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Synthesis and biological evaluation of stilbene-based pure estrogen antagonists

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Abstract—Replacement of one of the ethyl substituents in diethylstilbestrol by side chains with functional groups converted this potent estrogen into pure antiestrogens with the potential for the treatment of breast cancer. These agents completely suppressed estrogen receptor-mediated gene activation and inhibited the growth of estrogen-sensitive MCF-7 breast cancer cells in submicromolar concentrations. The most potent derivative displayed similar activity as fulvestrant (ICI 182,780) in vitro and in the mouse uterine weight test. Obviously, the stilbene structure can act as a substitute for estradiol in the development of pure estrogen antagonists.

Breast cancer is the most important malignancy among women. In postmenopausal patients, the majority of these tumors are estrogen-dependent and respond to endocrine therapies such as the administration of estrogen antagonists, as demonstrated by the successful application of tamoxifen. Since many patients relapse under the treatment with tamoxifen as the result of the partial agonist character of this drug, the use of pure antiestrogens might be an effective alternative.[1] In previous studies[2,3] we have shown that the introduction of alkyl side chains with functional elements such as amino or sulfone groups can completely abrogate agonist activity of nonsteroidal ligands of the estrogen receptor. These agents block ER-mediated gene activation and inhibit the growth of estrogen-sensitive human MCF-7 breast cancer cells in vitro. In vivo they also display antiestrogenic activity but the potencies are less pronounced than those found for partial antagonists of the SERM type. In this respect, the nonsteroidal pure antiestrogens resemble fulvestrant[4] (Fig. 1), a steroidal antagonist without residual estrogenic activity. For therapeutic purposes, it cannot be given orally but has to be administered to patients by intramuscular injections.[4] The reason for the low bioavailability has not been well understood and might be due to the structure of the side chain. Our previous studies with 2-phenylindole-based pure antiestrogens revealed good in vitro effects but rather low activity in vivo, especially when these compounds were dosed orally. These results can be taken as a hint that the structure of the carrier molecule also plays a role in bioactivity. Therefore, we searched for different basic structures with improved activity in vivo.

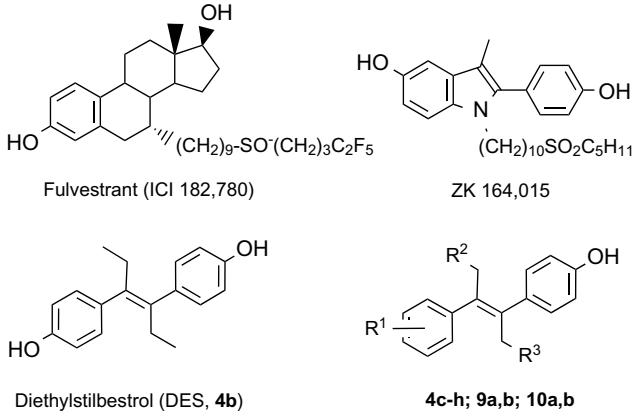

Figure 1. Structures of the stilbene-based antiestrogens and reference drugs.

Diethylstilbestrol (DES, Fig. 1) is a drug, which is as potent as 17β-estradiol (E2) both in vitro and in vivo. Its use in women, however, has become obsolete after it had been associated with transplacental carcinogenesis leading to clear-cell adenocarcinoma of the vagina and

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cervix in daughters of women receiving DES during pregnancy.[5] The good pharmacokinetic profile and high binding affinity for the estrogen receptor (ER) make DES attractive as the starting point for the development of antiestrogens for clinical use. The main question we wanted to answer in this study was whether it is possible to convert this potent estrogen into an estrogen antagonist devoid of any agonist activity. To achieve this goal we replaced one of the ethyl groups by long alkyl chains that incorporate functional groups. Previous studies have shown that sulfur functions in a distance of 10 carbon atoms from the core of the molecule are appropriate for this purpose.[2]

