective ω-bromo-substitued side chain (4.3 mmol) in 20 ml of dry DMF was added dropwise. The cooling bath was removed and stirring continued for 2 h. The excess of NaH was destroyed by adding water dropwise. EtOAc and water (30 ml) was added. The organic layer was separated and the aqueous phase extracted twice with EtOAc. The combined organic layers were washed with water and saline, and dried ($MgSO_4$). After evaporation of the solvent, the product was purified by chromatography (SiO_2) with mixtures of CH_2Cl_2 and petroleum ether.

*6,11-Dihydro-3,8-dimethoxy-11-[10-(pentylthio)decyl]-5H-benzo[a]carbazole (**4a**)*

From **1** and **7a**. Yellow fluorescent oil, yield 73%.– ^{1}H-NMR ($CDCl_3$) δ = 0.90 (t, ^3J = 7.0 Hz, 3H, C*H*$_3$-CH_2), 1.27–1.41 (m, 16H, CH_3(C*H*$_2$)$_2$, -$(CH_2)_6$-), 1.51–1.70 (m, 4H, -S-CH_2-C*H*$_2$-), 1.82–1.91 (m, 2H, -N-CH_2-C*H*$_2$-), 2.47–2.53 (m, 4H, S$(CH_2)_2$), 2.81–2.98 (m, 4H, Ar-$(CH_2)_2$-Ar), 3.85 (s, 3H, -OCH_3), 3.87 (s, 3H, -OCH_3), 4.30 (t, ^3J = 7.9 Hz, 2H, -N-CH_2-), 6.81–6.86 (m, 2H, Ar-H^{9+2}), 6.90 (d, ^4J = 2.7 Hz, 1H, Ar-H^4), 6.98 (d, ^4J = 2.4 Hz, 1H, Ar-H^7), 7.22 (d, ^3J = 8.8 Hz, 1H, Ar-H^{10}), 7.44 (d, ^3J = 8.5 Hz, 1H, Ar-H^1).

*6,11-Dihydro-3,8-dimethoxy-11-[10-(pentylsulfonyl)decyl]-5H-benzo[a]-carbazole (**4b**)*

From **1** and **8a**. Colorless solid, yield 72%, mp. 93–94 °C.– Anal. ($C_{33}H_{47}NO_4S$).– ^{1}H NMR ($CDCl_3$) δ = 0.92 (t, ^3J = 7.0 Hz, 3H, C*H*$_3$-CH_2-), 1.28–1.49 (m, 16H, CH_3(C*H*$_2$)$_2$-, -$(CH_2)_6$-), 1.76–1.90 (m, 6H, -SO_2-CH_2-C*H*$_2$-, N-CH_2-C*H*$_2$-), 2.81–2.99 (m, 8H, $SO_2(CH_2)_2$, Ar-$(CH_2)_2$-Ar), 3.85 (s, 3H, -OCH_3^A), 3.87 (s, 3H, -OCH_3^B), 4.30 (t, ^3J = 7.8 Hz, 2H, N-CH_2-), 6.81–6.86 (m, 2H, Ar-H^{9+2}), 6.90 (d, ^4J = 2.7 Hz, 1H, Ar-H^4), 6.98 (d, ^4J = 2.4 Hz, 1H, Ar-H^7), 7.22 (d, ^3J = 8.8 Hz, 1H, Ar-H^{10}), 7.44 (d, ^3J = 8.5 Hz, 1H, Ar-H^1).

*6,11-Dihydro-3,8-dimethoxy-11-[10-(4,4,5,5,5-pentafluoropentylsulfonyl)-decyl]-5H-benzo[a]carbazole (**4c**)*

From **1** and **8b**. Yellow solid, mp. 84 °C (EtOH), yield 73%.– Anal. ($C_{33}H_{42}F_5NO_4S$).– ^{1}H-NMR ($CDCl_3$) δ = 1.28–1.34 (m, 12H, -$(CH_2)_6$-), 1.77–1.90 (m, 4H, -SO_2-CH_2-C*H*$_2$-), 2.10–2.38 (m, 4H, -N-CH_2-C*H*$_2$-, -CH_2-C_2F_5), 2.82–3.07 (m, 8H, Ar-$(CH_2)_2$-Ar, $SO_2(CH_2)_2$), 3.85 (s, 3H, -OCH_3), 3.88 (s, 3H, -OCH_3), 4.31 (t, ^3J = 7.8 Hz, 2H, -N-CH_2-), 6.81–6.86 (m, 2H, Ar-H^{9+2}), 6.90 (d, ^4J = 2.7 Hz, 1H, Ar-H^4), 6.98 (d, ^4J = 2.4 Hz, 1H, Ar-H^7), 7.22 (d, ^3J = 8.8 Hz, 1H, Ar-H^{10}), 7.44 (d, ^3J = 8.5 Hz, 1H, Ar-H^1).

