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2-Phenylindoles with Sulfur Containing Side Chains. Estrogen Receptor Affinity, Antiestrogenic Potency, and Antitumor Activity

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The 2-phenylindole system has been identified as a suitable structure for the design of non-steroidal pure estrogen antagonists [E. von Angerer *et al.*, *J. Steroid Biochem. Molec. Biol.* 49 (1994) 51–62]. Derivatives with an amide function in the side chain antagonized the stimulatory effect of estrogens both *in vitro* and *in vivo*, and showed no agonistic activity when given alone. The findings of other groups who studied steroidal antiestrogens prompted us to replace the amide function by sulfide, sulfoxide, sulfone, sulfonamide and related groups. The compounds with polar sulfur functions retained the high binding affinity for the calf uterine estrogen receptor (RBA: 1–5% of estradiol; ICI 182,780; 6.2%). The estrogenic effect was quantified in a transcription assay using HeLa cells cotransfected with the expression vector HEG0 for the human estrogen receptor and a reporter plasmid that harbored a Vit. A2 ERE and the luciferase gene driven by a thymidine kinase promotor. Pentylsulfide, -sulfinyl, and -sulfonyl groups, linked to the indole nitrogen by a decamethylene spacer, were devoid of any transcriptional activity. These results were confirmed in the mouse uterine weight test. The sulfone (ZK 164,015) completely abolished the effect of a standard dose of estrone at a daily dose of 7 mg/kg. This compound strongly inhibited the growth of hormone-sensitive human MCF-7 breast cancer cells with an IC_{50}-value close to 1 nM. Similar activity was found for the steroidal sulfoxide ICI 182,780. We were also able to demonstrate significant antineoplastic activity *in vivo* for some of these new 2-phenylindole derivatives. Copyright © 1996 Elsevier Science Ltd.

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INTRODUCTION

The nonsteroidal antiestrogen tamoxifen is an effective treatment for postmenopausal women with estrogen receptor positive mammary carcinoma as first-line therapy. About 50% of these patients with advanced disease are likely to respond to this treatment with a remission [1] which is often of rather short duration and tumors resume growth under therapy. It is assumed that carcinomas that have become resistant to tamoxifen might have developed a dependency on tamoxifen which has considerable estrogenic potency [2, 3]. Experimental studies have demonstrated that mammary tumor cells can use tamoxifen for maintaining growth after a process of adaption [4, 5]. Subsequent treatment with a pure antiestrogen such as ICI 182,780 can arrest the growth of tamoxifen-resistant tumors [6]. A recent phase II clinical trial with this pure antiestrogen as second-line treatment after tamoxifen confirmed these experimental findings [7]. However, a pure estrogen antagonist when given as a first-line drug might increase the duration of remission in patients liable to respond to antiestrogens.

For several years, we have synthesized and characterized potent antiestrogens based on non-steroidal structures. The first drug that entered clinical trials was the 2-phenylindole derivative zindoxifene [8]. Its endocrine profile was that of a partial antagonist or, more precisely, a mixed agonist/antagonist. A considerable improvement of the antiestrogenic potency was reached after an amino function had been introduced into the side chain [9]. ZK 119,010, a 2-phenylindole

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Abbreviations: DMF, dimethyl formamide, ER, estrogen receptor, ERE, estrogen response element, FCS, fetal calf serum, RBA, relative binding affinity

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derivative with a pyrrolidinohexyl group in position 1, was a pure antiestrogen in mice [9]. In rats, however, some estrogenic activity was noticed at high doses [10]. This was confirmed in a transcription assay that we have developed [11].

In our continuing search for pure antiestrogens based on heterocyclic systems we varied the side chain structure of the 2-phenylindole system and introduced amide functions separated by spacer groups of varying lengths from the indole nucleus [11]. From these studies the derivative ZK 169,978 (5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[11-(methyl-propylcarbamoyl)undecyl]indole (Fig. 1) with all the characteristics of a pure antiestrogen emerged [11]. In many respects it resembled the estradiol derivative ICI 164,384 which was described earlier by Wakeling and Bowler [12] as pure antiestrogen. In a more recent study Wakeling *et al.* [13] showed that the replacement of the amide function by a sulfoxide group increases the potency of the steroid as estrogen antagonist. These findings prompted us to modify the functional group incorporated in the long alkyl side chain. We replaced the amide function by the sulfonamide fragment and by sulfur in various oxidation states. A similar modification has been described by Nique *et al.* in a series of 11β-substituted estradiol derivatives [14]. We also modified the alkyl spacer by replacing one methylene group by sulfur, a modification that was also performed by Labrie's group [15, 16]. For comparison we also introduced the side chain of ICI 182,780 into the 2-phenylindole structure.

All of these 2-phenylindole derivatives with functional side chains were tested for binding affinity for the calf uterine estrogen receptor and transactivation of estrogen-regulated genes. This *in vitro*-system for determination of residual estrogenic activity has recently been established in our laboratory [11, 17]. HeLa cells are cotransfected with the expression vector for the human wild type estrogen receptor (HEG0) described by Chambon and his group [18] and a reporter plasmid which contains the estrogen response element from the Vitellogenin A2 gene [19] and the luciferase gene for quantifying transcription. Estrogen receptor mediated antitumor activity was monitored in human MCF-7 breast cancer cells. The *in vivo* activities of some selected derivatives were determined in the uterine weight test in mice (antiestrogenic activity) and in the mouse MXT mammary tumor model (antineoplastic activity).

MATERIALS AND METHODS

Syntheses

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Solid compounds were analyzed for C, H, and N. The results were within $\pm$ 0.4% of the calculated values. IR and [^{1}H]-NMR spectra were consistent with the assigned structures. The synthesis of ZK 169,978 has been described previously [11]. The starting material for the side chain of compounds **5f** and **5g** was a generous gift from Dr R. Bohlmann, Schering AG. The syntheses of the new 2-phenylindole derivatives are outlined in Fig. 2. Usually, the sulfur containing side chains which had been synthesized by conventional methods were directly introduced into 5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**1a**) via alkylation (*i*) and ether cleavage by boron tribromide (*vii*, derivatives **5a,c,e,g,h,l**). Since the sulfoxides decomposed under ether cleavage conditions, ether cleavage of **1a** was performed prior to alkylation followed by protection of the phenolic hydroxy groups with 3,4-dihydro-2H-pyran (*viii, ix*). Removal of the protective groups (*x*) gave derivatives **5b,d,f**. The synthesis of the bifunctional compounds **5j** and **5k** was accomplished by introduction of 1,ω-dibromoalkanes into **1a** (*ii*) and substitution of the remaining halide by a thiol group via the isoalkylthiouronium salt pathway (*v*; **3j,3k**). Alkylation of the thiols **3j** and **3k** with the corresponding ω-bromoalkanoic amides led to the thioethers **4j** and **4k** (*vi*), which were submitted to ether cleavage. The sulfonamido derivative **5i** was prepared by the following route: **1a** was alkylated with 11-bromoundecanoic N-methylamide (*i*), followed by reduction by lithium aluminium hydride (*iii*, **3i**), reaction with propylsulfonylchloride (*iv*, **4i**), and ether cleavage.

General procedure for N-alkylation of 5-methoxy-2-(4-methoxyphenyl)-3-methylindole

Under a nitrogen atmosphere, 5-methoxy-2-(4-methoxyphenyl)-3-methyl-indole (**1a**) (13 mmol) in 75 ml of dry DMF was added slowly to an ice-cold suspension of sodium hydride (18 mmol) in 20 ml of dry DMF. After stirring for 30 min at 0°C, a solution of the corresponding ω-bromo substituted side chain (13 mmol) in 60 ml of dry DMF was added slowly to the indole anion solution. After stirring for 2 h at room

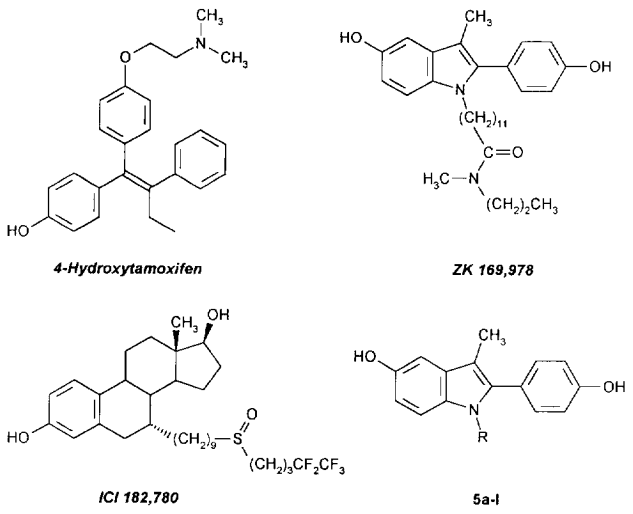

Fig. 1. Chemical structures of compounds used in this study.

