[CANCER RESEARCH 51, 3867–3873, August 1, 1991]

A Potent Specific Pure Antiestrogen with Clinical Potential

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ABSTRACT

Previous studies from this laboratory have described a series of 7α-alkylamide analogues of estradiol with pure antiestrogenic activity, exemplified by ICI 164,384. A new compound, 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]estra-1,3,5(10)-triene-3,17β-diol (ICI 182,780) has now been identified which has significantly increased antiestrogenic potency and retains pure estrogen antagonist activity. The antiuterotrophic potency of ICI 182,780 in the immature rat was more than 10-fold greater than that of ICI 164,384 (50% effective doses of 0.06 and 0.9 mg/kg, respectively). This order of magnitude increase of *in vivo* potency was also reflected, in part, by intrinsic activity at the estrogen receptor. The relative binding affinities of ICI 182,780 and ICI 164,384 were 0.89 and 0.19, respectively, compared with that of estradiol (1.0). Similarly, the *in vitro* growth-inhibitory potency of ICI 182,780 exceeded that of ICI 164,384 in MCF-7 human breast cancer cells, where 50% inhibitory concentrations of 0.29 and 1.3 nM, respectively, were recorded. ICI 182,780 was a more effective inhibitor of MCF-7 growth than 4′-hydroxytamoxifen, producing an 80% reduction of cell number under conditions where 4′-hydroxytamoxifen achieved a maximum of 50% inhibition. This increased efficacy was reflected by a greater reduction of the proportion of cells engaged in DNA synthesis in ICI 182,780-treated cell cultures compared with tamoxifen-treated cells.

Sustained antiestrogenic effects, following a single parenteral dose of ICI 182,780 in oil suspension, were apparent in both rats and pigtail monkeys. *In vivo*, antitumor activity of ICI 182,780 was demonstrated with xenografts of MCF-7 and Br10 human breast cancers in nude mice. A single injection of ICI 182,780 provided antitumor efficacy equivalent to that of daily tamoxifen treatment for at least 4 weeks.

The properties of ICI 182,780 identify this pure antiestrogen as a prime candidate with which to evaluate the potential therapeutic benefits of complete estrogen withdrawal in endocrine-responsive human breast cancer.

INTRODUCTION

Nonsteroidal antiestrogens, exemplified by tamoxifen [ICI 46,474 (Nolvadex)], have been used extensively, and with great success, in the therapy of breast cancer (1, 2). Antiestrogens compete with endogenous estrogens for binding to ER[2] but a complete description of the mode of action of these molecules remains elusive (3, 4). In particular, it is difficult to account for the diversity of biological actions which range between full agonist, estrogen-like trophic effects, through partial agonism to complete blockade of estrogen action. This diversity was first apparent in species differences of organ response (5) but remarkably extends to differential effects of tamoxifen on estrogen-responsive genes within target cells (6). Because of the potential of nonsteroidal antiestrogens to manifest stimulatory activity it remains unclear whether their clinical activity is in any way limited compared with that which might be achieved by complete endocrine ablation.

Received 4/10/91; accepted 5/21/91.

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[2] The abbreviations used are: ER, estrogen receptor(s); ICI 164,384, *N*-*n*-butyl-*N*-methyl-11-(3,17β-dihydroxyestra-1,3,5(10)-triene-7α-yl)undecanamide; ICI 182,780, 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]estra-1,3,5(10)-triene-3,17β-diol; OVX, ovariectomized; IC_{50}, 50% inhibitory concentration; ED_{50}, 50% effective dose.

Considerations of the kind outlined above led us to search for novel molecules which would bind ER with high affinity without activating any of the normal transcriptional hormone responses and consequent manifestations of estrogen action. Such molecules would be clearly distinguished from tamoxifen-like ligands and would be pure antiestrogens. The rationale for the design and testing of novel putative pure antagonists has been described elsewhere (7, 8), as have the first examples of such compounds (9–11). The prototype pure antiestrogen, ICI 164,384, a 7α-alkylamide analogue of estradiol, is devoid of stimulatory activity and blocks completely the trophic actions of estrogens and of the partial antiestrogens in all estrogen-responsive cell and animal models examined to date (see Ref. 12 for a review).

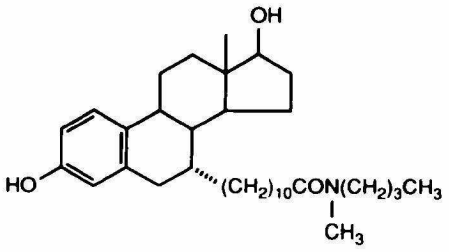
In this report we describe some of the properties of a new pure antiestrogen, ICI 182,780. ICI 182,780 is a potent and specific inhibitor of estrogen action and demonstrated excellent growth-inhibitory effects in both cell and animal models of human breast cancer. Such properties identify ICI 182,780 as a prime candidate with which to explore the therapeutic potential of pure antiestrogens in the treatment of breast cancer.