*6,11-Dihydro-11-[4-[4-(pentylsulfonyl)butoxy]benzyl]-3,8-di-(tetrahydro-pyran-2-yloxy)-5H-benzo[a]carbazole (**5d**)*

From **3** and **12a**. Orange resin, yield 44%.– ^{1}H-NMR ($CDCl_3$) δ = 0.87–0.94 (m, 3H, C*H*$_3$-CH_2-), 1.11–2.08 (m, 22H, CH_3(C*H*$_2$)$_3$-, -(C*H*$_2$)$_2$-CH_2-OAr, -(C*H*$_2$)$_3$-CH_2-O-), 2.83–3.06 (m, 8H, Ar-$(CH_2)_2$-Ar, $SO_2(CH_2)_2$), 3.57–4.07 (m, 6H, -CH_2-O-, -CH_2-OAr), 5.40–5.46 (m, 4H, -O-(CH-)O-, -N-CH_2-), 6.78–7.19 (m, 6H, Ar-H), 6.82 and 7.10 (AA′BB′, ^3J = 8.7 Hz, 4H, Ar-H).

*6,11-Dihydro-11-[4-[4-(4,4,5,5,5-pentafluorpentylsulfonyl)butoxy]benzyl]-3,8-di-(tetrahydropyran-2-yl-oxy)-5H-benzo[a]carbazole (**5e**)*

Prepared from **3** and **12b** and purified by chromatography (Al_2O_3) with CH_2Cl_2 /Petroleum ether (4 : 1) as eluent to give an orange resin (51%) that was reacted without further characterization.

General Procedure for the Cleavage of the Methoxy Groups

Under N_2, a solution of the dimethoxybenzo[*a*]carbazole derivative (0.54 mmol) in 10 ml of CH_2Cl_2 was added slowly to a stirred solution of 1.16 mmol of BBr_3 in 10 ml of CH_2Cl_2 at –10 °C. After addition, the cooling bath was removed and the mixture was stirred for 3 h at room temperature. The ice-cold mixture was treated with small portions of a cold saturated $NaHCO_3$ solution (60 ml). After addition of ethyl acetate (50 ml), the mixture was stirred for 15 min. The organic layer was removed and the aqueous phase extracted with EtOAc. The combined organic layers were washed with $NaHCO_3$ solution and water, and dried ($MgSO_4$). After removal of the solvent, the crude product was purified by chromatography and recrystallized if possible.

*6,11-Dihydro-3,8-dihydroxy-11-[10-(pentylthio)decyl]-5H-benzo[a]carbazole (**6a**)*

Colorless crystals, mp 115–116 °C (CH_2Cl_2/n-hexane 1 : 1), yield 63%.– Anal. ($C_{31}H_{43}NO_2S$).– ^{1}H-NMR ($CDCl_3$) δ = 0.87–0.93 (m, 3H, C*H*$_3$-CH_2-), 1.25–1.65 (m, 20H, -(C*H*$_2$)$_7$-, CH_3(C*H*$_2$)$_3$-), 1.83–1.86 (m, 2H, -N-CH_2-C*H*$_2$-), 2.51 (t, ^3J = 7.4 Hz, 4H, S(CH_2-)$_2$), 2.84 (m, 4H, Ar-(CH_2)$_2$-Ar), 4.28 (t, ^3J = 7.8 Hz, 2H, -N-CH_2-), 4.63 (s, br, 1H, Ar-OH), 5.12 (s, br, 1H, Ar-OH), 6.73–6.78 (m, 2H, Ar-H^{2+9}), 6.83 (d, ^4J = 2.5 Hz, 1H, Ar-H^4), 6.92 (d, ^4J = 2.3 Hz, 1H, Ar-H^7), 7.17 (d, ^3J = 8.5 Hz, 1H, Ar-H^{10}), 7.38 (d, ^3J = 8.1 Hz, 1H, Ar-H^1).