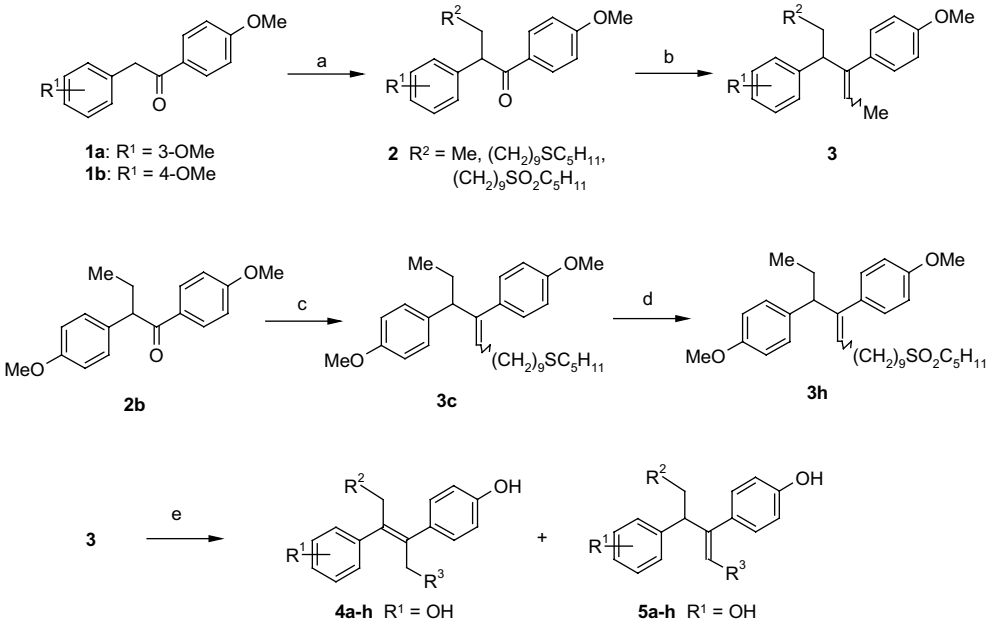
The synthesis of the new DES derivatives started from the corresponding desoxyanisoins **1** (Scheme 1). The first side chain was introduced by deprotonation and subsequent reaction with ethyl bromide or the bromo alkane with the respective sulfur function to give the ketones **2**. The second substituent was introduced by a Grignard reaction, which led to the formation of a double bond with orientation towards the side chain (**3**). Since the acidity of the sulfone prevented its direct use as the Grignard reagent, the thio ether function had to be oxidized with *m*-CPBA after the Grignard reaction to give **3h**. Cleavage of the methoxy groups in **3** led to mixtures of the stereoisomeric phenols **4** and **5** with a preference for the stilbene structure **4**. For the preparation of DES derivatives **9** and **10** with side chains containing an additional methylamino group the synthesis had to be modified (Scheme 2): First the desoxyanisoin **1b** was reacted with ethyl 6-bromohexanoate to give **6**, followed by the Grignard reaction with EtMgBr to afford the ester **7**, which was then reacted with the appropriate amine to give **8**. Deprotection with BBr_3 resulted in double bond migration from a styrene-like to stilbene-like position. In the last step, the phenolic stilbenes **9** were reduced with $LiAlH_4$ to yield the amines **10**.

All of the stilbenes were obtained as *E*/*Z* mixtures with the *E*-stereoisomer as the dominant product. HPLC studies revealed a ratio of *E*-**4** to *Z*-**4** of approximately 85:15. When the stereoisomers had been separated by HPLC they rapidly isomerized to give the original ratio of isomers. Thus, no attempt was made to study the stereoisomers separately.