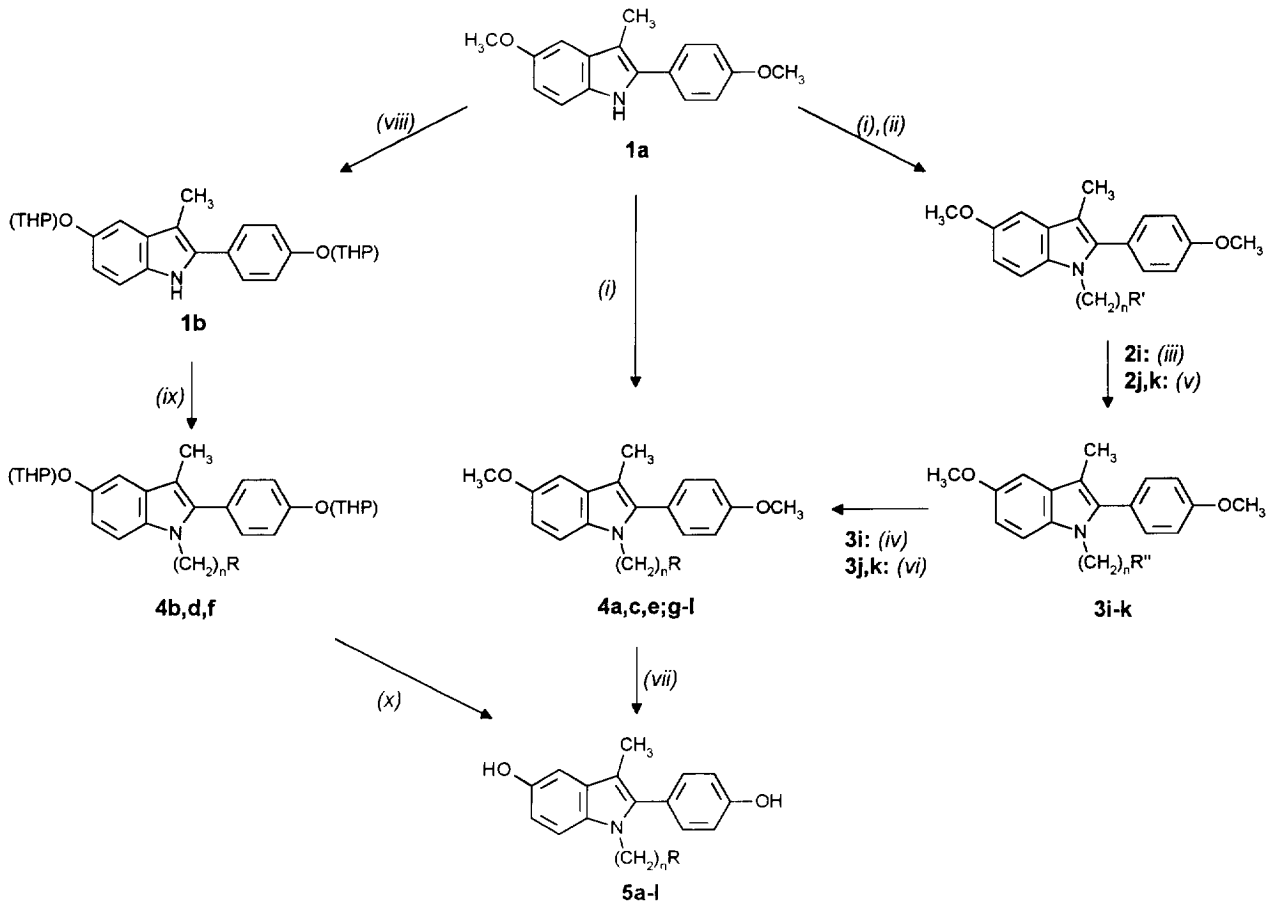

Fig. 2. Synthesis of 1-substituted 5-hydroxy-2-(4-hydroxyphenyl)-3-methylindoles. (THP) = α-tetrahydropyranyl. *(i)* $Br(CH_2)_nX$, NaH, DMF; *(ii)* $Br(CH_2)_nBr$ (4-fold excess), NaH, DMF; *(iii)* 1. $LiAlH_4$, THF; 2. H_2O; *(iv)* $CH_3(CH_2)_2SO_2Cl$, NEt_3; *(v)* 1. thiourea, THF; 2. aq. NaOH; *(vi)* $Br(CH_2)_mY$, NaH, DMF, 60°C; *(vii)* BBr_3, CH_2Cl_2, 25°C, 3h; *(viii)* 1. BBr_3, CH_2Cl_2, 40°C, 5h; 2. 3,4-dihydro-2H-pyran, EtOAc, HCl; *(ix)* same procedure as *(i)*, NaH stoichiometrically; *(x)* aq. oxalic acid, MeOH.

temperature, water was added to destroy the excess of sodium hydride. The product was extracted with EtOAc. The organic layer was washed with water and dried ($MgSO_4$). After evaporation of the solvent *in vacuo* the residue was purified by column chromatography (SiO_2; CH_2Cl_2/ligroin or CH_2Cl_2/EtOAc mixtures).

The following compounds were obtained by this method: 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[10-(methylcarbamoyl)decyl]indole (**2i**); m.p. (hexane/ether, 1:1) 88°C; 75% yield; $C_{29}H_{40}N_2O_3$ (464.6); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.86–1.91 (m; 16H; $-(CH_2)_8-$), 2.13 (t; $^3J = 7$ Hz; 2H; $-CH_2-CON-$), 2.20 (s; 3H; Ar-CH_3), 2.78 (d; $^3J = 5$ Hz; 3H; $-CONH-CH_3$), 3.88 (s; 6H; $-OCH_3$), 3.96 (t; $^3J = 7$ Hz; 2H; ArN-CH_2-), 6.73–7.41 (m; 7H; Ar-H); 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[10-(pentylthio)decyl]indole (**4a**); greenish oil; 91% yield; $C_{32}H_{47}NO_2S$ (509.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.70–1.94 (m; 25H; $-(CH_2)_8-$, $-(CH_2)_3CH_3$), 2.19 (s; 3H; Ar-CH_3), 2.32–2.64 (m; 4H; $S(CH_2-)_2$), 3.85 (s; 6H; $-OCH_3$), 3.91 (t; $^3J = 7$ Hz; 2H; ArN-CH_2-), 6.60–7.33 (m; 7H; Ar-H); 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]indole (**4c**); m.p. (EtOH) 69–70°C; 82% yield; $C_{32}H_{47}NO_4S$ (541.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.72–2.15 (m; 25H; $-(CH_2)_8-$, $-(CH_2)_3CH_3$), 2.20 (s; 3H; Ar-CH_3), 2.75–3.16 (m; 4H; $SO_2(CH_2-)_2$), 3.88 (s; 6H; $-OCH_3$), 3.97 (t; $^3J = 7$ Hz; 2H; ArN-CH_2-), 6.69–7.45 (m; 7H; Ar-H); 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[12-(propylsulfonyl)dodecyl]indole (**4e**); light yellow oil; 92% yield; $C_{32}H_{47}NO_4S$ (541.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.83–2.25 (m; 25H; $-(CH_2)_{10}-$, $-CH_2CH_3$), 2.30 (s; 3H; Ar-CH_3), 2.83–3.17 (m; 4H; $SO_2(CH_2-)_2$), 4.01 (s; 6H; $-OCH_3$), 4.08 (t; $^3J = 7$ Hz; 2H; ArN-CH_2-), 6.73–7.35 (m; 7H; Ar-H); 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[9-(4,4,5,5,5-pentafluoropentylsulfonyl)nonyl]indole (**4g**); m.p. (hexane) 87.5–89°C; 58% yield; $C_{31}H_{40}F_5NO_4S$ (617.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.92–2.39 (m; 16H; $-(CH_2)_7-$, $-CH_2-$), 2.08–2.35 (m; 2H; $-CH_2CF_2-$), 2.17 (s; 3H; Ar-CH_3), 2.77–3.25 (m; 4H; $SO_2(CH_2-)_2$), 3.88 (s; 6H; $-OCH_3$), 3.97 (t; $^3J = 7$ Hz; 2H; ArN-CH_2-), 6.75–7.44 (m; 7H; Ar-H); 1-[10-(butyl-methylsulfamoyl)decyl]-5-

methoxy-2-(4-methoxyphenyl)-3-methylindole (**4h**); colorless oil; 23% yield; $C_{32}H_{48}N_2O_4S$ (556.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.75–2.12 (m; 23H; —$(CH_2)_8$—, —$(CH_2)_2CH_3$), 2.21 (s; 3H; Ar-CH_3), 2.73–3.33 (m; 4H; —CH_2-SO_2N-CH_2—), 2.86 (s; 3H; —SO_2N-CH_3), 3.91 (s; 6H; —OCH_3), 3.99 (t; ^3J = 7 Hz; 2H; ArN-CH_2—), 6.78–7.48 (m; 7H; Ar-H); 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[10-(1-methylimidazol-2-yl-thio)-decyl]indole (**4l**); greenish oil; 88% yield; $C_{31}H_{41}N_3O_2S$ (519.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.88–1.86 (m; 16H; —$(CH_2)_8$—), 2.20 (s; 3H; Ar-CH_3), 2.90–3.21 (m; 2H; —CH_2-S-(imidazol-2-yl)), 3.61 (s; 3H; (1-imidazolyl)-CH_3), 3.91 (s; 6H; —OCH_3), 3.97 (t; ^3J = 7 Hz; 2H; ArN-CH_2—), 6.76–7.44 (m; 9H; Ar-H).