*6,11-Dihydro-3,8-dihydroxy-11-[10-(pentylsulfonyl)decyl]-5H-benzo[a]carbazole (**6b**)*

Beige powder, mp 129–130 °C ($CHCl_3$), yield 70%.– ^{1}H-NMR (DMSO-d_6) δ = 0.87 (t, ^3J = 7.0 Hz, 3H, C*H*$_3$-CH_2-), 1.20–1.33 (m, 16H, -(CH_2)$_8$-, CH_3(C*H*$_2$)$_2$-), 1.58–1.65 (m, 6H, -SO_2-CH_2-C*H*$_2$-, -N-CH_2-C*H*$_2$-), 2.72 (m, 4H, Ar-(CH_2)$_2$-Ar), 3.03 (t, ^3J = 7.9 Hz, 4H, SO_2(CH_2-)$_2$), 4.28 (t, ^3J = 7.9 Hz, 2H, -N-CH_2-), 6.60 (dd, ^3J = 8.7 Hz, ^4J = 2.2 Hz, 1H, Ar-H^9), 6.70 (dd, ^3J = 8.3Hz, ^4J = 2.3 Hz, 1H, Ar-H^2), 6.75 (d, ^4J = 2.3 Hz, 1H, Ar-H^4), 6.76 (d, ^4J = 2.2 Hz, 1H, Ar-H^7), 7.21 (d, ^3J = 8.7 Hz, 1H, Ar-H^{10}), 7.39 (d, ^3J = 8.3 Hz, 1H, Ar-H^1), 8.67 (s, 1H, Ar-OH), 9.47 (s, 1H, Ar-OH).– MS (70 eV) *m/z* (%) = 526 (100) [M$^+$], 390 (13) [M$^+$-(SO_2-C_5H_{11})], 264 (55, [M$^+$-(CH_2)$_9$-SO_2-C_5H_{11}].

*6,11-Dihydro-3,8-dihydroxy-11-[10-(4,4,5,5,5-pentafluorpentylsulfonyl)-decyl]-5H-benzo[a]carbazole (**6c**)*

Brownish amorphous solid, yield 70%.– ^{1}H-NMR ($CDCl_3$) δ = 1.22–1.46 (m, 12H, -(CH_2)$_6$-), 1.78–1.91 (m, 4H, -SO_2-CH_2-C*H*$_2$-), 2.17–2.39 (m, 4H, -N-CH_2-C*H*$_2$-, -CH_2-C_2F_5), 2.77–2.93 (m, 4H, Ar-(CH_2)$_2$-Ar), 2.97–3.10 (m, 4H, SO_2(CH_2-)$_2$), 4.31 (t, ^3J = 7.7 Hz, 2H, -N-CH_2-), 4.58 (s, br, 1H, Ar-OH), 5.28 (s, br, 1H, Ar-OH), 6.72–6.78 (m, 2H, Ar-H^{2+9}), 6.84 (d, ^4J = 2.1 Hz, 1H, Ar-H^4), 6.93 (d, ^4J = 2.4 Hz, 1H, Ar-H^7), 7.18 (d,^3J = 8.7 Hz, 1H, Ar-H^{10}), 7.39 (d, ^3J = 8.4 Hz, 1H, Ar-H^1).

General Procedure for the Cleavage of the Tetrahydropyranyl Ethers

Under N_2, 10 ml of aqueous oxalic acid (8 %) was added dropwise to a solution of the (tetrahydropyran-2-yl)phenyl ether in 15 ml MeOH and 15 ml THF. Then, the mixture was heated at 60–70 °C for 3.5 h. After cooling, the mixture was neutralized with $NaHCO_3$ solution, stirred for additional 15 min, extracted several times wth 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. The following compounds were obtained by this method:

*6,11-Dihydro-3,8-dihydroxy-11-[4-[4-(pentylsulfonyl)butoxy]benzyl]-5H-benzo[a]carbazole (**6d**)*