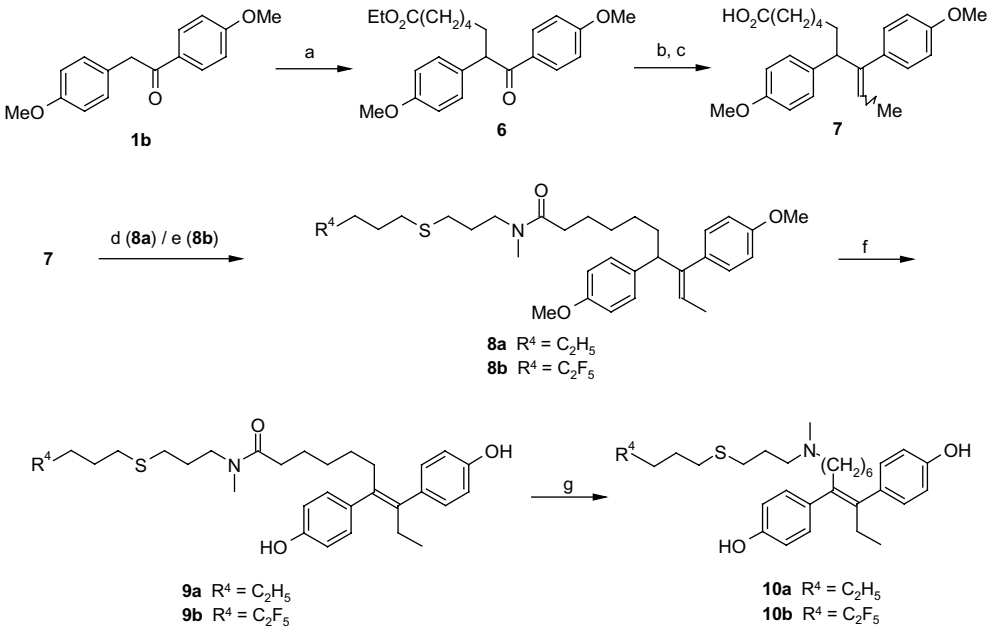
The first step in the biological characterization of the new stilbene derivatives was the determination of the binding affinities for the ER. Calf uterine cytosol was used as receptor source as described previously.[6] The introduction of long side chains reduced the affinity considerably (Table 1). A similar observation was made with steroids, for example, fulvestrant.[7] The data showed that the affinity is mainly dependent on the type of side chain used. The most favorable conditions are provided by the bifunctional side chains as demonstrated by derivatives **9** and **10**. Their values are close to those found for fulvestrant and the indole derivative ZK 164,015.

Estrogenic and antiestrogenic activities were determined in vitro[6] using ER+ human MCF-7/2a cells[8] stably transfected with a luciferase reporter gene under the control of an ERE. Since pure antiestrogens should be devoid of any agonist action by definition, all new stilbene derivatives were evaluated for agonism. At a concentration of 10^{-6}M they exhibited values for luciferase activity below that of control cells grown in steroid depleted medium (Fig. 2). Luciferase activities below baseline are characteristic for pure antiestrogens and indicate the blockade of ligand-independent activation of the ER, responsible for the basal luciferase activity in control cells.[8]

Antiestrogenic activity was determined in a similar assay by simultaneous treatment of the cells with

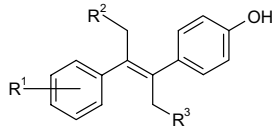


Scheme 1. Reagents and conditions: (a) 1. NaH, DMF, 0 °C; 2. R^2CH_2Br, rt, 2 h, 62–86%; (b) EtMgBr, Et_2O, reflux, 2 h, 54–76%; (c) $H_{11}C_5S(CH_2)_{10}MgBr$, Et_2O, reflux, 2 h, 33%; (d) *m*-CPBA, $CHCl_3$, rt, 2 h, 76%; (e) BBr_3, CH_2Cl_2, −5 °C–rt, 7 h, 52–95%.



Scheme 2. Reagents and conditions: (a) 1. NaH, DMF, 0 °C; 2. $Br(CH_2)_5CO_2Et$, rt, 2 h, 79%; (b) EtMgBr, Et_2O/THF, reflux, 2 h, 72%; (c) KOH, H_2O, EtOH, reflux, 4 h, 95%; (d) 1. PCl_5, 60 °C, 1 h; 2. $MeNH(CH_2)_3SC_5H_{11}$, NaOH, 79%; (e) $MeNH(CH_2)_3S(CH_2)_3C_2F_5$, DCC, CH_2Cl_2, rt, 4 h, 73%; (f) BBr_3, CH_2Cl_2, −5 °C–rt, 7 h, 80–95%; (g) $LiAlH_4$, THF, reflux, 1 h, 21–24%.

