Eur J Cancer Clin Oncol, Vol. 21, No. 4, pp. 531–537, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00

The Inhibitory Effect of 5-Acetoxy-2-(4-acetoxyphenyl)-1-ethyl-3-methylindole (D 16726) on Estrogen-dependent Mammary Tumors*

ERWIN VON ANGERER,†‡ JELICA PREKAJAC† and MARTIN BERGER§

†*Institut für Pharmazie, Universität Regensburg, Universitätsstraße 31, D-8400 Regensburg, F.R.G. and*
§*Institut für Toxikologie und Chemotherapie, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, F.R.G.*

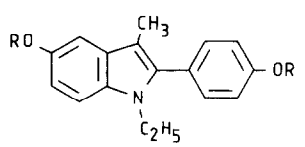
Abstract—*The antineoplastic activity of the antiestrogen 5-acetoxy-2-(4-acetoxyphenyl)-1-ethyl-3-methylindole (D 16726) was determined in several estrogen-dependent mammary tumor models. The growth of the DMBA-induced rat mammary carcinoma was inhibited by doses ranging from 1 to 12 mg/kg. The maximum decrease of tumor area was 67% (control + 635%). D 16726 was also active against MNU-induced rat mammary tumors and transplanted MXT tumors of the mouse. The growth of estrogen receptor-positive MCF-7 breast cancer cells was inhibited by the hydroxy derivative D 15414 (10^{-8}–10^{-5} M). Because of the high binding affinity of D 15414 for the estrogen receptor (RBA 6.7–10.0) and the lack of activity against hormone-independent MDA-MB 231 breast cancer cells, a specific mode of action involving the estrogen receptor is likely.*

INTRODUCTION

THE ENDOCRINE pharmacotherapy with antiestrogens has become an established method in the management of advanced breast cancer. The response rates which can be achieved with these drugs in estrogen receptor positive patients are similar to those obtained with various ablative endocrine manipulations [1–3], suggesting that they inhibit estrogen-dependent pathways to the same extent as the withdrawal of the hormone. The advantages of the therapy with estrogen antagonists are opposed by the fact that more than one-third of the patients do not respond to this treatment despite the presence of estrogen receptors in the malignant tissue [4].

Therefore we have searched for agents with higher efficacy for this application. Since the failures in therapy with the antiestrogens which are presently in clinical use might be associated with their triphenyl ethylene structure, we looked for new non-steroidal structures [5, 6]. This search was successful in the 2-phenylindole series [7, 8]. Derivatives with short alkyl groups at the nitrogen and two oxygen functions in appropriate positions bind strongly to the calf uterine estrogen receptor. They are capable of interfering with estrogen receptor-mediated processes, e.g. the growth stimulation of hormone-dependent mammary tumors [7].

From a large number of 2-phenylindole derivatives which we have studied [7, 8], the title compound 5-acetoxy-2-(4-acetoxyphenyl)-1-ethyl-3-methylindole (D 16726) was selected for evaluation as a mammary tumor inhibiting drug. Its activity was determined in a variety of hormone-dependent tumor models including the 7,12-dimethylbenz[a]anthracene (DMBA)- and methylnitrosourea (MNU)-induced mammary tumors in the rat, the transplantable MXT-tumor of the mouse and human MCF-7 breast cancer cells.



R = $COCH_3$ D 16726 (NSC 341952)
R = H D 15414

Fig. 1. Structural formulas of D 16726 and D 15414.

Accepted 25 September 1984.
*Supported by a grant of the Deutsche Forschungsgmeinschaft.
‡To whom correspondence should be addressed.

MATERIALS AND METHODS

Chemicals

5-Acetoxy-2-(4-acetoxyphenyl)-1-ethyl-3-methylindole (D 16726) and its free hydroxy derivative D 15414 were synthesized as described previously [8]. Tamoxifen citrate was a gift from ICI, Plankstadt, F.R.G. [2, 4, 6, 7-^{3}H]Estradiol (110 Ci/mmol) and [^{3}H]thymidine (80 Ci/mmol) were obtained from New England Nuclear, Dreieich, F.R.G. 7,12-Dimethylbenz[a]anthracene (DMBA), hormones and biochemicals were purchased from Sigma, München, F.R.G.