General procedure for the introduction of ω-bromoalkanes into 5-methoxy-2-(4-methoxyphenyl)-3-methylindole

The procedure described above was applied with one modification: a fourfold excess of 1,ω-dibromoalkane was used to suppress the formation of bis-alkylation products.The following compounds were obtained by this method: 1-(6-bromohexyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**2j**); m.p. (EtOH) 58-59°C; 81% yield; $C_{23}H_{28}BrNO_2$ (430.4); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.92–1.92 (m; 8H; —$(CH_2)_4$—), 2.18 (s; 3H; Ar-CH_3), 3.24 (t; ^3J = 7 Hz; 2H; —CH_2-Br), 3.85 (s; 6H; —OCH_3), 3.94 (t; ^3J = 7 Hz; 2H; ArN-CH_2—), 6.68–7.36 (m; 7H; Ar-H); 1-(10-bromodecyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**2k**); greenish oil; 78% yield; $C_{27}H_{36}BrNO_2$ (486.5); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.88–2.10 (m; 16H; —$(CH_2)_8$—), 2.17 (s; 3H; Ar-CH_3), 3.39 (t; ^3J = 7 Hz; 2H; —CH_2-Br), 3.87 (s; 6H; —OCH_3), 3.96 (t; ^3J = 7 Hz; 2H; ArN-CH_2—), 6.74–7.45 (m; 7H; Ar-H).

*Reduction of 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[10-(n-methylcarbamoyl)-decyl]indole (**2i**)*

Under a nitrogen atmosphere, 10.3 mmol of **2i** in 250 ml of dry THF was added slowly to a boiling suspension of 25.8 mmol of $LiAlH_4$ in 150 ml of dry THF. After 1 h, the mixture was cooled to room temperature and stirring was continued overnight. Hydrolysis of the excess of $LiAlH_4$ and the intermediate product was carried out by cautious addition of water. The organic layer was separated from the precipitate by filtration. The solution was concentrated *in vacuo*, and the residue extracted with EtOAc. The organic layer was washed with water and dried over $MgSO_4$. After removal of the solvent, the remaining oil was purified by column chromatography (SiO_2; MeOH/NEt_3, 3:1). Compound **3i**, 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[11-(methylamino)undecyl]indole, was obtained as a yellow oil in 77% yield; $C_{29}H_{42}N_2O_2$ (450.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.82–1.80 (m; 18H; —$(CH_2)_9$—), 2.17 (s; 3H; Ar-CH_3), 2.28–2.70 (m; 2H; —CH_2-N—), 2.40 (s; 3H; —N-CH_3), 3.85 (s; 6H; —OCH_3), 3.92 (t; ^3J = 7 Hz; 2H; ArN-CH_2—), 6.69–7.39 (m; 7H; Ar-H).

*Synthesis of 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[11-(methyl-propylsulfonamido)-undecyl]indole (**4i**)*

n-Propylsulfonylchlorid (34.8 mmol) in 18 ml of dry $CHCl_3$ was added to a solution of 3.48 mmol of **3i** and 10 ml NEt_3 in 30 ml of dry $CHCl_3$. The reaction temperature was kept below 15°C by means of a cooling bath. After stirring for 2 h at room temperature, the mixture was diluted with EtOAc and CH_2Cl_2 and twice washed with sat. $NaHCO_3$ solution. The organic layer was dried ($MgSO_4$) and the solvents removed *in vacuo*. The residue was purified by column chromatography (SiO_2; CH_2Cl_2/EtOAc, 50:1) to give a dark red oil; 86% yield; $C_{32}H_{48}N_2O_4S$ (556.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.75–2.11 (m; 23H; —$(CH_2)_9$—, —CH_2CH_3); 2.20 (s; 3H; Ar-CH_3), 2.63–3.43 (m; 4H; —CH_2-NSO_2-CH_2—), 2.87 (s; 3H; —N-CH_3), 3.91 (s; 6H; —OCH_3), 3.97 (t; ^3J = 7 Hz; 2H; ArN-CH_2—), 6.78–7.47 (m; 7H; Ar-H).

General procedure for the introduction of thiol groups

A solution of 1.72 mmol 1-(ω-bromoalkyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**2j,2k**) and 5.15 mmol thiourea in 25 ml of dry THF was heated for 24 h under reflux. A solution of 34.4 mmol NaOH in 25 ml H_2O was added, and heating was continued for another 3 h. After the solvent had been removed under reduced pressure, the mixture was extracted with CH_2Cl_2. The organic layer was washed with 1 N HCl and water. After drying over $MgSO_4$, the solvent was evaporated and the residue chromatographed (SiO_2; CH_2Cl_2).

The following compounds were synthesized by this method: 1-(6-mercaptohexyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**3j**); light yellow oil; 69% yield; $C_{23}H_{29}NO_2S$ (383.6); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.94–1.78 (m; 4H; —$(CH_2)_4$—), 2.20 (s; 3H; Ar-CH_3), 2.19–2.63 (m; 2H; —CH_2-SH), 3.87 (s; 6H; —OCH_3), 3.98 (t; ^3J = 7 Hz; 2H; ArN-CH_2—), 6.74–7.46 (m; 7H; Ar-H); 1-(10-mercaptodecyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**3k**); greenish oil; 63% yield; $C_{27}H_{37}NO_2S$ (439.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.90–1.86 (m; 16H; —$(CH_2)_8$—), 2.20 (s; 3H; Ar-CH_3), 2.30–2.77 (m; 2H; —CH_2-SH), 3.91 (s; 6H; —OCH_3), 3.99 (t; ^3J = 7 Hz; 2H; ArN-CH_2—), 6.72–7.53 (m; 7H; Ar-H).

*General procedure for the synthesis of thioethers **4j** and **4k)***

Under a nitrogen atmosphere, 2.61 mmol 1-(ω-mercaptoalkyl)-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**3j,k**) in 15 ml of dry DMF were added slowly to a suspension of sodium hydride (3.13 mmol) in 5 ml of dry DMF. After stirring for 30 min, a solution of 2.61 mmol of the corresponding ω-bromalkanoic amide in 10 ml of dry DMF was added slowly. After stirring for 2 h at 50–60°C, a small amount of water was

added cautiously. The mixture was diluted with EtOAc, washed with water and dried ($MgSO_4$). After evaporation of the solvent *in vacuo* the crude product was purified by column chromatography (SiO_2; CH_2Cl_2/EtOAc mixtures).

The following compounds were obtained by this procedure: 1-[6-[3-(butyl-methylcarbamoyl)-propyl-thio]hexyl]-5-methoxy-2-(4-methoxyphenyl)-3-methylindole (**4j**); colorless oil; 93% yield; $C_{32}H_{46}N_2O_3S$ (538.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.69–2.71 (m; 23H; —$(CH_2)_5$-S-$(CH_2)_3$-CON—, —$(CH_2)_2CH_3$), 2.20 (s; 3H; Ar-CH_3), 2.90, 2.94 (s; 3H; —CON-CH_3), 3.09–3.60 (m; 2H; —CON-CH_2—), 3.88 (s; 6H; —OCH_3), 3.98 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 6.76–7.48 (m; 7H; Ar-H); 5-methoxy-2-(4-methoxyphenyl)-3-methyl-1-[10-[(*i*-propyl-methyl carbamoyl)-methylthio]decyl]indole (**4k**); colorless oil; 30% yield; $C_{33}H_{48}N_2O_3S$ (552.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.92–1.85 (m; 22H; —$(CH_2)_8$—, —CON-CH(—C<u>H</u>$_3)_2$), 2.18 (s; 3H; Ar-CH_3), 2.66 (t; ^{3}J = 7Hz; —CH_2-S—), 2.71–3.00 (m; 3H; —CON-CH_3), 3.23, 3.30 (s; 2H; —S-CH_2-CON), 3.65–4.20 (m; 1H; —CON-C<u>H</u>(-$CH_3)_2$), 3.87 (s; 6H; —OCH_3), 3.93 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 6.70–7.40 (m; 7H; Ar-H).