Table 1. Relative binding affinities of stilbene-based antiestrogens and reference drugs for the estrogen receptor

Compd	R^1	R^2	R^3	RBA[a]
				
17β-estradiol (E2)				100
4a[b]	3-OH	Me	Me	37
4b (DES)	4-OH	Me	Me	72
4c	3-OH	$–(CH_2)_9–S–C_5H_{11}$	Me	0.03
4d[b]	4-OH	$–(CH_2)_9–S–C_5H_{11}$	Me	0.11
4e[b]	3-OH	Me	$–(CH_2)_9–S–C_5H_{11}$	0.07
4f[b]	3-OH	$–(CH_2)_9–SO_2–C_5H_{11}$	Me	0.87
4g[b]	4-OH	$–(CH_2)_9–SO_2–C_5H_{11}$	Me	2.4
4h[b]	3-OH	Me	$–(CH_2)_9–SO_2–C_5H_{11}$	1.7
9a[b]	4-OH	$(CH_2)_4CONMe–(CH_2)_3SC_5H_{11}$	Me	2.9
9b[b]	4-OH	$(CH_2)_4CONMe–(CH_2)_3S(CH_2)_3C_2F_5$	Me	1.9
10a[b]	4-OH	$(CH_2)_5NMe–(CH_2)_3SC_5H_{11}$	Me	8.6
10b[b]	4-OH	$(CH_2)_5NMe–(CH_2)_3S(CH_2)_3C_2F_5$	Me	2.2
Fulvestrant				5.2
ZK 164,015				5.0

[a] Relative binding affinities for the calf uterine estrogen receptor, determined by incubation at 4 °C for 20 h.
[b] Mixture of *E*- and *Z*-isomers in dynamic equilibrium.

1 nM E2 and the respective antiestrogen in various concentrations. All DES derivatives inhibited E2-stimulated luciferase expression in submicromolar concentrations (Table 2). The lowest IC_{50} values were recorded for the amines **10a** and **10b** (10 and 11 nM) and were close to the value for fulvestrant (5 nM).

Since the envisaged application of these agents is the treatment of hormone-dependent breast cancer, their antiproliferative activities were evaluated using wild-type MCF-7 breast cancer cells and for comparison ER- human MDA-MB 231 mammary carcinoma cells. The growth of hormone-independent cells was not affected by the DES derivatives up to 1 μM. At 10 μM,

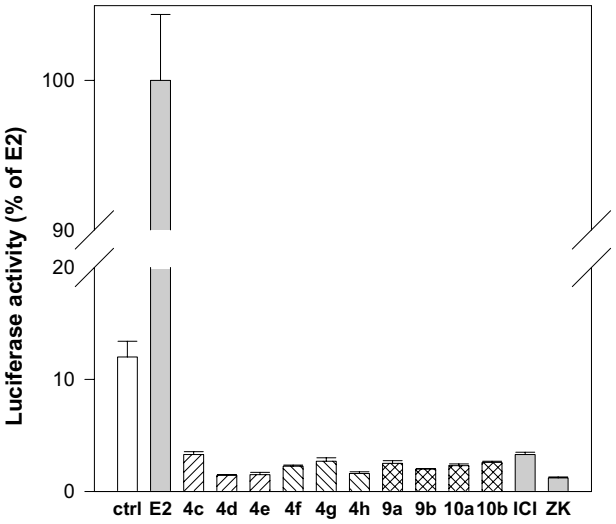


Figure 2. Estrogenic activity in MCF-7/2a breast cancer cells. Concentrations were 1 μM except for E2 (1 nM). ICI = fulvestrant, ZK = ZK 164,015. Note the breaks in the *y*-axis.