Colorless foam, yield 72%.– ^{1}H-NMR ($CDCl_3$) δ = 0.88–2.12 (m, 13H, CH_3-(CH_2)$_3$-, -(C*H*$_2$)$_2$-CH_2-OAr), 2.83–3.07 (m, 8H, Ar-(CH_2)$_2$-Ar, SO_2-(CH_2-)$_2$), 3.92–4.00 (m, 2H, -CH_2-O-), 4.50 (s, br, 1H, Ar-OH), 4.76 (s, br, 1H, Ar-OH), 5.45 (s, 2H, -N-CH_2-), 6.58 (dd, ^3J = 8.4 Hz, ^4J = 2.7 Hz, 1H, Ar-H^9), 6.68 (dd, ^3J = 8.7 Hz, ^4J = 2.5 Hz, 1H, Ar-H^2), 6.82 (d, ^4J = 2.5 Hz, 1H,Ar-H^4), 6.84, 7.13 (AA′BB′, ^3J = 8.7 Hz, Ar-H), 6.98 (d, ^4J = 2.7 Hz, 1H, Ar-H^7), 7.00 (d, ^3J = 8.7 Hz, 1H, Ar-H^{10}), 7.10 (d, ^3J = 8.4 Hz, 1H, Ar-H^1).

*6,11-Dihydro-3,8-dihydroxy-11-[4-[4-(4,4,4,5,5-pentafluorpentylsulfonyl)-butoxy]benzyl]-5H-benzo[a]carbazole (**6e**)*

Green foam, yield 64%.– ^{1}H-NMR ($CDCl_3$) δ = 1.26–2.38 (m, 8H, -(CH_2)$_2$-C_2F_5, -SO_2-CH_2-(C*H*$_2$)$_2$-), 2.86–2.93 (m, 4H, Ar-(CH_2)$_2$-Ar), 3.03–3.12 (m, 4H, SO_2-(CH_2-)$_2$), 3.96–4.01 (m, 2H, -CH_2-O-), 5.45 (s, 2H, -N-CH_2-), 6.58 (dd, ^3J = 8.4 Hz, ^4J = 2.7 Hz, 1H, Ar-H^9), 6.68 (dd, ^3J = 8.6 Hz, ^4J = 2.4 Hz, 1H, Ar-H^2), 6.82 (d, ^4J = 2.4 Hz, 1H, Ar-H^4), 6.83, 7.10 (AA′BB′, ^3J = 8.7 Hz, Ar-H), 6.97 (d, ^4J = 2.7 Hz, 1H, Ar-H^7), 7.00 (d,^3J = 8.7 Hz, 1H, Ar-H^{10}), 7.13 (d, ^3J = 8.4 Hz, 1H, Ar-H^1).

*Syntheses of New Side Chains: 1-Bromo-10-(4,4,5,5,5-pentafluoropentylthio)decane (**7b**)*

A solution of 4,4,5,5,5-pentafluoropentyl thioacetate (14.6 mmol) in 30 ml of dry MeOH was added dropwise under N_2 to a solution of 78 mmol sodium methylate in 60 ml of dry MeOH. After heating to 60–70 °C for 30 min the excess of sodium methylate was destroyed by careful addition of water. The mixture was acidified with 2N HCl and extracted four times with *n*-hexane. The combined organic layers were washed with water, dried ($MgSO_4$), diluted with 80 ml of anhydrous DMF, and added under N_2 slowly to a stirred suspension of NaH (10.8 mmol) in 10 ml of anhydrous DMF. After the addition stirring was continued for 30 min. The mixture was transferred to a dropping funnel and the bottom DMF-layer was added dropwise to a solution of 1,10-dibromodecane (36.1 mmol) in 25 ml of dry DMF. The mixture was heated to 50–60 °C for 2 h. After cooling, water was added until a clear solution was obtained. After the addition of 100 ml EtOAc and 150 ml of water the organic layer was separated and the aqueous phase extracted three times with EtOAc. The combined organic layers were washed with saline and dried ($MgSO_4$). After evaporation of the solvent the residue was chromatographed (SiO_2/CH_2Cl_2-petroleum ether 1 : 5) to give the desired product as the second of three fractions. Slightly yellow oil which crystallized at 17–20 °C, yield 41%.– ^{1}H-NMR ($CDCl_3$) δ = 1.21–2.51 (m, 20H, -(CH_2)$_8$-, -(CH_2)$_2$-C_2F_5), 2.57–3.05 (m, 4H, S(CH_2-)$_2$), 3.57 (t, ^3J = 7.0 Hz, 2H, -CH_2Br).