General procedure for the ether cleavage

Under a nitrogen atmosphere, 7.50 mmol of the methoxy derivative **4** in 120 ml dry CH_2Cl_2 was added to 30 mmol of boron tribromide in 120 ml of dry CH_2Cl_2 at −20°C. After stirring for 4h at room temperature, sat. $NaHCO_3$ solution was added slowly with cooling. When the vigorous reaction had ceased the mixture was extracted several times with EtOAc. The organic layer was washed with water and brine, dried ($MgSO_4$) and the solvent removed *in vacuo*. The crude products were purified by column chromatography (SiO_2; CH_2Cl_2/EtOAc mixtures).

The following compounds were synthesized by this method: 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[10-(pentylthio)decyl]indole (**5a**); wax-like solid; 81% yield; $C_{30}H_{43}NO_2S$ (481.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.68–1.90 (m; 25H; —$(CH_2)_8$—, —$(CH_2)_3CH_3$), 2.16 (s; 3H; Ar-CH_3), 2.37–2.70 (m; 4H; S$(CH_2$-$)_2$), 3.97 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 5.22 (s, br; 2H; —OH), 6.69–7.40 (m; 7H; Ar-H); 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[10-(pentylsulfonyl)decyl]indole (**5c**); m.p. ($CHCl_3$) 115–117°C; 64% yield; $C_{30}H_{43}NO_4S$ (513.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.65–2.14 (m; 25H; —$(CH_2)_8$—, —$(CH_2)_3CH_3$), 2.18 (s; 3H; Ar-CH_3), 2.82–3.19 (m; 4H; $SO_2(CH_2$—$)_2$), 3.97 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 4.88 (s, br; 1H; —OH), 6.18 (s, br; 1H; —OH), 6.67–7.41 (m; 7H; Ar-H); 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[12-(propylsulfonyl) dodecyl] indole (**5e**); light yellow resin; 76% yield; $C_{30}H_{43}NO_4S$ (513.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.77–2.12 (m; 25H; —$(CH_2)_{10}$—, —CH_2CH_3), 2.13 (s; 3H; Ar-CH_3), 2.76–3.18 (m; 4H; $SO_2(CH_2$-$)_2$), 3.95 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2-), 6.66–7.40 (m; 7H; Ar-H); 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[9-(4,4,5,5,5-pentafluoropentyl)nonyl]-indole (**5g**); greenish resin; 63% yield; $C_{29}H_{36}F_5NO_4S$ (589.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.71–2.42 (m; 16H; —$(CH_2)_7$—, —CH_2—), 1.99–2.34 (m; 2H; —CH_2-C_2F_5), 2.13 (s; 3H; Ar-CH_3), 2.83–3.28 (m; 4H; $SO_2(CH_2$—$)_2$), 3.97 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 4.97 (s, br; 1H; -OH), 6.10 (s, br; 1H; —OH), 6.70–7.41 (m; 7H; Ar-H); 1-[10-(butylmethylsulfamoyl) decyl]-5-hydroxy-(4-hydroxyphenyl)-3-methylindole (**5h**); greenish resin; 94% yield; $C_{30}H_{44}N_2O_4S$ (528.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.66–2.27 (m; 23H; —$(CH_2)_8$—, —$(CH_2)_2CH_3$), 2.14 (s; 3H; Ar-CH_3), 2.74–3.33 (m; 4H; —CH_2-SO_2N-CH_2—), 2.88 (s; 3H; —SO_2N-CH_3), 3.97 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 5.10 (s, br; 1H; —OH), 6.33 (s, br; 1H; —OH), 6.69–7.40 (m; 7H; Ar-H); 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[11-(N-methyl-propylsulfonamido)undecyl]indole (**5i**); yellow resin; 75% yield; $C_{30}H_{44}N_2O_4S$ (528.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.85–2.22 (m; 23H; —$(CH_2)_9$—, —CH_2CH_3), 2.12 (s; 3H; Ar-CH_3), 2.74–3.30 (m; 4H; —CH_2-NSO_2-CH_2—), 2.85 (s; 3H; —SO_2N-CH_3), 3.91 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 4.85 (s, br; 1H; —OH), 6.09 (s, br; 1H; —OH), 6.59–7.28 (m; 7H; Ar-H); 1-[6-[3-(butyl-methylcarbamoyl)propylthio]hexyl]-5-hydroxy-2-(4-hydroxyphenyl)-3-methylindole (**5j**); light yellow resin; 93% yield; $C_{30}H_{24}N_2O_3S$ (510.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.64–2.77 (m; 23H; —$(CH_2)_5$-S-$(CH_2)_3$-CON—, —$(CH_2)_2CH_3$), 2.14 (s; 3H; Ar-CH_3), 2.98 (s; 3H; —CON-CH_3), 3.11–3.62 (m; 2H; —CON-CH_2—), 3.95 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 5.49 (s, br; 1H; —OH), 6.62–7.34 (m; 7H; Ar-H); 8.49 (s, br; 1H; —OH); 1-[10-[(*i*-propyl-methylcarbamoyl) methylthio]decyl]-5-hydroxy-2-(4-hydroxyphenyl)-3-methylindole (**5k**); light yellow resin; 74% yield; $C_{31}H_{44}N_2O_3S$ (524.8); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.85–2.02 (m; 16H; —$(CH_2)_8$—), 1.12, 1.20 (d; ^{3}J = 7 Hz; 6H; —CON-CH(—C<u>H</u>$_3)_2$), 2.13 (s; 3H; Ar-CH_3), 2.66 (t; ^{3}J = 7Hz; —CH_2-S—), 2.84, 2.91 (s; 3H; —CON-CH_3), 3.33, 3.40 (s; 2H; —S-CH_2-CON—), 3.73–4.22 (m; 1H; —CON-C<u>H</u>(-$CH_3)_2$), 3.95 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 5.72 (s, br; 1H; —OH), 6.64–7.31 (m; 7H; Ar-H), 8.33 (s, br; 1H; —OH); 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[10-(1-methylimidazol-2-yl-thio)decyl]indole (**5l**); colourless foam; 81% yield; $C_{29}H_{37}N_3O_2S$ (491.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.80–1.88 (m; 16H; —$(CH_2)_8$-), 2.14 (s; 3H; Ar-CH_3), 3.03 (t; ^{3}J = 7 Hz; 2H; —CH_2-S-(imidazol-2-yl)), 3.62 (s; 3H; (1-imidazolyl)-CH_3), 3.97 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 6.63–7.30 (m; 7H; Ar-H).

Procedure for the synthesis of 3-methyl-5-(2-tetrahydropyranyloxy)-2-[4-(2-tetrahydropyranyloxy)phenyl]indole (**1b**)

Under a nitrogen atmosphere, a solution of 20.3 mmol of the indole **1a** in 120 ml of dry CH_2Cl_2 was

added slowly to a cooled solution of 122 mmol boron tribromide (−10°C) in 250 ml of dry CH_2Cl_2. The cooling bath was removed and the mixture heated for 5 h to 40°C. Then, it was poured in small portions into 600 ml of ice-cold saturated $NaHCO_3$ solution with vigorous stirring. After addition of 150 ml of EtOAc stirring was continued for 10 min. The product was extracted with EtOAc, the organic layer washed with $NaHCO_3$ solution and water, and dried ($MgSO_4$). The solvent was evaporated *in vacuo*. The residue was dissolved in 90 ml EtOAc, and 26 ml 3,4-dihydro-2H-pyran was added. Under nitrogen, 4.61 ml of EtOAc saturated with hydrogen chloride was added to the mixture and stirring was continued for 1.5 h. After neutralization with $NaHCO_3$-solution the product was extracted with EtOAc. The organic layer was washed with $NaHCO_3$-solution and water, dried ($MgSO_4$) and brought to dryness. The crude product was dissolved in hot CCl_4. Cooling of the solution to 4°C furnished a light yellow, amorphous powder (m.p. 169°C; 58% yield). An analytical sample was crystallized from EtOH/hexane (1:5) to give white crystals (m.p. 175–178°C); $C_{25}H_{29}NO_4$ (407.5); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.39–2.20 (m; 12H; —$(CH_2)_3$—), 2.35 (s; 3H; Ar-CH_3), 3.35–4.29 (m; 4H; —CH_2-O—), 5.30–5.54 (m; 2H; —O-(CH-)-O—), 6.76–7.55 (m; 7H; Ar-H), 7.83 (s, br; 1H; ArN-H).

General procedure for the introduction of side chains carrying a sulfinyl group

The procedure as described under *(i)* was applied with one modification: the amount of sodium hydride was reduced to a stoichiometrical one in order to prevent reduction of the sulfoxide. Crude products were purified by chromatography (SiO_2; EtOAc/MeOH mixtures).