Table 2. Antiestrogenic and antiproliferative activities in E2-stimulated breast cancer cells

Compd	Antiestrogenic activity[a] IC_{50} (μM)	Antiproliferative activity[b] IC_{50} (μM)
4c	1.2	2.1
4d	0.79	1.4
4e	0.20	0.59
4f	0.37	0.27
4g	0.13	0.10
4h	0.050	0.11
9a	0.60	1.9
9b	0.31	0.7
10a	0.010	0.010
10b	0.011	0.007
Fulvestrant	0.005	0.004
ZK 164,015	0.025	0.051

[a] Inhibition of luciferase activity in ER+ MCF-7/2a breast cancer cells, transfected with the EREwtc luc plasmid and stimulated by E2 (10^{-9} M).

[b] Growth inhibition of wild-type MCF-7 breast cancer cells stimulated by E2 (10^{-9} M).

however, both the sulfones and the amines inhibited the growth of these cells (Table 3), a property, which they share with the parent drug DES and which appears to be the result of receptor-independent cytotoxicity.

When these compounds were tested in estrogen-sensitive MCF-7 cells under the same conditions a strong inhibitory effect was observed with IC_{50} values in the nanomolar range (Table 3). The differences in activity—more than three orders of magnitude—clearly show that all of these compounds act via the ER. In order to compare the antiproliferative activities of the new DES derivatives with the antiestrogenic effects measured in transfected MCF-7/2a cells, wild-type MCF-7 cells were treated with the test compounds in the presence of 1 nM E2 (Table 2). The addition of E2 led to an average increase of the IC_{50} values by a factor of 20. The results of this assay are in agreement with the data obtained in

Table 3. Antiproliferative activity of stilbene-based antiestrogens

Compd	MDA-MB 231 cells[a]		MCF-7 cells[b]
	% T/C at 5 μM	% T/C at 10 μM	IC_{50} (nM)
4f	96 ± 3	−7 ± 1[c]	49
4g	94 ± 7	−4 ± 1[c]	5.1
4h	90 ± 8	32 ± 8[c]	6.1
10a	75 ± 6[c]	−25 ± 1[c]	0.75
10b	−15 ± 1[c]	−15 ± 1[c]	0.22
Fulvestrant	93 ± 6	95 ± 6	0.21
ZK 164.015	84 ± 5[c]	−2 ± 2[c]	1.4

[a] Growth inhibition of ER- MDA-MB 231 breast cancer cells, incubated for 96 h.

[b] Growth inhibition of ER+ MCF-7 breast cancer cells, incubated for 200 h.

[c] Significant inhibition; $p < 0.01$.

MCF-7/2a cells and make an antiestrogenic mode of action likely. The strongest inhibitory effects were achieved in both assays with derivatives that carry an amino group in the side chain (**10a**,**b**). The potency of these compounds (10 and 7 nM) was close to that of the steroidal reference drug fulvestrant (4 nM).

The aim of this study was the development of pure antiestrogens with sufficient bioavailability for therapeutic application. In a preliminary test, we determined the antiuterotrophic effect of the most active derivative **10b** in immature mice in comparison with fulvestrant. Both compounds completely suppressed the uterotrophic effect of 10 μg E2/kg bodyweight (Fig. 3). When these compounds were given alone in a dose of 6 and 12 mg/kg, respectively, no agonist activity was noticed.

Since all of the new stilbenes with free hydroxy groups bind to the ER, a binding mode similar to that of DES[9] has to be assumed. It is anticipated that the binding pocket of the bovine ER is rather similar to the one in the human ERα. Thus, hydrogen bridges are formed between the phenolic groups and histidine, equivalent to