Estradiol receptor binding assay

The relative binding affinity (RBA) of the test compound was determined in a competitive binding assay with [^{3}H]estradiol. The previously described procedure was used with modifications [9]. Cytosols from different target tissues were incubated for 18 hr at 4°C with different concentrations of competitor and 2×10^{-9} M [^{3}H]estradiol. After incubation, dextran-coated charcoal was added to adsorb unbound ligand (90 min, 4°C) and, after centrifugation, radioactivity was determined in the supernatant using 100 μl aliquots. Six concentrations of competitor were chosen to provide values between 10 and 90% bound radioactivity. A semilogarithmic plot of bound radioactivity vs concentration was used to determine the relative binding affinity given as a ratio of molar concentration of estradiol and test compound required to decrease the amount of bound radioactivity by 50%, multiplied by 100.

DMBA-induced rat mammary tumors

Female Sprague-Dawley rats (Zentralinstitut für Versuchstierzucht, Hannover, F.R.G.), 50 days old, were administered by gavage a single dose of 20 mg DMBA dissolved in 1 ml of olive oil. The rats were examined for tumor masses by palpation twice weekly, beginning 30 days after feeding of DMBA; those without tumors by day 70 were discarded. Animals were assigned randomly to experimental groups when the tumor area per animal exceeded 140 mm^2. The tumor area was determined by caliper measurements of two perpendicular axes, one across the largest diameter. Analysis revealed an approximately equal distribution of tumors of different latencies, tumor number and total tumor area among each of the treatment and control groups.

The drug was dissolved in olive oil (1 ml/kg body weight) and administered perorally. From Monday to Thursday a single dose, and on Friday a double dose, was administered. Tumor size and body weight were measured twice weekly. Criteria for determining tumor response to the drug included change in tumor area per animal, change in size of individual tumors $\geqslant$50% (increased, decreased) or $<$50% (static), appearance of new tumors and proportion of tumors regressing to nonpalpability. Significance of difference was determined by the *U* test according to Mann and Whitney [10].

MNU-induced mammary tumors

Tumors were induced in virgin female Sprague-Dawley rats (Zentralinstitut für Versuchstierkunde, Hannover, F.R.G.) by three i.v. injections of 50 mg/kg methylnitrosourea (MNU) into the tail vein on days 50, 71 and 92 of life. Beginning 4 weeks after the first injection of MNU, the animals were weighed and palpated twice weekly over the whole experimental period to record tumor manifestation. The tumor size was estimated according to a volume-weight calibration curve by comparison with prefabricated plasticine models. Rats with a total tumor volume of 0.8–1.2 cm^3 were randomly allocated to treatment groups and therapy was started immediately thereafter. Animals were injected subcutaneously with D 16726 or tamoxifen dissolved in olive oil (2%; w/v) or with vehicle alone. Total tumor volumes per animal were used for the statistical comparison of tumor growth curves. A nonparametric multivariate procedure [11] was applied for determining the significance of difference between experimental groups.

Transplanted MXT-mammary tumors of the mouse

The MXT-mammary tumor was generously provided by Dr A. E. Bogden, EG & G Mason Research Institute, Worcester, MA, U.S.A. and Dr G. Leclercq, Institute Jules Bordet, Brussels, Belgium. Tumors grew for 4–5 weeks in the host animals and 1-mm^3 pieces were serially transplanted into 8- to 9-week-old female B6D2F1-mice, obtained from Charles-River-Wiga, Sulzfeld, F.R.G. Animals were assigned randomly in groups of ten and treatment was started 24 hr after transplantation. Drugs were dissolved or suspended in olive oil (1 ml/kg body wt) and administered subcutaneously on Monday, Wednesday and Friday. After a 6-week period of treatment animals were killed and autopsied. Tumors were removed 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 20 hr. Uteri were freed from connective tissue, washed with saturated alcoholic solution of LiCl, dried at 100°C for 24 hr and weighed.