The following compounds were prepared by this method: 3-methyl-1-[10-(pentylsulfinyl)-decyl]-5-(α-tetrahydropyranyloxy)-2-[4-(α-tetrahydropyranyloxy) phenyl]indole (**4b**); greenish oil; 83% yield; $C_{40}H_{59}NO_5S$ (666.0); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.7–2.4 (m; 37H; —$(CH_2)_8$—, —$(CH_2)_3CH_3$, —$(CH_2)_3$—), 2.17 (s; 3H; Ar-CH_3), 2.45–2.82 (m; 4H; $SO(CH_2\text{-})_2$), 3.33–4.24 (m; 4H; —CH_2-O—), 3.92 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 5.29–5.55 (m; 2H; —O-(CH-)-O—), 6.79–7.37 (m; 7H; Ar-H); 3-methyl-1-[12-(propylsulfinyl)dodecyl]-5-(α-tetrahydropyranyloxy)-2-[4-(α-tetrahydropyranyloxy)phenyl]indole (**4d**); brownish oil; 90% yield; $C_{40}H_{59}NO_5S$ (666.0); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.9–2.3 (m; 37H; —$(CH_2)_{10}$—, —CH_2CH_3, —$(CH_2)_3$—), 2.16 (s; 3H; Ar-CH_3), 2.49–2.81 (m; 4H; $SO(CH_2\text{-})_2$), 3.36–4.36 (m; 6H; ArN-CH_2—, —CH_2-O—), 5.32–5.56 (m; 2H; —O-(CH-)-O—), 6.82–7.39 (m; 7H; Ar-H); 3-methyl-1-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]-5-(α-tetrahydropyranyloxy)-2-[4-(α-tetrahydropyranyloxy) phenyl]-indole (**4f**); greenish oil that solidified after some time; 86% yield; $C_{39}H_{52}F_5NO_5S$ (741.9); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.8–2.6 (m; 30H; —$(CH_2)_7$—, —$(CH_2)_2C_2F_5$, —$(CH_2)_3$—), 2.17 (s; 3H; Ar-CH_3), 2.49–2.88 (m; 4H; $SO(CH_2\text{-})_2$), 3.33–4.23 (m; 4H; —CH_2-O—), 3.92 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 5.30–5.54 (m; 2H; —O-(CH-)-O—), 6.79–7.38 (m; 7H; Ar-H).

General procedure for the cleavage of the tetrahydropyranyl ether

After addition of 10 ml of an aqueous oxalic acid solution (8%) the solution of 1.10 mmol indole **4b,4d** or **4f** in 30 ml MeOH was heated to 60–70°C for 3.5 h under a nitrogen atmosphere. After cooling saturated $NaHCO_3$ solution was added, and stirring was continued for 15 min. The product was extracted with EtOAc, the organic layer washed with water and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography (SiO_2; EtOAc/MeOH mixtures).

In this study, the following compounds were synthesized by this method: 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[10-(pentylsulfinyl)decyl]indole (**5b**); greenish resin; 88% yield; $C_{30}H_{43}NO_3S$ (497.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.63–2.24 (m; 25H; —$(CH_2)_8$—, —$(CH_2)_3CH_3$), 2.10 (s; 3H; Ar-CH_3), 2.52–2.95 (m; 4H; $SO(CH_2\text{-})_2$), 3.92 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 5.95 (s, br; 1H; —OH), 6.64–7.30 (m; 7H; Ar-H), 8.47 (s, br; 1H; —OH); 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[12-(propylsulfinyl)dodecyl] indole (**5d**); brownish resin; 83% yield; $C_{30}H_{43}NO_3S$ (497.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.80–2.26 (m; 25H; —$(CH_2)_{10}$—, —CH_2CH_3), 2.12 (s; 3H; Ar-CH_3), 2.52–2.96 (m; 4H; $SO(CH_2\text{-})_2$), 3.95 (t; ^{3}J = 7 Hz; 2H; ArN-CH_2—), 6.67–7.35 (m; 7H; Ar-H); 5-hydroxy-2-(4-hydroxyphenyl)-3-methyl-1-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]indole (**5f**); light yellow resin; 84% yield; $C_{29}H_{36}F_5NO_3S$ (573.7); ^{1}H-NMR ($CDCl_3$): δ (ppm) = 0.84–3.05 (m; 18H; —$(CH_2)_7$—, —$(CH_2)_2C_2F_5$), 2.12 (s; 3H; Ar-CH_3), 2.55–2.97 (m; 4H; $SO(CH_2\text{-})_2$), 3.95 (t; 3J = 7 Hz; 2H; ArN-CH_2—), 6.64–7.36 (m; 7H; Ar-H).

Materials and reagents for bioassays

[^{3}H]17β-estradiol was purchased from New England Nuclear (Dreieich, Germany); all other biochemicals including tamoxifen were obtained from Sigma (Munich, Germany). 4-Hydroxytamoxifen was a gift from Professor P.W. Jungblut, Hannover (Germany). ICI 182,780 was generously provided by Dr M.R. Schneider, Berlin (Germany). The luciferase reporter plasmid EREwtc luc harboring the luciferase gene from *Photinus pyralis* under the control of an estrogen response element (ERE) was synthesized from pGEM/luc (Promega) and EREwtc [20] (generously provided by Dr. Klein-Hitpaß, Essen, Germany) in the authors' laboratory as described [17]. The estrogen receptor expression vector HEG0 [18] was generously provided by Professor P. Chambon, Strasbourg. HBS buffer was

prepared from a solution of 32.0 g NaCl, 1.48 g KCl, 0.5 g $Na_2HPO_4 \cdot 2H_2O$, 4.0 g D-(+)-glucose, 20.0 g HEPES in 200 ml of water, adjusted to pH 7.5 with NaOH, which was diluted by a factor of 10 and adjusted to pH 7.08.

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} to 10^{-5} M competing ligand (in 100 μl buffer), 100 μl of calf uterine cytosol, and buffer. The mixture was incubated for 18 h at 4°C, after which 0.5 ml of dextran-coated charcoal (DCC) slurry (0.8% charcoal Norit A and 0.008% dextran in buffer) was added to the tubes, and the contents were mixed. The tubes were incubated for 90 min at 4°C and then centrifuged at 700 x ***g*** 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.

Luciferase assay in cotransfected HeLa cells

HeLa cells were grown in DMEM supplemented with 10% FCS, 100 U penicilline, 100 μg streptomycine, and 150 mg L-glutamine in 500 ml of medium without phenol red. One week before the start of the experiment, the medium was replaced by one containing DCC-treated FCS (ctFCS). Shortly before confluence, the medium was removed and cells were washed with 10 ml of PBS. Cells were gently shaken for a few seconds with trypsin-EDTA solution (4 ml) and after removal of the solution incubated for 2 min at 37°C. After addition of 10 ml of medium, the cell suspension (0.5 ml per well) was transferred to 6-well plates containing 2 ml of medium. Cells were grown until the density of the monolayer was about 50% (1–2 days) followed by addition of 2 μg of the reporter plasmid EREwtc luc and 0.01 μg of the estrogen receptor expression vector HEG0 per well. For a successful transfection it is necessary to generate a very fine precipitate of the DNA by subsequent dilution with 45% water, 5% 2.5 M $CaCl_2$, and 50% HBS buffer and continuous shaking. After 20 min at room temperature an opalescent solution should be obtained.

After addition of the DNA solution, medium was removed and cells were washed with 2 ml of PBS, followed by treatment with glycerol (15% in PBS) for 2 min. After washing with PBS, fresh medium containing the test substances was added. The maximum of luciferase expression was reached 18 h after addition of the transfection solution. At that time, medium was removed and cells were washed with PBS. Cell lysis and quantification of luminescence was performed according to the procedure described in luciferase assay system E1500 of Promega (Serva, Heidelberg, Germany). Luminescence was measured in a luminometer Lumat LB 9501 (Berthold, Wildbad, Germany) as relative light units (RLU) which were converted into fg luciferase by a calibration curve. Protein concentration was determined by the Bradford method [22].