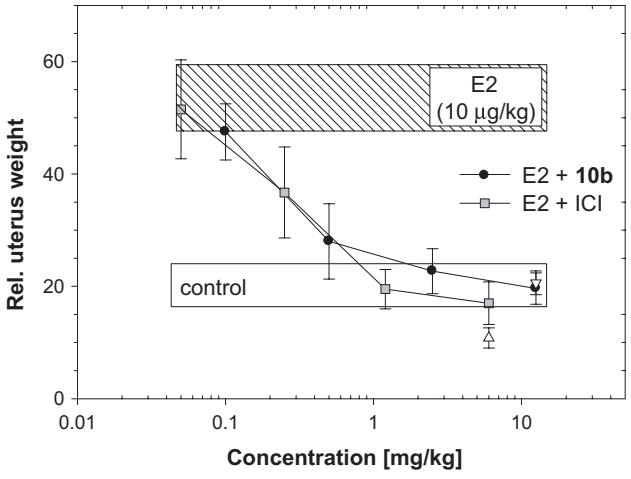


Figure 3. Antiuterotrophic effects of **10b** and fulvestrant given s.c. simultaneously with 10 μg E2/kg bodyweight to immature mice. Open triangles show the effects of **10b** (12 mg; ▽) and fulvestrant (6 mg; △) alone on uterus weight.

His524, and glutamate/arginine, equivalent to Glu353/Arg394 in the human ER, respectively. The long side chain reduces the binding affinity considerably, but it prevents helix 12 from occupying the agonist conformation.[10] In this respect, these compounds resemble other antiestrogens such as raloxifen, which interfere with the receptor–coactivator interaction by changing the position of helix 12.[11]

The results of this study clearly show that it is possible to convert the potent estrogen DES into pure antiestrogens by the introduction of appropriate side chains into the molecule. The most favorable conditions for antagonism are a tertiary amino group in the side chain, separated by a hexamethylene spacer group from the stilbene core, an additional thio ether function in the remaining chain, and fluorination of the terminal positions as demonstrated by compound **10b**. This kind of side chain appears to be equivalent to that in fulvestrant. Since both agents display similar activities both in vitro and in vivo equivalence of the carrier molecules has to be assumed. The complete antagonism can be rationalized by an ER destabilization as observed with other pure antiestrogens.[12] When administered subcutaneously to mice a 100-fold excess of stilbene **10b** or fulvestrant was necessary to block the stimulatory effect of 10 μg E2 completely. The rather low bioactivity can be explained by the reduced binding affinities of both agents for the ER when compared with E2.

References and notes

1. Jones, S. E. *Semin. Oncol.* **2003**, *30*, 14.
2. Biberger, C.; von Angerer, E. *J. Steroid Biochem. Mol. Biol.* **1996**, *58*, 31.
3. (a) Leichtl, S.; von Angerer, E. *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 283; (b) von Angerer, E.; Biberger, C.; Holler, E.; Koop, R.; Leichtl, S. *J. Steroid Biochem. Mol. Biol.* **1994**, *49*, 51.
4. Morris, C.; Wakeling, A. *Endocr. Relat. Cancer* **2002**, *9*, 267.
5. Melnick, S.; Cole, P.; Anderson, D.; Herbst, A. *N. Engl. J. Med.* **1987**, *316*, 514.
6. Golob, T.; Liebl, R.; von Angerer, E. *Bioorg. Med. Chem.* **2002**, *10*, 3941.
7. Biberger, C.; von Angerer, E. *J. Steroid Biochem. Mol. Biol.* **1998**, *64*, 277.
8. Hafner, F.; Holler, E.; von Angerer, E. *J. Steroid Biochem. Mol. Biol.* **1996**, *58*, 385.
9. Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. *Cell* **1998**, *95*, 927.
10. Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engström, O.; Öhman, L.; Greene, G. L.; Gustafsson, J.-A.; Carlquist, M. *Nature* **1997**, *389*, 753.
11. Edwards, D. P. *J. Mammary Gland Biol. Neoplasia* **2000**, *5*, 307.
12. Hoffmann, J.; Bohlmann, R.; Heinrich, N.; Hofmeister, H.; Kroll, H.; Kunzer, H.; Lichtner, R. B.; Nishino, Y.; Parczyk, K.; Sauer, G.; Gieschen, H.; Ulbrich, H. F.; Schneider, M. R. *J. Natl. Cancer Inst.* **2004**, *96*, 210.