MCF-7 human breast cancer cells

Ther MCF-7 cell line was kindly provided by Dr M. E. Lippman, NCI, Bethesda, MD, U.S.A.

Cells were grown in improved minimal essential medium (MEM), as modified by Richter *et al.* [12] (Biochrom, Berlin, F.R.G.), supplemented with glutamine (0.3 g/l), gentamycin (40 mg/l) and 5% newborn calf serum (NCS) (Gibco) or charcoal-treated NCC (CCS). CCS was prepared by incubation of 500 ml NCS with a dextran-coated charcoal pellet [13] for 4 hr in a shaker at 0-4°C. The procedure was repeated with a fresh pellet. After each incubation, the charcoal was removed by centrifugation. The serum was sterilized through a 0.20 μm filter (Sartorius, Göttingen, F.R.G.) and stored at -20°C. Cells were grown in a humidified incubator in 5% CO_2 at 37°C.

Two weeks before start of the experiment, cells were switched from NCS to CCS and received two additional media changes before they were harvested with 0.05% trypsin-0.02% EDTA in 0.15 M NaCl. They were syringed gently to prevent clumping, and approximately 2×10^4 cells in 2 ml were plated in duplicate in six-well dishes (Costar). One day later cells were switched to a medium containing the substances and 0.1% ethanol, in which the compounds had been dissolved. The medium of control wells contained an equal volume of ethanol. On day 4 the media were changed. Three days later cells were labeled with 1 μCi [^{3}H]thymidine per well for 2 hr. Cells were washed with cold PBS and harvested in PBS containing 0.02% EDTA. After centrifugation the cell pellet was resuspended in 1 ml of PBS and divided in two equal parts. One part was counted in ZI Coulter counter, the other was sonicated. After addition of 4 ml of 10% trichloroacetic acid (TCA), the acid-insoluble fraction was collected on a 0.45 μm filter (Metricel, Gelman) and counted after addition of 10 ml scintillation liquid (Quickszint 212, Zinsser) and an LS 8000 Beckman scintillation counter.

MDA-MB 231 human breast cancer cells

The MDA-MB 231 cell line was also generously provided by Dr M. E. Lippman. Cells were grown in McCoy 5a medium (Boehringer, Mannheim, F.R.G.) supplemented with 10% NCS and gentamycin (40 μg/ml). The experiments were performed as described for the MCF-7 cells with one exception: the incubation period was reduced from 6 to 2 days.

RESULTS

Estrogen receptor affinity

A prerequisite for the interference of a drug in hormonal events is the ability to bind to the hormone receptor. Therefore we determined the binding affinity for the estrogen receptor in various target tissues. Since we knew that the phenolic acetate was readily cleaved to the free hydroxy derivative D 15414 *in vivo*, we performed all of the *in vitro* experiments with this compound, which is probably the active metabolite. In order to demonstrate the hydrolytic cleavage of the acetate we kept the indole D 16726 in stabilized blood at room temperature. After 1 hr, only the hydroxy derivative D 15414 and a minor amount of the monoacetate were detected by HPLC.

The relative binding affinity of D 15414 for the calf uterine estrogen receptor was 9.5 (17β-estradiol = 100). A similar value was found using a cytosol from human MCF-7 breast cancer cells (RBA = 10.0). In rats the binding affinity was somewhat less: rat uteri: RBA = 6.8; DMBA-induced mammary carcinoma: RBA = 6.7.