Determination of cytostatic activity in MCF-7 human breast cancer cells

Hormone-sensitive human MCF-7 breast cancer cells were obtained from American Type Culture Collection (ATCC, Rockville, MD, U.S.A.). Cells were grown in improved Minimal Essential Medium (MEM), as modified by Richter *et al.* [23] (Biochrom, Berlin, FRG), supplemented with glutamine (0.3 g/l), gentamycin (60 mg/l) and 10% DCC-treated newborn calf serum (ctNCS) (Gibco). The serum was sterilized through a 0.20 μm filter (Sartorius, Göttingen, Germany) and stored at – 20°C. Cells were grown in a humidified incubator in 5% CO_2 at 37°C and harvested with 0.05% trypsin – 0.02% EDTA in 0.15 M NaCl. At the start of the experiment, the cell suspension was transferred to 96-well microplates (100 μl/well). After growing them for 3 days in a humidified incubator with 5% CO_2 at 37°C, medium was replaced by one containing the test compounds. Control wells (16/plate) contained 0.1% of DMF that was used for the preparation of the stock solution. The initial cell density was determined by addition of glutaric dialdehyde (1% in PBS; 100 μl/well). After incubation for 4–7 days, medium was removed and 100 μl of glutaric aldehyde in PBS (1%) were added for fixation. After 15 min, the solution of aldehyde was decanted. Cells were stained by treating them for 25 min with 100 μl of an aqueous solution of crystal violet (0.02%). After decanting, cells were washed several times with water to remove adherent dye. After addition of 100 μl of ethanol (70%), plates were gently shaken for 2 h. Optical density of each well was measured in a microplate autoreader EL 309 (Bio-tek) at 578 nm.

Mice uterine weight tests

Immature female mice (20 days old, of the NMRI strain) from Charles River Wiga, Sulzfeld, Germany, were randomly divided into groups of six to ten animals. To determine estrogenic activity, compounds were dissolved in olive oil (100 μl/animal) and injected subcutaneously on three consecutive days. Control animals received the vehicle alone. Twenty-four hours after the last injection, the animals were killed by

cervical dislocation and weighed. Uteri were dissected free of fat and fixed in Bouin solution (saturated aqueous picric acid—40% formaldehyde—glacial acetic acid 15:5:1 by vol.) for 2 h. Uteri were freed from connective tissue, washed with ethanol, dried at 100°C for 18 h, and weighed. The relative uterus weight was calculated by the formula: uterine dry weight (mg)/body weight (g), multiplied by 100.

To determine the antiestrogenic activity, injections contained a standard dose (0.4 μg) of estrone and increasing doses of the compounds. The inhibition (%) of the estrone-stimulated uterine growth was estimated by the formula: $100 - [(W_{S,T} - W_V)/(W_S - W_V) \times 100]$ ($W_{S,T}$ = rel. uterus weight of animals treated with estrone standard (0.4 μg) + test compound; W_V = rel. uterus weight of control animals; W_S = rel. uterus weight of animals treated with estrone standard).

Transplanted MXT-mammary tumors of the mouse

The MXT-M 3.2 mammary tumors were generously provided by Dr A. E. Bogden, EG and G Mason Research Institute, Worcester, MA, U.S.A. Hormone-sensitive tumors grew for 4–5 weeks in the host animals before transplantation. Tumor pieces of 1 mm^2 were serially transplanted into 8- to 10-week-old femal $B_6D_2F_1$-mice, obtained from Charles River Wiga (Sulzfeld, Germany). Animals were assigned randomly in groups of ten and treatment was started 24 h after transplantation. Drugs were dissolved in olive oil and administered subcutaneously on Monday, Wednesday and Friday. After a 5-week period of treatment, animals were killed and autopsied. Tumors were removed and weighed. The uterine dry weight was determined as described above. The change of body weight between start and end of therapy was recorded in order to detect obvious toxicity. Significance of differences was determined by the U-test according to Wilcoxon, modified by Mann and Whitney.

RESULTS

Estrogen receptor binding affinity

All new derivatives were first tested for their ability to bind to the estrogen receptor. As in previous studies, we used the calf uterine cytosol as a convenient source of estrogen receptors. The RBA values obtained with calf estrogen receptors are generally somewhat lower than those from other species. Therefore, various reference compounds were included for comparison. The RBA values of the new compounds ranged from 0.3 to 4.7 (Table 1). The rather low value for the sulfide **5a** can be rationalized by the low polarity of the functional group which is only a very weak hydrogen bridge acceptor. The comparison of the 2-phenylindole based sulfoxides (**5b, 5d, 5f**) with the steroidal sulfoxide ICI 182,780 revealed somewhat lower binding affinities for the nonsteroidal carrier. The difference between sulfoxide and sulfone was variable and not very pronounced. The compounds with an amide function showed binding affinities slightly lower than the sulfoxides, an observation which was also made with 7α-substituted estradiol derivatives [11].

Transcription activation

Figure 3 summarizes the effects of various 2-phenylindole derivatives on the estrogen receptor mediated transcription of the luciferase gene in HeLa cells. All compounds were applied in a concentration of 10^{-6} M except 17β-estradiol (10^{-8} M) which served as reference agonist. All values are based on that of

Table 1. Binding affinities of 1-substituted 5- hydroxy-2-(4-hydroxyphenyl)-3-methylindoles and reference compounds for the estrogen receptor

Compound	R*	RBA†
5a	$-(CH_2)_{10}-S-C_5H_{11}$	0.27
5b	$-(CH_2)_{10}-SO-C_5H_{11}$	3.4
5c	(ZK 164,015)$-(CH_2)_{10}-SO_2-C_5H_{11}$	4.7
5d	$-(CH_2)_{12}-SO-C_3H_7$	2.9
5e	$-(CH_2)_{12}-SO_2-C_3H_7$	1.9
5f	$-(CH_2)_9-SO-(CH_2)_3-C_2F_5$	2.4
5g	$-(CH_2)_9-SO_2-(CH_2)_3-C_2F_5$	1.4
5h	$-(CH_2)_{10}-SO_2-N(CH_3)-C_4H_9$	2.2
5i	$-(CH_2)_{11}-N(CH_3)-SO_2-C_3H_7$	1.8
5j	$-(CH_2)_6-S-(CH_2)_3-CO-N(CH_3)-C_4H_9$	1.6
5k	$-(CH_2)_{10}-S-CH_2-CO-N(CH_3)-CH(CH_3)_2$	1.7
5l	$-(CH_2)_{10}-S-$[(1-methyl)-imidazol-2-yl]	1.7
Tamoxifen		0.36
4-Hydroxytamoxifen		6.8
ICI 182,780		6.2
ZK 169,978		2.1

*R refers to the general structure in Fig. 1.

†Relative binding affinities for the calf uterine estrogen receptor; value for ß-estradiol = 100.

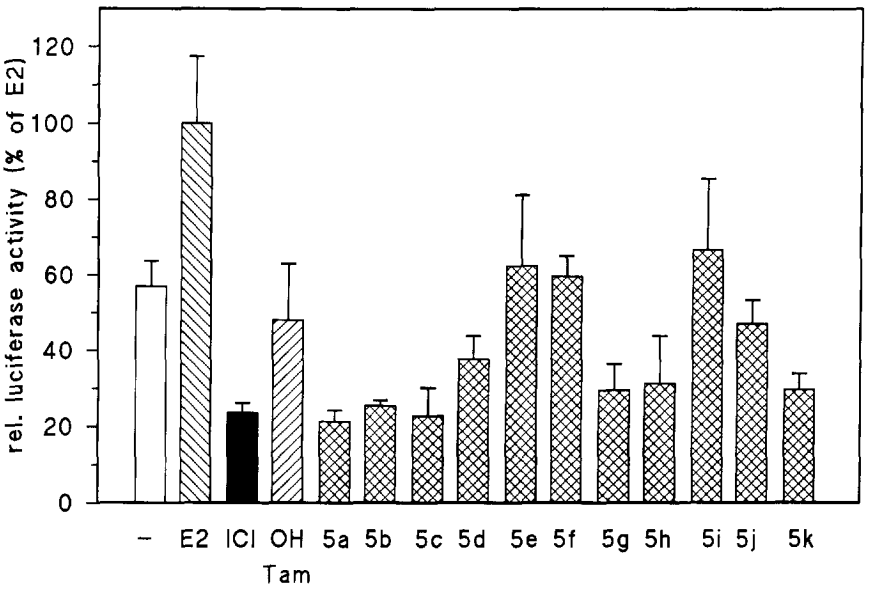

Fig. 3. Luciferase expression in HeLa cells cotransfected with the reporter plasmid EREwtc luc and the estrogen receptor expression vector HEG0, and treated with 17β-estradiol (E2, 10 nM), ICI 182,780 (ICI, 1 μM), 4-hydroxytamoxifen (OHTam, 1 μM), and 2-phenylindole derivatives 5a-k (1 μM). Values are means of three independent experiments ± SD.

estradiol which was set 100%. The control values for untreated cells were consistently higher than that for the reference drug ICI 182,780. The reason is probably the presence of growth factors in the serum which were not completely be removed by DCC-treatment. The activation of ER-mediated transcription by a variety of peptides has been reported [24–26]. The lowest luciferase levels were recorded for the steroid ICI 182,780, for 2-phenylindole derivatives in which the sulfur in all three different oxidation states was linked to the indole by a a decamethylene spacer group, and for the sulfon **5g**. Some of the derivatives showed an increase of luciferase activity to values close to that of 4-hydroxytamoxifen. The increased luciferase expression observed for the compound **5f** was unexpected because it carries the same side chain as ICI 182,780.