MATERIALS AND METHODS

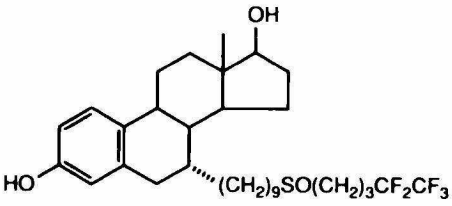
Reagents. The antiestrogens tamoxifen (ICI 46474; *trans*-1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene), the *trans*-4′-hydroxy metabolite of tamoxifen, ICI 164,384, and ICI 182,780 were synthesized in Chemistry Department I, ICI Pharmaceuticals. Structures of ICI 164,384 and ICI 182,780 are illustrated in Fig. 1. Stock solutions of these agents were prepared in ethanol, stored at 4°C, and diluted as required. 17β-[^{3}H]estradiol, 85–110 Ci/mmol, and sodium ^{125}I-iodide, IMS 30, were obtained from the Radiochemical Centre, Amersham, England. Materials for gonadotropin assays were obtained from the National Institute for Arthritis, Metabolism, and Digestive Diseases, Bethesda, MD except for ovine luteinizing hormone for iodination, supplied by L. E. Reichert, Emory University, Atlanta, GA. 17β-Estradiol benzoate, insulin, and materials for flow cytometry (propidium iodide, bromodeoxyuridine, anti-mouse IgG fluorescein isothiocyanate conjugate; F 0257) were obtained from Sigma Chemical Company, Poole, Dorset, England, except for purified mouse anti-bromodeoxyuridine monoclonal antibody (No. 7580) which was from Becton Dickinson, Mountain View, CA. All materials for cell culture were from Gibco, Paisley, Scotland, with the exception of Costar flasks which were from Northumbria Biologicals, Cramlington, England. MCF-7 cells were obtained from Dr. C. M. McGrath, Michigan Cancer Foundation, Detroit, MI, and BT20 cells were from Dr. J. Taylor, Imperial Cancer Research Fund, London, England.

Estrogen Receptor Binding Assay. The method used for competitive binding assays to measure the relative affinity of antiestrogens for rat uterine ER has been described elsewhere (13) except that competitor dilutions were prepared in tris:dimethylformamide (1:1) (14) and mixed together with 17β-[^{3}H]estradiol and then with cytosol at a ratio of 1:20.

Cell Proliferation and Flow Cytometry Studies. The methods used for MCF-7 cell culture and growth inhibition assays have been detailed elsewhere (15). Briefly, cells were cultured in multiwell plates (24-well, seeding density 4×10^4) in minimal essential medium containing phenol red, insulin (10 μg/ml), and 5% charcoal-stripped fetal calf serum without estradiol. Antiestrogens and/or estradiol were added at 1000-fold dilution from ethanol stock, in fresh medium 2 days after seeding. Cultures were maintained for 5 days with one further medium change and growth was assessed by measurement of total cell protein at the



ICI 164,384



ICI 182,780

Fig. 1. Structure of pure antiestrogens.

beginning and end of treatment and compared with that of controls treated with ethanol (0.1%) alone. BT-20 cells were treated similarly.

The measurement of antiestrogen effects on cell cycle and population distribution of MCF-7 cells using two parameter flow cytometry followed the method described previously (16).

Assays of Estrogenic/Antiestrogenic Effects. The rat uterine weight assay for the measurement of estrogenic and antiestrogenic activity has been described elsewhere (17). Details of doses, route of administration, and duration of treatment are reported in individual figure legends. ICI 182,780 and 17β-estradiol benzoate were prepared for administration by diluting an ethanol stock solution into the required volume of arachis oil with gentle warming (60°C). Tamoxifen was prepared for administration p.o. as a dispersion in aqueous 0.5% Tween 80. For immature and mature rats the dose volumes were 0.5 and 0.1 ml/100 g body weight, respectively. In studies with intact rats, blood samples were collected terminally for measurement of luteinizing hormone, follicle-stimulating hormone, and prolactin. Plasma gonadotropin concentrations were determined by a modification of the double antibody technique described by Niswender *et al.* (18).