Response of DMBA-induced rat mammary tumors

In a preliminary study we found that some of the 2-phenylindole derivatives inhibit the growth of DMBA-induced hormone-dependent mammary carcinoma of the rat following subcutaneous administration of the drug [8]. The title compound D 16726 reduced the average tumor area by 69% after 4 weeks of treatment with a daily dose of 4 mg/kg. This result prompted us to study the dose-dependence of the antitumor activity of D 16726 using a peroral route of administration. The effect of three different doses of D 16726 on the tumor area of established tumors are illustrated in Fig. 2. The inhibition was dose-

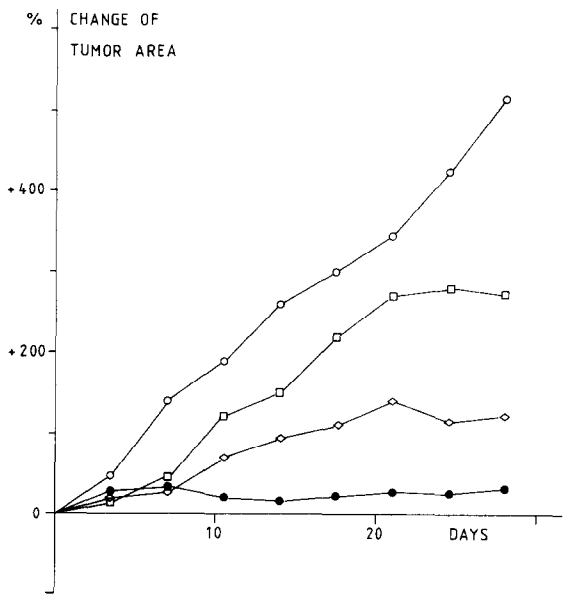


Fig. 2. The effect of various doses of D 16726, dissolved in olive oil, on the tumor area of SD rats bearing DMBA-induced mammary carcinoma. Administration schema: from Monday to Thursday, a single daily dose; on Friday, a double dose p.o. Control, vehicle alone (○–○); *D* 16726, 1 mg/kg (□–□), 2 mg/kg (◇–◇), 4 mg/kg (●–●).

dependent and significant for the 2- and 4-mg doses. When the dose was increased to 12 mg tumor growth was not only completely inhibited but the tumor area was considerably reduced. In both experiments the tumor area of untreated animals grew to more than 600% of the starting area. The inhibitory effect of D 16726 was accompanied by the remission of large fraction of tumors (Table 1). Also, the number of tumors appearing during therapy was considerably diminished.

Response of MNU-induced rat mammary tumors

The vast majority of MNU-induced rat mammary tumors have been found to be hormone-responsive [14] but they are different from those induced by DMBA. The difference appears to be due to a diminished dependence on prolactin [15]. Therefore MNU-induced mammary tumors may resemble human breast cancer more closely than DMBA tumors. In the experiment shown in Table 2, D 16726 was compared with tamoxifen, a drug which is widely used in the chemotherapy of breast cancer. Tumor growth was delayed by both drugs. The inhibition by D 16726 was significant ($P < 0.01$) while the effect of tamoxifen was not significant.

Effect on transplantable MXT-mammary tumors of the mouse

Since some antiestrogens like tamoxifen evoke different estrogenic responses in rat and mouse we studied the effect of D 16726 on the ovarian-dependent transplantable MXT-tumor of the mouse. Tamoxifen served as reference drug. A marked growth inhibition of the transplanted tumors was observed, but the difference between the two drugs was statistically not significant (Table 3). Despite the fact that tamoxifen is an estrogen in mice [16] and D 16726 an impeded agonist [8], no stimulation of uterine growth was observed. Tumor growth inhibition induced by estrogens like diethylstilbestrol is accompanied by an increase of more than 100% of uterine dry weight (data not shown).