Effect on the growth of human MCF-7 breast cancer cells

All compounds of this study strongly inhibited cellular growth in a dose-dependent manner (Figs 6 and 7). The IC_{50}-values of the most active derivatives **5b**, **5c**, **5f**, and **5h** were in the nanomolar range. Since the inhibitory effects of sulfoxides and sulfones did not differ much data for **5d** and **5g** were not shown. Interestingly, the sulfide **5a** was also very active despite its low binding affinity for the estrogen receptor. Possibly an interconversion of various oxidation states of the sulfur can take place under the conditions of this assay. With exception of **5h**, all derivatives with an additional nitrogen function in the side chain were less active than compounds furnished solely with a sulfur function. The cytostatic activities of most of these compounds were higher than that of 4-hydroxytamoxifen. The most potent 2-phenylindole derivatives showed inhibitory effects close to that of the steroidal antiestrogen ICI 182,780.

Endocrine activity in mice

Based on the data from the transcription assays we only submitted a limited number of these compounds (**5a**, **5c**, **5f**, **5j**) to the mouse uterine weight test. As expected, the derivatives **5a** and **5c** that were devoid of stimulatory activity in the luciferase assay displayed no estrogenic activity in immature mice and suppressed the estrone-stimulated uterine growth completely (Fig. 4). The same observation was made with the derivative **5f** which carries the same side chain as ICI 182,780 (Fig. 5). The forth compound **5j** was completely inactive in this test model. A possible explanation could be poor resorption or rapid metabolic deactivation.

Antitumor activity in mice bearing mammary tumors

The growth of transplanted mouse MXT mammary tumors is hormone-dependent and is strongly inhibited by tamoxifen in early passages (Fig. 8, left hand side). After several passages the hormone-dependency is gradually lost. Only two of the 2-phenylindole derivatives inhibited the growth of these tumors significantly when adminstered in a subcutaneous 30 mg/kg dose three times per week. The steroidal antiestrogen ICI 182,780 was inactive. The 2-phenylindole **5f** with the same side chain was given in two schedules, the normal one and a bolus injection (250 mg/kg) at the beginning of the experiment. The reason for the administration of a high single dose was the adaption to the phase II clinical trial in which ICI 182,780 was given as intramuscular injection every four

weeks [7]. The latter schedule increased the inhibitory effect although it did not turn significant.

DISCUSSION

The major objective of this paper was the improvement of the potency of non-steroidal antiestrogens with a 2-phenylindole-based structure. Several new 2-phenylindole derivatives with amide functions linked by a long alkyl spacer to the heterocycle have recently been identified as estrogen antagonists without residual estrogenic activity [11]. Preliminary *in vivo* data on antitumor activity showed that their potency might not be sufficient for application as drugs for treatment of mammary carcinomas and other estrogen-related diseases. Based on findings by Wakeling and collegues we replaced the amide function by sulfur in various oxidation states. Other modifications of the side chain comprised the introduction of a sulfonamide group in two different orientations and the replacement of one of the methylene groups in the previously described amides by a sulfur atom. All of these new 2-phenylindole derivatives bind to the calf uterine estrogen receptor with RBA values comparable to those of estradiol derivatives with functional side chains in 7α position.

Since the lack of estrogenic activity is a prerequisite for our further investigations we established a rapid and convenient *in vitro*-system for the determination of estrogenic effects [11, 16, 27]. HeLa cells which do not express the estrogen receptor where cotransfected with the expression vector (HEG0) for the human estrogen receptor and a reporter plasmid. This plasmid (EREwtc luc) contains the cognate element for the estrogen receptor (ERE) and the luciferase as reporting enzyme. Agonistic activity can easily be quantified by measuring the luciferase catalyzed emission of light.

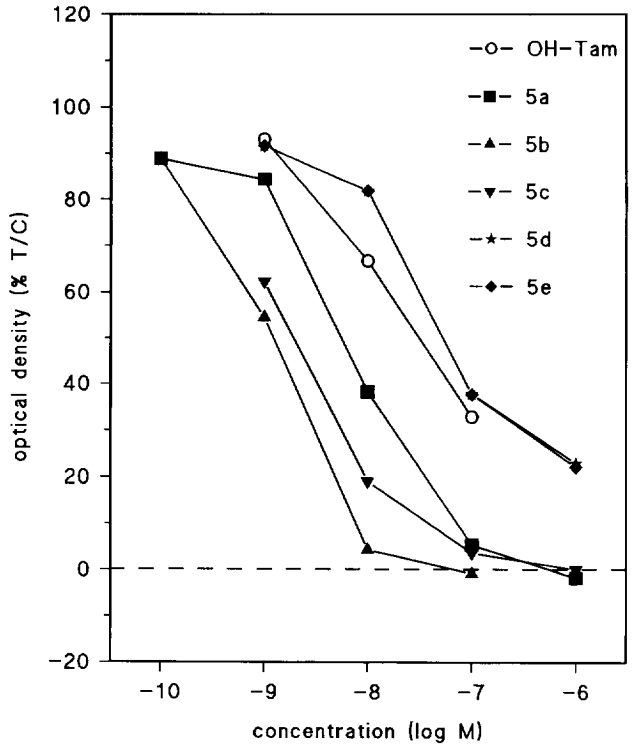

Fig. 4. Effect of 2-phenylindoles 5a, 5b, 5c, 5e, and 4-hydroxytamoxifen (OH-Tam) on the growth of hormone-sensitive human MCF-7 breast cancer cells, shown as optical densities following crystal violet staining of viable cells. Values are means of 16 replicates; SD are in the range of 10–20%.

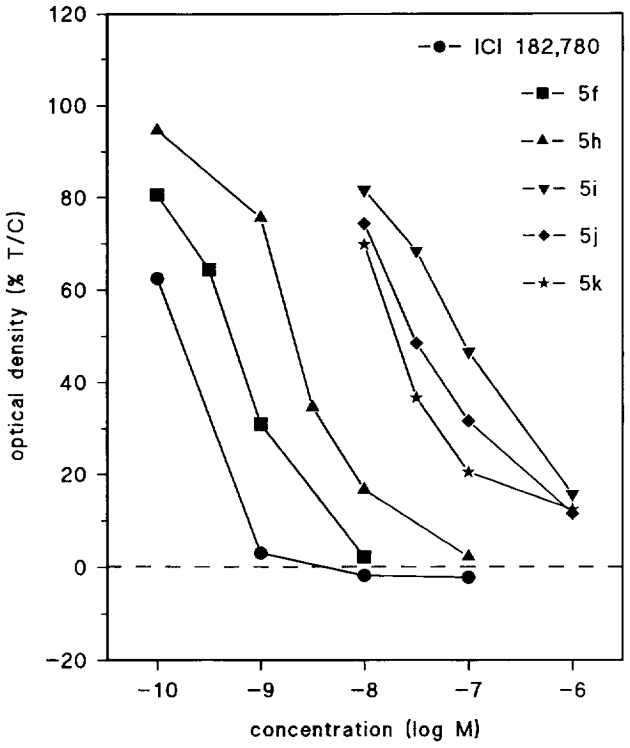

Fig. 5. Estrogenic and antiestrogenic activity of 5f (upper panel) and 5j (for details see Fig. 4).

For the quantification of the activating effects, the value of the pure antiestrogen ICI 182,780 has to be considered as base line rather than the untreated controls which are very variable depending on the batches of charcoal-treated serum used. Under this assumption, most of the compounds studied stimulated luciferase expression with a maximum effect of about 50% of that of estradiol. Derivatives with a sulfur function linked by 10 methylene groups to the heterocycle and a terminal pentyl group (**5a, 5b, 5c,**) were devoid of agonistic activity independently from the oxidation state of the sulfur. It appears that the $-(CH_2)_{10}-SO_{11}-C_5H_{11}$ element prevents the estrogen receptor from activation.

This and related studies should provide a basis for the rational design of new antiestrogens as drugs for the therapy of estrogen-dependent malignancies such as breast cancer. A widely used *in vitro*-model for the evaluation of cytostatics acting via the estrogen receptor are human MCF-7 breast cancer cells. Highest activity was observed for the derivatives that contained the $-(CH_2)_{10}-SO_{(2)}$ element (**5b, 5c, 5f, 5h**) which also proved to be the most potent antiestrogens *in vivo*. The

IC_{50}-values of these compounds were below 10^{-8} molar. It can be assumed that the antitumor activity in these cells is receptor mediated because the growth of ER-negative cells such as MDA-MB 231 breast cancer cells is not inhibited by micromolar concentrations (data not shown). Since the binding affinities of theses compounds vary only little with one exception (thioether **5a**), their influence on growth inhibition could not be evaluated. Binding to receptor is prerequisitive for specific cytostatic activity in MCF-7 cells, but structural elements of the side chain modulate the activity of these compounds. This is in agreement with results from 7α-substituted estradiol derivatives [27].