In studies with OVX rats, surgical preparation was performed at least 2 weeks before treatment began. To measure the duration of action of a single large dose of ICI 182,780, OVX rats were treated with a daily s.c. dose of 0.5 μg of estradiol benzoate beginning on the day of ICI 182,780 administration and continued until vaginal smears showed evidence of cornification. At that point the experiment was terminated and uterine weight was recorded. The arachis oil formulation used in these single dose duration of action studies contained 50 mg ICI 182,780/ml.

For studies of the duration of action of ICI 182,780 in monkeys, adult female pigtail macaques (*Macaca nemestrina*) weighing 6–8 kg were ovariectomized not less than 6 months before treatment. Preliminary studies established that daily s.c. treatment of OVX monkeys with 5 μg estradiol benzoate/kg induced perineal swelling in a reproducible manner with individual maxima being attained after 11 days. The magnitude of the estrogenic effect was assessed visually on an arbitary scale of 1–6. Groups of five monkeys were treated s.c. once daily for 10 days with 0.1–1.0 mg/kg ICI 182,780, or with a single dose of 10 mg/kg, 10 days before beginning estradiol treatment. Perineal size was estimated daily and the time taken for initiation of swelling (mean score, 2) and attainment of maximum (score, 4–6) was recorded.

Tumor Growth Inhibition Assays. MCF-7 cells were suspended in culture medium (no serum) and inoculated s.c. into the flank of adult female nude mice (0.1 ml/approximately 5×10^6 cells). Mice were maintained in a clean environment and were given sterile food and water. Estrogen supplementation was provided by ethynyl estradiol at 1 μg/ml in the water. Antiestrogen treatment was initiated when tumor diameter attained a minimum of 0.5 cm. The Br10 tumor at passage 49 was obtained from Dr. N. Brunner (Copenhagen, Denmark) and established by implantation of 1–2-mm^3 tumor fragments into the flank of anesthetized intact adult female nude mice. After 3 passages a reproducible pattern of growth was established without additional estrogen supplementation. Approximately two-thirds of animals established progressively growing tumors which attained measurable size (area, $\geq$70 mm^2) after 6–7 weeks. Antiestrogen treatment was initiated at the time of transplantation. Tamoxifen was administered once daily p.o. at a dose of 10 mg/kg (1 ml/100 g body weight of aqueous dispersion in 0.5% Tween 80) and ICI 182,780 as a single s.c. injection of 5 mg/mouse (50 mg/ml in arachis oil). Tumor size was assessed weekly as the product of caliper measurements of the largest diameter and the axis perpendicular to it.

RESULTS

Estrogen Receptor Interaction

The capacity of ICI 182,780 to compete with 17β-[^{3}H]estradiol for binding to rat uterine ER was evaluated and compared with that of estradiol and the previously reported pure antiestrogen ICI 164,384 (Fig. 2). Each antiestrogen displaced 17β-[^{3}H]estradiol in a concentration-dependent manner and the displacement curves were parallel to that of estradiol. IC_{50} values of 0.83, 0.94, and 4.48×10^{-8} M were recorded for estradiol, ICI 182,780, and ICI 164,384, respectively. Relative binding affinities calculated from these IC_{50} values were 0.89 and 0.19 for ICI 182,780 and ICI 164,384, respectively, compared with that of estradiol (1).

Estrogenic/Antiestrogenic Effects

When administered alone, parenterally (s.c.), to immature female rats ICI 182,780 was devoid of uterotropic activity and, when coadministered with estradiol, it effectively blocked the uterotropic action of estradiol in a dose-dependent manner [ED_{50} 0.06 mg/kg/day s.c. (Fig. 3*A*)]. Complete antagonism of estrogen action was achieved with a dose of 0.5 mg ICI 182,780/kg/day s.c. The effects of ICI 182,780 administered p.o. were qualitatively similar but potency was reduced by an order of

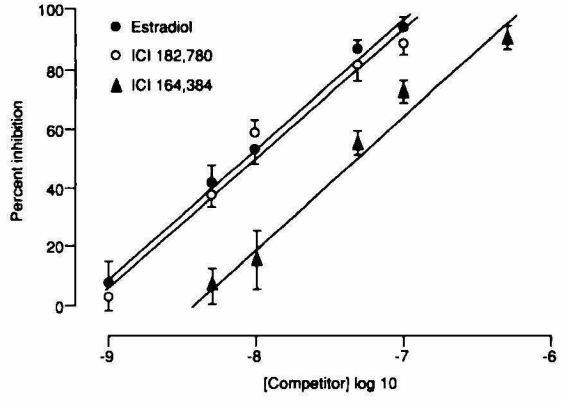


Fig. 2. Competition for binding of 5×10^{-9} M [^{3}H]17β-estradiol to rat uterine estrogen receptor by unlabeled 17β-estradiol, ICI 182,780, and ICI 164,384. Percent inhibition refers to specific binding corrected by subtraction from total 17β-[^{3}H]estradiol bound, the nonspecific component recorded in the presence of 5×10^{-7} M unlabeled 17β-estradiol. *Points*, mean of 9 observations in three different experiments; *bars*, SEM. IC_{50} values were calculated by linear regression analysis of percentage of inhibition *versus* $\log_{10}$[competitor].