*1-Bromo-10-(4,4,5,5,5-pentafluoropentylsulfonyl)decane (**8b**)*

A solution of *meta*-chloroperbenzoic acid (10.6 mmol) in 85 ml $CHCl_3$ was added within 30 min to a stirred solution of **10b** (4.95 mmol) in 240 ml $CHCl_3$. After stirring for 2.5 h at room temperature, the solution was poured into 240 ml of sat. $NaHCO_3$ solution. After stirring for 10 min the organic layer was separated, washed with water, and dried ($MgSO_4$). After evaporation of the solvent the product was purified by crystallization from EtOH to afford colorless crystals, mp 85–86 °C, yield 68%.– Anal. ($C_{15}H_{26}BrF_5O_2S$).– ^{1}H-NMR ($CDCl_3$) δ = 1.30–2.71 (m, 20H, -(CH_2)$_8$-, -(CH_2)$_2$-C_2F_5), 3.12–3.60 (m, 4H, SO_2(CH_2-)$_2$), 3.75 (t, ^3J = 7.0 Hz, 2H, -CH_2Br).

*Methyl 4-[4-(4,4,5,5,5-Pentafluoropentylthio)butoxy]benzoate (**9b**)*

From 4,4,5,5,5-pentafluoropentylthiol and an equimolar amount of methyl 4-(4-bromobutoxy)benzoate by a method similar to that described for the synthesis of **7b**. Yellow oil, yield 73%.– ^{1}H-NMR ($CDCl_3$) δ = 1.61–2.51 (m, 8H, -(CH_2)$_2$-C_2F_5, -CH_2-(C*H*$_2$)$_2$-CH_2-), 2.65–3.08 (m, 4H, S(CH_2-)$_2$), 4.16 (s, 3H, -OCH_3), 4.31 (t, ^3J = 6.0 Hz, 2H, -OCH_2-), 7.11, 8.15 (AA′BB′, ^3J = 9.0 Hz, 4H, Ar-H).

*4-[4-(4,4,5,5,5-Pentafluoropentylthio)butoxy]benzyl Alcohol (**10b**)*

Under N_2, a solution of the ester **9b** (6.4 mmol) in 10 ml of dry THF was added dropwise to a refluxing solution of 4.2 mmol of $LiAlH_4$ in 20 ml of dry Et_2O. After addition, the reaction mixture was heated under reflux for 7 h. After standing overnight under exclusion of moisture, the mixture was carefully hydrolyzed by adding water dropwise. After the vigorous reaction had ceased, an excess of water was added. The mixture was acidified with 10 % H_2SO_4 in order to dissolve the precipitate. The organic layer was separated and the aqueous phase extracted with EtOAc. The combined organic layers were washed with water and dried ($MgSO_4$). After evaporation of the solvent, the product was separated from unreacted ester and purified by chromatography (SiO_2) to give a yellow oil (78%).– ^{1}H-NMR ($CDCl_3$) δ = 1.63–2.53 (m, 8H, -$(CH_2)_2$-C_2F_5, -CH_2-(CH_2)$_2$-CH_2-, -CH_2-O*H*), 2.74–3.10 (m, 4H, S(CH_2-)$_2$), 4.23–4.53 (m, 2H, -CH_2-OAr), 5.00 (s, 2H,-C*H*$_2$-OH), 7.22, 7.63 (AA′BB′, ^3J = 9.0 Hz, 4H, Ar-H).

*4-[4-(4,4,5,5,5-Pentafluoropentylsulfonyl)butoxy]benzyl Alcohol (**11b**)*

Prepared by a method similar to that described for **8b** to give a colorless amorphous powder, mp 92–95 °C, yield 68%.– Anal. ($C_{16}H_{21}F_5O_4S$).– ^{1}H-NMR (DMSO-d_6) δ = 1.83–2.01 (m, 6H, -C*H*$_2$-CH_2-SO_2-, -$(CH_2)_2$-C_2F_5, -CH_2-O*H*), 2.30–2.45 (m, 2H, -C*H*$_2$-CH_2-O-), 3.21–3.27 (m, 4H, SO_2(CH_2-)$_2$), 3.98 (s, br, 2H, -CH_2-Ar), 4.41 (s, 2H, -C*H*$_2$-OH), 6.88, 7.21 (AA′BB′, ^3J = 8.5 Hz, 4H, Ar-H).