Table 1. The effect of peroral administration of D 16726 on the DMBA-induced mammary carcinoma of the Sprague-Dawley rat

Dose,* mg	No. of animals	No. of tumors†	New tumors	Compl. remn.,‡ %	Part. remn.,§ %	Static tumors,‖ %	Progr. tumours,¶ %	Change of tumor area** median†† %	range†† %
0	9	26	26	8	2	13	77	632	-43-885
1	9	18	23	17	10	32	41	193‡‡	-87-1003
2	9	25	16	23	12	23	52	120	-100-383
4	9	21	12	36	15	15	34	20‡‡	-100-344
0	8	20	40	7	3	23	67	635	91-1010
4	8	22	17	33	16	28	23	-30‡‡	-71-392
12	7	17	8	44	24	16	16	-67‡‡	-100-308

*Dose per kg body wt, dissolved in olive oil. The animals received a single dose daily from Monday to Thursday and a double dose on Friday, p.o.
‡At the beginning of the test.
‡Tumor not palpable.
§Reduction of initial tumor size ⩽50%.
‖Tumor size 51-150% of the initial size.
¶Tumor size >150% of the initial size.
**Average on the 28th day of therapy. The *U* test according to Mann and Whitney was used to determine the significance.
††Valucs without sign are understood to be positive.
‡‡Significant ($P \leqslant 0.01$).

Table 2. The effect of D 16726 and tamoxifen on the growth of MNU-induced mammary tumors of the rat

Drug	Dose* (mg)	No. of animals	Median tumor vol. (cm^3) (95% confidence limits) day 1	day 35	Change of body wt,†%	Dead/*n*‡
-	-	15	1.2 (0.9-2.0)	39.7 (21.0-51.0)	+11	4/15
D 16726	5.7	15	1.2 (0.8-2.2)	19.7 (8.0-58.8)§	-13	5/15
Tamoxifen	6.0	15	1.3 (0.9-3.1)	29.6 (7.1-48.5)‖	-5	9/15

*Dose per kg body wt, dissolved in olive oil. The animals received a single dose daily from Monday to Friday, s.c.
†Difference in weight between day 35 and day 1.
‡At day 35.
§Significant vs control, $P < 0.01$.
‖Not significant.

Table 3. The effect of D 16726 and tamoxifen on transplanted MXT mammary tumors of the mouse

Drug	Dose* (mg)	No. of animals	Tumor* weight (mg)†	Uterotrophic activity‡
-	-	9	692	68 ± 22
D 16726	6	8	192	60 ± 13
D 16726	12	9	55	60 ± 9
Tamoxifen	6	9	18	64 ± 7

*Dose per kg body wt, dissolved in olive oil. The animals received a single dose on Monday, Wednesday and Friday, s.c.

†Mean value tumor weight was determined after 6 weeks of treatment. The tumor inhibition of treated animals was significant ($P < 0.01$). Differences between treatment groups were insignificant.

‡Uterus dry wt (mg)/body wt (g) × 100, determined at the end of therapy; mean ± S.D.

Table 4. The effect of D 15414 and tamoxifen on the growth of MCF-7 cells

Compound	Concentration, M	Cell No./dish* × 10^3	(% T/C)	[^{3}H]Thymidine incorporation counts/min,† × 10^3	(% T/C)
Control		1.16 ± 0.4		42.6 - 7.3	
D 15414	10^{-8}	0.84 ± 0.19	(72)	30.0 ± 9.3‡	(70)
	10^{-7}	0.57 ± 0.35‡	(49)	18.6 ± 2.7‡	(44)
	10^{-6}	0.37 ± 0.15‡	(32)	11.0 ± 2.9‡	(26)
	10^{-5}	0.07 ± 0.02‡	(6)	1.0 ± 0.3‡	(2)
Tamoxifen	10^{-6}	0.59 ± 0.01‡	(51)	11.6 ± 1.8‡	(27)

*Cell No. based on coulter counts on day 7, mean of 6 dishes ± S.D.

†Radioactivity/dish; mean of 6 dishes ± S.D.

‡Inhibition is significant ($P < 0.05$).