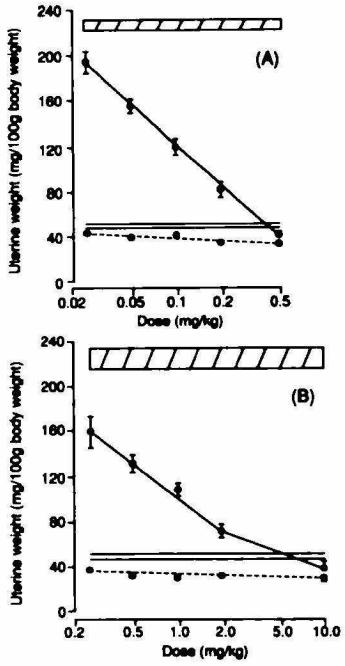


Fig. 3. Effects of ICI 182,780 on uterine weight of immature rats. Animals received daily, a single dose of arachis oil vehicle alone (□), 0.5 μg 17β-estradiol benzoate s.c. alone (▨), or the indicated doses of ICI 182,780 alone (----) or together with estradiol (——), for 3 days. *A*, parenteral (s.c.) administration; *B*, p.o. administration. *Points*, means for a minimum of 10 observations in at least 2 different experiments. In this and succeeding figures *bars* on each *point* represent the SEM. Where no *bar* is present errors were smaller than the symbols.

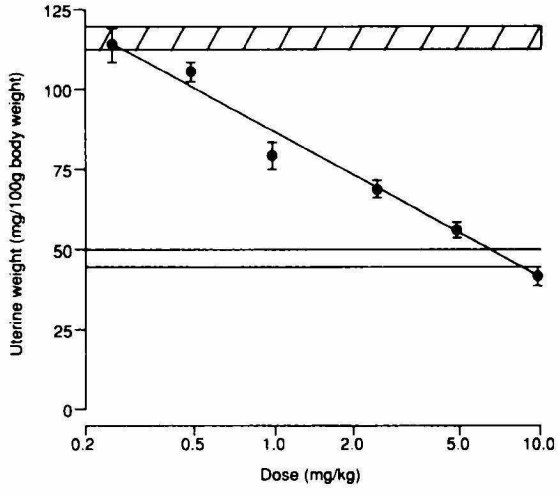


Fig. 4. Antagonism of the uterotropic effect of tamoxifen by ICI 182,780. Immature rats were treated as described in the legend to Fig. 3, except that the ▨ represents the effect of 1 mg tamoxifen/kg alone and —— is the effect of the indicated doses of ICI 182,780 together with tamoxifen. *Points*, mean for at least 5 observations; *bars*, SEM.

magnitude compared with s.c. dosing [*cf.* ED_{50} 0.46 and complete antagonism at 5 mg/kg/day p.o. (Fig. 3*B*)]. Similarly, the uterotropic action of tamoxifen was also blocked in a dose-dependent manner by coadministration of ICI 182,780 (Fig. 4). Complete blockade of tamoxifen action required an approximately 5-fold dose ratio. Similar studies of uterotropic and antiuterotropic activity in immature mice and in ovariectomized adult rats and mice provided confirmation of the pure antagonist profile of ICI 182,780 (data not shown).

Chronic daily parenteral (s.c.) treatment of intact adult female rats with increasing doses of ICI 182,780 reduced the weight of the uterus in a dose-dependent fashion (Fig. 5). At the highest dose in this study, 1 mg/kg/day, involution of the uterus after 14 days approached that following ovariectomy. Cyclical vaginal cornification was blocked partially (0.1 mg/kg/day) or completely (0.3 mg/kg/day) but body weight gain and serum gonadotropin concentrations were largely unaffected (Table 1). The p.o. antiuterotropic activity of ICI 182,780 in intact rats was substantially less than its parenteral potency; at 10 mg/kg/day for 14 days an effect approximating 50% that of ovariectomy was recorded.