The results from the transfection assays have demonstrated that the regulation of transcription is a very sensitive instrument for monitoring the influence of small alterations in the side chain structure of ligands bound to the estrogen receptor. In a previous study [11] we have shown that the data from this assay are in good agreement with results from *in vivo*-experiments in a series of indole derivatives with an amide function. Similar results were obtained with compounds of this study, e.g. **5a** and **5c** which can be considered pure antiestrogens in mice. However, some exceptions were noted for compounds that stimulated luciferase expression. Derivative **5f** with the same side chain as ICI 182,780 also was—not unexpectedly—a pure antagonist and **5j** was completely inactive *in vivo*, possibly due to low bioavailability.

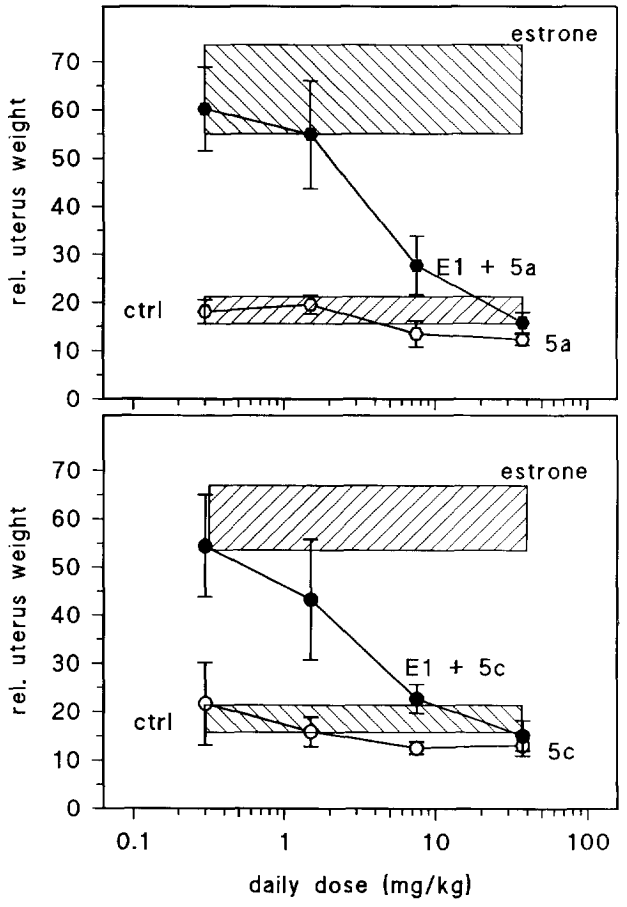

Fig. 6. Estrogenic and antiestrogenic activity of 5a (upper panel) and 5c in the mouse uterine weight test. Animals were injected daily with the drug alone (open symbols) or together with a standard dose of 0.4 μg estrone/animal (closed symbols) for three days. Rel. uterus weight (= uterus dry weight (mg)/body weight (g) ×100) was determined 24 h after the last injection; mean of 6 animals ± SD. Hatched boxes indicate values for control and estrone-treated animals ± SD.

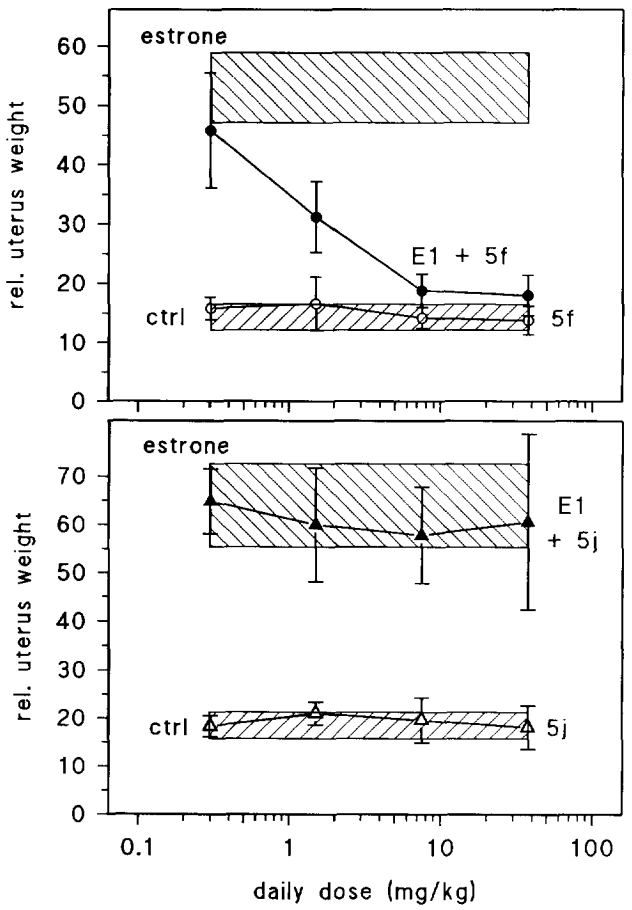

Fig. 7. Effect of 2-phenylindoles 5f, 5h-k, and ICI 182,780 on the growth of hormone-sensitive human MCF-7 breast cancer cells, shown as optical densities following crystal violet staining of viable cells. Values are means of 16 replicates; SD are in the range of 10–20%.

For determination of antitumor activity *in vivo* we used hormone-dependent MXT mammary tumors implanted into female BDF-1 mice, a widely used model for studying the antineoplastic effects of hormonally active compounds and cytotoxic receptor ligands [28–30]. The hormone-sensitivity of these tumors was proved by their response to tamoxifen and to ovariectomy. From a theoretical point of view complete receptor blockade by a pure antiestrogen should exert the same effect as ovariectomy. However, when these antiestrogens were administered to the animals in rather high doses (3 × 30 mg/kg bodyweight per week) only a minor often non-significant inhibition of tumor growth was observed. This applies for the 2-phenylindole derivatives as well as for the steroid ICI

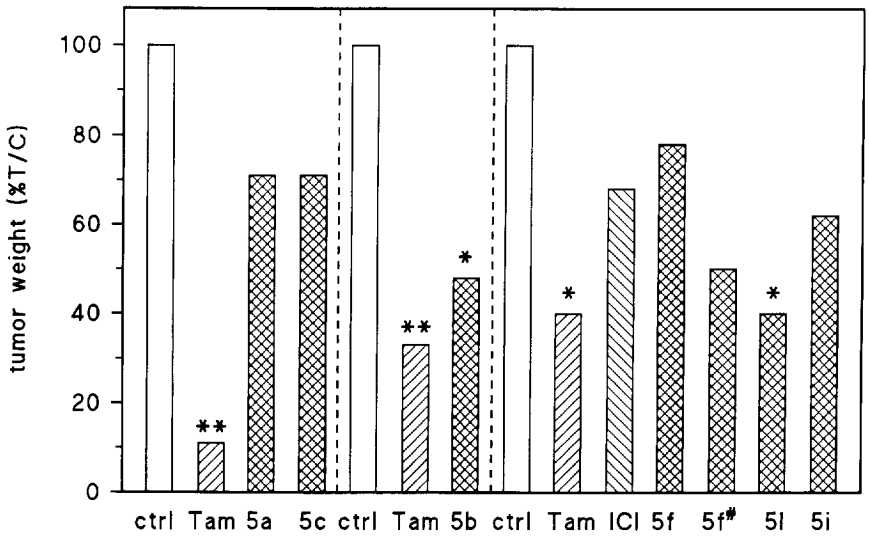

Fig. 8. Inhibitory effect of various 2-phenylindole derivatives, tamoxifen (Tam), and ICI 182,780 on the growth of transplanted, hormone-dependent MXT mouse mammary tumors. Six animals per group were treated for 6 weeks with 3 × 30 mg/kg body weight per week except for tamoxifen (3 × 8.8 mg/kg) and 5f#, which was given as a 250 mg/kg bolus injection at the beginning of the experiment. Treatment was started 1 day after transplantion. After 6 weeks, animals were killed, tumors removed and weighed. Significant differences between control and treated animals are indicated by one ($p < 0.05$) or two ($p < 0.01$) asterisks.

182,780. The reason for this unsatisfying response is unclear because the uteri respond very well to these antiestrogens. The main difference between these two different *in vivo*-experiments is the age of the animals which are immature in the uterine weight test and adult in the tumor assay. In the first case, both agonist and antagonist were given simultaneously, whereas in the latter case, the antagonist, given every second day, has to compete with the continuously produced sex hormones. This raises the question whether the MXT mammary tumor which simulates the premenopausal situation is the adequate model. Maybe, a model for postmenopausal breast cancer as it is used for the evaluation of aromatase inhibitors [31, 32] would be more appropriate for these compounds than the MXT tumor.

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