Table 5. The effect of D 15414 and tamoxifen on the growth of MDA-MB 231 cells

Compound	Concentration, M	Cell No./dish* × 10^3	(% T/C)	[^{3}H]Thymidine incorporation counts/min,†, × 10^3	(% T/C)
Control		2.30 ± 0.39		2.77 ± 0.11	
D 15414	10^{-8}	2.25 ± 0.44	(98)	2.80 ± 0.23	(101)
	10^{-7}	2.18 ± 0.28	(95)	2.91 ± 0.30	(105)
	10^{-6}	2.08 ± 0.56	(90)	2.56 ± 0.14	(92)
	10^{-5}	2.13 ± 0.63	(93)	2.73 ± 0.34	(99)
Tamoxifen	10^{-6}	2.20 ± 0.99	(96)	2.77 ± 0.16	(100)

*Cell No. based on counts counts on day 3, mean of 6 dishes ± S.D.

†Radioactivity/dish; mean of 6 dishes ± S.D.

Effect on breast cancer cells in vitro

Estrogen receptor affinity and endocrine activity make a mode of action involving the estrogen receptor very likely but do not exclude a general cytostatic action of D 16726. Therefore, we studied the *in vitro* effect of the indole derivative D 15414 on human breast cancer cells which contain estrogen receptors (MCF-7) or are devoid of them (MDA-MB 231). Cell growth was estimated in terms of cell number and [^{3}H]thymidine incorporation.

The effects of different concentrations of D 15415 on cell growth of MCF-7 cells are shown in Table 4. Concentrations of 10^{-7} M and higher caused a significant decrease in cell number and thymidine incorporation after 6 days of incubation. When assessed under identical experimental conditions D 15414 and tamoxifen (10^{-6}M) had no significant effect on MDA-MB 231 cells after 2 days. The differences in the treatment period is due to the different doubling times of the two cell lines. Obviously, estrogen receptors in the cells are required for a growth inhibiting effect of D 15414.

DISCUSSION

Endocrine pharmacotherapy of hormone-dependent mammary tumors has become an important alternative to the treatment with cytostatic agents. Up to now, the choice of drugs has been restricted to strong estrogens and some antiestrogens of the triphenylethylene type, mainly tamoxifen. Our rationale for developing new drugs was not only to increase the number of therapeutic agents for the treatment of estrogen-dependent malignancies but also to find drugs which are effective against estrogen receptor-positive tumors which do not respond to triphenylethylene antiestrogens. One possible way to reach this goal is the introduction of new active structures. Our development was based on the 2-phenylindole system.

The antineoplastic activity of the indole derivative D 16726 was evaluated in a broad spectrum of experimental mammary tumors. We found that this agent is active against hormone-dependent tumors independently of the route of administration and the kind of species used as host. Preliminary results indicate that the latter applies also for human estrogen receptor-positive breast cancer xenografts in nude mice [17]. Since the 2-phenylindole derivative is active both *in vivo* and *in vitro*, a central mode of action is rather unlikely. Therefore we assume that D 16726 acts after hydrolysis to D 15414 via the cellular estrogen receptor system. We have not found any hints that unspecific cytotoxic effects contribute to the antitumor effect of D 16726, as in the case of tamoxifen [18, 19] and of a similar chlorine-substituted indole derivative [7]. From the uterine weight test [8] we knew that D 16726 is an estrogen antagonist in the mouse. In this respect, the indole derivative is different from tamoxifen, which acts as an estrogen in the mouse [16]. The estrogenic activity of D 16726 is rather small at doses used for the treatment of tumor-bearing animals, but it cannot be excluded that estrogenic effects are responsible for the tumor inhibition. This reservation has to be made for all antiestrogens which are presently in clinical use.

Acknowledgements—The authors wish to thank Dr A. E. Bogden and Dr G. Leclercq for providing the MXT tumors, Dr M. E. Lippman for providing the tumor cell lines, Dr M. R. Schneider for his advice concerning the MXT tumor experiments, R. Brunner, G. Seidl, A. Schmitz and K. Schmitz for technical assistance, and the Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