Following the precedent that many steroids administered parenterally in oil have a sustained duration of action, the effect of ICI 182,780 administered as a single s.c. bolus dose in oil suspension was tested in adult ovariectomized rats. The initiation of vaginal cornification and uterine growth by daily administration of 0.5 μg of estradiol benzoate was blocked for more than 6 weeks by 10 mg of ICI 182,780. Uterine weights 42 days after ICI 182,780 for ovariectomized controls, estrogen-treated controls, and ICI 182,780 plus estrogen-treated rats were 60.6 ± 3.5 (SEM), 311 ± 26, and 63.3 ± 0.6 mg ($n = 5$), respectively. Similar treatment of intact adult females completely blocked

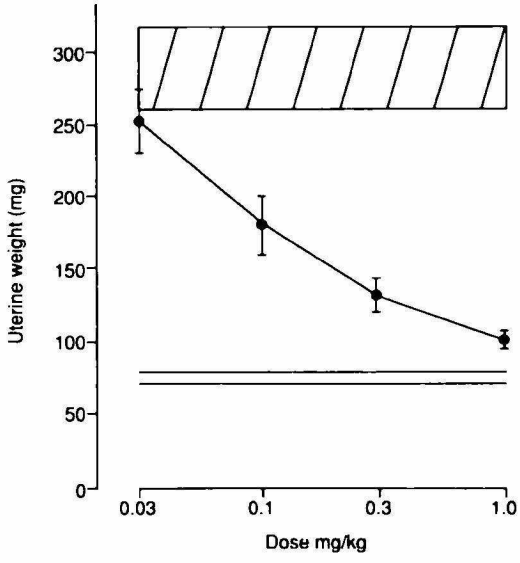


Fig. 5. Effect of ICI 182,780 on the uterus of intact adult rats. Groups of 5 adult rats with regular 4-day estrous cycles were treated once daily, for 14 days, with arachis oil vehicle alone (▨) or the indicated doses of ICI 182,780 s.c. Uterine weight was also recorded for vehicle-treated animals ovariectomized at the beginning of the study (□).

Table 1 *Effect of ICI 182,780 on body weight and plasma gonadotropins of adult female rats*

Values are mean ± SEM; $n = 5$. All ICI 182,780 values differ from corresponding OVX controls, at $P < 0.001$.

Treatment	Body wt gain (g)	Gonadotropins (ng/ml)		
		Luteinizing hormone	Follicle-stimulating hormone	Prolactin
Intact control	40.0 ± 2.5	2.4 ± 0.6	3.0 ± 0.4	25.3 ± 3.3
OVX control	64.8 ± 1.9[a]	19.7 ± 2.2[a]	24.0 ± 0.5[a]	3.7 ± 0.7[a]
ICI 182,780 (mg/kg)				
0.03	43.6 ± 2.5	2.1 ± 0.2	2.2 ± 0.2	18.2 ± 8.9
0.1	44.6 ± 1.7	1.2 ± 0.1	2.5 ± 0.6	19.1 ± 6.4
0.3	45.8 ± 2.0	1.0 ± 0.1	2.5 ± 0.5	28.8 ± 17.2
1.0	42.6 ± 2.1	2.3 ± 0.3	3.6 ± 0.6	6.0 ± 2.2[b]

[a] $P < 0.001$ *versus* intact control.
[b] $P < 0.05$ *versus* intact control.

Table 2 *Antiestrogenic action of ICI 182,780 in ovariectomized estrogen-treated monkeys*

Treatment	Days from start of estrogen treatment to reach perineal score of >2	Days from start of estrogen treatment to reach perineal score of 4–6 (maximum)
Control (estradiol benzoate alone)	5	11
ICI 182,780 10 days: pretreatment with		
0.1 mg/kg s.c.	13	17
0.5 mg/kg s.c.	18	23
1.0 mg/kg s.c.	41	47
10.0 mg/kg s.c.	23	33

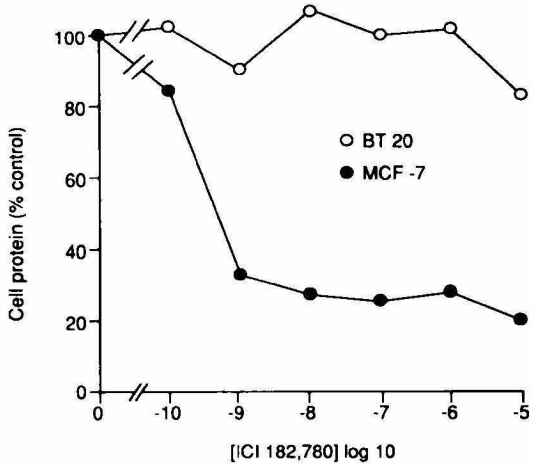


Fig. 6. Effects of ICI 182,780 on the proliferation of MCF-7 and BT-20 human breast cancer cells. Cells were plated in 24-well dishes (4×10^4/well) and cultured for 2 days in minimal essential medium with 5% charcoal-stripped fetal calf serum containing phenol red and insulin but no additional estrogens. One dish was assayed for total protein (Lowry) as day 0 control; remaining dishes received fresh medium with (treated) or without (control) the indicated concentrations of ICI 182,780 added in ethanol (1 μg/ml medium). Cells were grown for a further 5 days with fresh medium added after 3 days. Cell growth is represented as the difference between the increase of total protein in control and treated wells between day 0 and day 5. *Points*, mean of quadruplicate observations where SEM was less than 5%.