*4-[4-(4,4,5,5,5-Pentafluoropentylsulfonyl)butoxy]benzyl Bromide (**12b**)*

Under N_2 a solution of the benzyl alcohol **11b** (3.18 mmol) in 5 ml of dry CH_2Cl_2 was cooled to 0 °C. With vigorous stirring 3.5 mmol of PBr_3 in 5 ml of dry CH_2Cl_2 was added slowly. After stirring for 30 min the mixture was poured into ice-water followed by the addition of 100 ml of EtOAc. The layers were separated and aqueous phase extracted twice with EtOAc. The combined organic layers were washed with $NaHCO_3$ solution and saline, and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by crystallization with an Et_2O/EtOAc (10:1) mixture to afford colorless crystals, mp 110°C, yield 54%.– Anal. ($C_{16}H_{20}BrF_5O_3S$).– ^{1}H-NMR ($CDCl_3$) δ = 1.91–2.39 (m, 8H, -C*H*$_2$-CH_2-SO_2-, -$(CH_2)_2$-C_2F_5, -C*H*$_2$-CH_2O-), 3.04–3.12 (m, 4H, SO_2(CH_2-)$_2$), 4.02 (t, ^3J=5.6 Hz, 2H, -CH_2-OAr), 4.50 (s, 2H, -C*H*$_2$-OH), 6.84 and 7.32 (AA′BB′, ^3J = 8.7 Hz, 4H, Ar-H).

*5-Hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[10-(4,4,5,5,5-pentafluoropentylsulfonyl)decyl]indole (**15c**)*

5-Methoxy-2-(4-methoxyphenyl)-3-methylindole (**13**) was deprotonated with NaH and reacted with the alkyl bromide **8b** to give 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[10-(4,4,5,5,5-pentafluorpentylsulfonyl)decyl]indole (**14c**) following the procedure described for the preparation of **4c**. After purification by chromatography (SiO_2) with CH_2Cl_2/EtOAc (40 : 1) as eluent, **14c** was obtained as a yellow resin, yield 89%.– ^{1}H-NMR ($CDCl_3$) δ = 0.96–2.36 (m, 20H, -$(CH_2)_8$-, -$(CH_2)_2$-C_2F_5), 2.20 (s, 3H, Ar-CH_3), 2.80–3.21 (m, 4H, SO_2(CH_2-)$_2$), 3.87 (s, 6H, OCH_3), 3.97 (t, ^3J = 7 Hz, 2H, ArN-CH_2-), 6.76–7.47 (m, 7H, Ar-H).

The product was used without further purification. Ether cleavage with BBr_3 was performed by a method similar to that described for the carbazole **6c** to give **15c** as a slightly yellow solid after purification by chromatography (SiO_2) with CH_2Cl_2/EtOAc (6 : 1) as eluent, yield 59%, mp 127–128 °C (CH_2Cl_2/n-hexane, 1 : 1).– Anal. ($C_{30}H_{38}F_5NO_4S$).– ^{1}H-NMR ($CDCl_3$) δ = 0.91–2.36 (m, 20H, -$(CH_2)_8$-, -$(CH_2)_2$-C_2F_5), 2.13 (s, 3H, Ar-CH_3), 2.81–3.22 (m, 4H, SO_2(CH_2-)$_2$), 3.94 (t, ^3J = 7 Hz, 2H, ArNCH_2-), 5.02 (s, br, 1H, -OH), 5.74 (s, br, 1H, -OH), 6.60–7.30 (m, 7H, Ar-H).– MS (70 eV) *m/z* (%) 604 (17) [M^++H], 603 (50) [M^+], 378 (6.8) [M^+-SO_2(CH_2)$_3$-C_2F_5], 252 (100) [M^+-$(CH_2)_9SO_2$-$(CH_2)_3C_2F_5$], 238 (19) [M^+-$(CH_2)_{10}SO_2(CH_2)_3C_2F_5$].

All of the bioassays including the estrogen receptor binding assay[4], the transcription assays with transiently transfected HeLa cells[4] and stably transfected MCF-7/2a cells[9], the proliferation assays with hormone-sensitive MCF-7 and hormone-independent MDA-MB 231 breast cancer cells[4], and the mouse uterine weight test[4] have been described previously.

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