1. Manni A, Pearson OH. Antiestrogen-induced remissions in premenopausal women with stage IV breast cancer: effects on ovarian function. *Cancer Treat Rep* 1980, **64**, 779–785.
2. Kiang DT, Frenning DH, Vosika GJ, Kennedy DJ. Comparison of tamoxifen and hypophysectomy in breast cancer treatment. *Cancer* 1980, **45**, 1322–1325.
3. Rubens RD, Hayward JC. Estrogen receptors and response to endocrine therapy and cytotoxic chemotherapy in advanced breast cancer. *Cancer* 1980, **46**, 2922–2924.
4. Maass H, Jonat W, Stolzenbach G, Trams G. The problem of nonresponding estrogen receptor-positive patients with advanced breast cancer. *Cancer* 1980, **46**, 2783–2787.
5. Angerer E von, Egginger G, Kranzfelder G, Bernhauer H, Schönenberger H. *N,N'*-Dialkyl-1,2-bis(hydroxyphenyl)ethylene diamines and *N,N'*-dialkyl-4,5-bis(4-hydroxyphenyl)imidazolidines: synthesis and evaluation of their mammary tumor inhibiting activity. *J Med Chem* 1982, **25**, 832–837.
6. Angerer E. von. The effect of *N,N'*-diethyl-1,2-bis(2,6-dichloro-4-hydroxyphenyl) ethylenediamines on the DMBA-induced mammary carcinoma of the rat. *J Med Chem* 1982, **25**, 1374–1377.
7. Angerer E von, Prekajac J. (Hydroxyphenyl)indoles: a new class of mammary tumor inhibiting compounds. *J Med Chem* 1983, **26**, 113.
8. Angerer E von, Prekajac J, Strohmeier J. 2-Phenylindoles. Relationship between structure, estrogen receptor affinity, and mammary tumor inhibiting activity in the rat. *J Med Chem* 1984, **27**, 1439–1447.
9. Kranzfelder G, Hartmann RW, Angerer E von, Schönenberger H, Bogden AE. 3,4-Bis(3'-hydroxyphenyl)hexane—a new mammary tumor-inhibiting compound. *J Cancer Res Clin Oncol* 1982, **103**, 165–180.
10. Mann HB, Whitney DR. On a test whether one or two random variables is stochastically larger than the other. *Am Math Statist* 1947, **18**, 50–60.
11. Koziol AJ, Donna AM. A distribution-free test for tumor growth curve analysis with application to an animal tumor immunotherapy experiment. *Biometrics* 1981, **37**, 383–390.
12. Richter A, Sandford KK, Evans VJ. Influence of a oxygen and culture medium on plating efficiency in some mammalian tissue culture cells. *JNCI* 1972, **49**, 1705–1712.

13. Scholl SM, Huff KK, Lippman ME. Antiestrogenic effects of LY 117018 in MCF-7 cells. *Endocrinology* 1983, **113,** 611-617.
14. Arafah BM, Gullino PM, Manni A, Pearson OH. Effect of ovariectomy on hormone receptors and growth of *N*-nitrosomethylurea-induced mammary tumors in the rat. *Cancer Res* 1980, **40,** 4628-4630.
15. Manni A, Raineri J, Arafah BM, Finegan HM, Pearson OH. Role of estrogen and prolactin in the growth and receptor levels of *N*-nitrosomethyl-urea-induced rat mammary tumors. *Cancer Res* 1982, **42,** 3492-3495.
16. Black LJ, Goode RL. Uterine bioassay of tamoxifen, trioxifene and a new estrogen antagonist (LY 117018) in rats and mice. *Life Sci* 1980, **26,** 1453-1458.
17. Angerer E von. Development of new drugs for endocrine tumour chemotherapy. *Cancer Treat Rev* 1984, **11** (Suppl. A), 147-153.
18. Taylor CM, Blanchard B, Zava DT. Estrogen receptor-mediated and cytotoxic effects of the antiestrogens tamoxifen and 4-hydroxy-tamoxifen. *Cancer Res* 1984, **44,** 1409-1414.
19. Bardon S, Vignon F, Derocq D, Rochefort H. The antiproliferative effect of tamoxifen in breast cancer cells: mediation by the estrogen receptor. *Mol Cell Endocrinol* 1984, **35,** 89-96.