cyclical vaginal cornification for at least 3 weeks and regressed the uterus (24% *cf.* intact control weight at 21 days after treatment).

The antiestrogenic activity of ICI 182,780 was also measured in OVX pigtail macaques. Maximum swelling is attained after 11 days of estrogen treatment (5 μg/kg/day). Pretreatment of monkeys with 0.1, 0.5, or 1 mg ICI 182,780/kg/day s.c. for 10 days prior to estrogen replacement produced an increasing delay in the onset of perineal swelling, of the order of 1, 2, and 5 weeks, respectively (Table 2). Administration of a single dose of 10 mg ICI 182,780/kg s.c. in oil suspension delayed the onset of perineal swelling by 3 weeks and the attainment of maximum swelling by in excess of 4 weeks (Table 2).

Breast Cancer Growth Inhibition

Human Breast Cancer Cells *in Vitro*. ICI 182,780 was an effective inhibitor of the growth of ER-positive MCF-7 human breast cancer cells but was without effect on the growth of ER-negative BT-20 human breast cancer cells (Fig. 6). ICI 182,780 was fully effective at 10^{-9} M on MCF-7 cells grown in medium containing phenol red but without added estradiol. Cell death was not observed in either MCF-7 or BT-20 cells exposed to 10^{-5} M ICI 182,780. The growth-inhibitory action of ICI 182,780 on MCF-7 cells was reversed in a competitive manner by estradiol (Fig. 7). In the presence of 10^{-8} M ICI 182,780, coincubation with 10^{-10} M estradiol had no effect but growth inhibition was reversed partially at 10^{-9} M and completely by 10^{-8} M estradiol. For MCF-7 cells grown in medium containing phenol red addition of estradiol alone provided a moderate, concentration-dependent growth stimulus (Fig. 7).

A comparison of the effect of ICI 182,780 with that of other antiestrogens on the growth of MCF-7 cells (Fig. 8) showed that it was significantly more potent than ICI 164,384 (IC_{50} = 0.29 and 1.3 nM, respectively) or 4′-hydroxytamoxifen. Also, like ICI 164,384, the maximum growth-inhibitory effect of ICI 182,780 exceeded that of 4′-hydroxytamoxifen [approximately 80% *cf.* 50% (Fig. 8)].

Flow cytometric analysis of cell cycle and population distribution of MCF-7 cells treated with tamoxifen or ICI 182,780 showed that both antiestrogens caused accumulation of cells in G_0/G_1 and also reduced the proportion of cells capable of continued DNA synthesis (Table 3). However, the maximal efficacy of ICI 182,780 compared with that of tamoxifen, when both compounds were used at optimum antiestrogenic (but not cytotoxic concentrations), was much greater. Thus, only 7% of cells were still potentially capable of division after 3–5 days of treatment with 10 nM ICI 182,780 compared with 37% in cultures treated with 4 μM tamoxifen.

Human Breast Tumors *in Vivo*. The effects of ICI 182,780 were compared with those of tamoxifen in two models of human breast cancer grown in nude mice. The growth of xenografts of MCF-7 human breast cancer cells, supported by continuous treatment with ethynyl estradiol, was blocked completely for at least 4 weeks by a single s.c. injection of 5 mg of ICI 182,780 in oil suspension (Fig. 9). The magnitude of this effect was comparable with that in animals treated continuously with a high dose of tamoxifen (10 mg/kg/day p.o.).

The growth of transplants of the Br10 human breast tumor was also suppressed effectively by ICI 182,780. Mice implanted with a 1–2-mm^3 tumor mass were given a single 5-mg s.c. injection of ICI 182,780 on the day of implantation or daily treatment for 8 weeks with tamoxifen (10 mg/kg/day p.o.). Tumor measurements (Fig. 10) showed a substantial and sustained reduction of tumor growth in ICI 182,780-treated mice

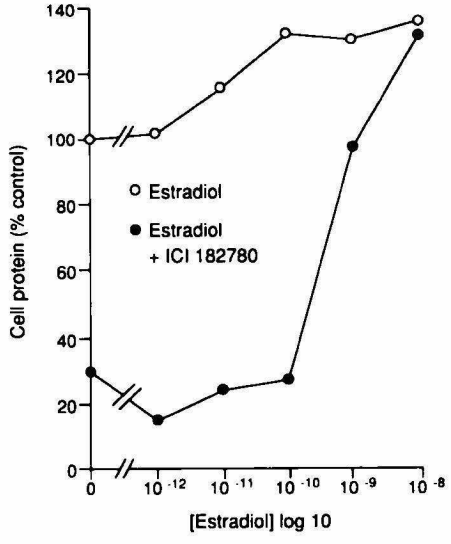


Fig. 7. Effects of 17β-estradiol on the growth of MCF-7 cells in the absence and presence of 10^{-8} M ICI 182,780. The experimental procedure was as described for Fig. 6.

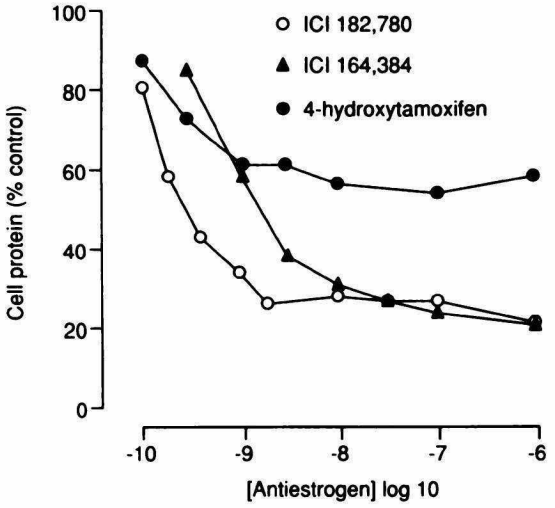


Fig. 8. Effects of different antiestrogens on the growth of MCF-7 cells. The experimental procedure was as described for Fig. 6. *Points*, mean derived from three or more different experiments with quadruplicate observations in each. SEM was less than 4%.

Table 3 *Effects of antiestrogens on population distribution of MCF-7 human breast cancer cells*

	% of cells			
	Cycling		Noncycling	
Treatment	G_0/G_1	$S + G_2 + M$	G_0/G_1	$S + G_2 + M$
Control	56	26	10	8
Tamoxifen				
0.4×10^{-6} M	41	7	41	12
1×10^{-6} M	35	11	44	11
2×10^{-6} M	30	8	52	10
4×10^{-6} M	27	10	50	13
ICI 182,780				
0.4×10^{-9} M	47	22	17	14
1×10^{-9} M	13	3	77	7
2×10^{-9} M	10	2	79	9
4×10^{-9} M	4	1	84	10
10×10^{-9} M	6	1	82	10

similar to that of high-dose tamoxifen treatment. Note that 2 weeks after the end of tamoxifen treatment tumor growth rate showed evidence of a return to control level whereas, even 3 months after a single dose of ICI 182,780, tumor growth rate remained below that of control. Ovariectomy of all animals after 3 months demonstrated the estrogen sensitivity of these tumors (Fig. 10).

DISCUSSION

The discovery of novel steroidal antiestrogens exemplified by ICI 164,384 (9, 10) provided for the first time pure estrogen antagonists which have shed new light on the physiology (11, 19–22) and the molecular mode of action of estrogens and antiestrogens (23–26). Among the initial series of 7α-alkylamide analogues of 17β-estradiol described previously (10) none was of sufficient potency *in vivo* to merit serious consideration as a candidate for clinical use. We therefore sought to identify more potent compounds which retained the potentially advantageous properties of ICI 164,384. For breast cancer treatment, these included high affinity for ER (27), the absence of estrogenic activity (9, 11–14), and more effective antiproliferative action on breast cancer cells than classical tamoxifen-like partial agonist antiestrogens (22–24). Further synthetic modification of the 7α side chain, in which the amide moiety was replaced by other polar groups and the terminal alkyl function was fluorinated (10), produced the pentafluoropentylsulfinyl compound ICI 182,780.

In receptor binding studies (Fig. 2) and as a specific and reversible inhibitor of MCF-7 breast cancer cell growth (Figs. 6–8), ICI 182,780 demonstrated an approximately 5-fold increase of intrinsic potency compared with ICI 164,384. This increased potency was clearly manifest *in vivo* where, in the rat antiuterotropic assay, ICI 182,780 [ED_{50} = 0.06 mg/kg (Fig. 3)] was at least an order of magnitude more potent than ICI

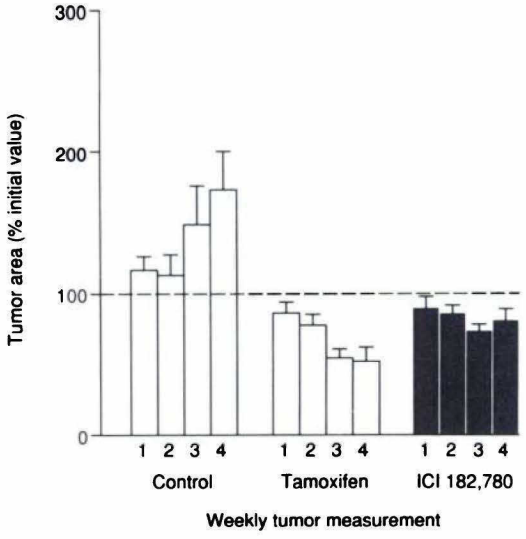


Fig. 9. Effect of ICI 182,780 and tamoxifen on the growth of established MCF-7-derived tumors in nude mice. *Columns*, means ($n \geq 5$) of tumor area normalized by reference to initial area preceding the 4-week treatment period; *bars*, SEM. All animals received continuous ethynyl estradiol (1 μg/ml in drinking water). Additionally, tamoxifen-treated animals were dosed daily (10 mg/kg p.o.) and ICI 182,780 once (5 mg/mouse s.c.) at the beginning of the 4-week measurement period.

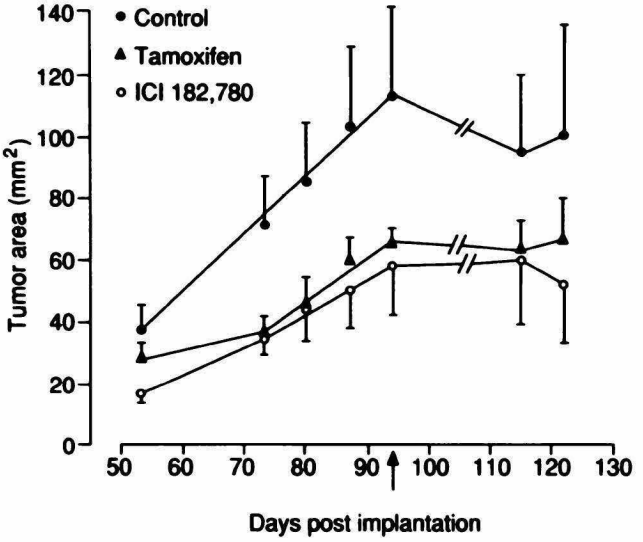


Fig. 10. Effect of ICI 182,780 and tamoxifen on the growth of Br10 human breast tumors in nude mice. Groups of 10 female nude mice bearing transplants of Br10 received either no treatment (control), daily tamoxifen (10 mg/kg p.o.) for 8 weeks beginning on the day of transplantation, or a single dose of ICI 182,780 (5 mg/mouse s.c.). Values are mean tumor area, (n = 6–8) for all tumors attaining measurable size by day 50 postimplantation; *bars*, SEM. Approximately 3 months postimplantation all mice were ovariectomized (*arrow*) and tumor measurements were continued for 1 month further.

164,384 [ED_{50} = 0.9 mg/kg; see Ref. 15]. The apparent 2-fold difference in potency ratio improvement between *in vitro* and *in vivo* assays for the two compounds is likely a reflection of differences in distribution and metabolism. Both *in vitro* and *in vivo* studies were consistent with a competitive interaction between ICI 182,780 and estradiol for binding to ER. The absence of a significant estrogenic activity of ICI 182,780 was clearly apparent in rodent uterotropic assays (*e.g.*, Fig. 3) and in its capacity to block completely the stimulatory action of tamoxifen (Fig. 4).

Of particular relevance to the therapeutic potential of ICI 182,780 are two observations reported here: (*a*) the enhanced efficacy compared with 4′-hydroxytamoxifen (or tamoxifen) on breast tumor cells (Fig. 8; Table 3); and (*b*) the excellent antiuterotropic action (Figs. 3–5; Table 2) achieved without affecting body weight and gonadotropin secretion (Table 1). The castration-like uterine involution achieved in intact animals in the absence of an effect on the latter indices of hypothalamic-pituitary function indicates that ICI 182,780 might be differentially active against peripheral and central targets of estrogen action, a property shared with ICI 164,384 (15). If translated to the clinical setting, this peripheral selectivity of action would obviate blockade of central negative estrogen feedback and consequent increases of estrogen production in the premenopausal patient. With respect to the enhanced efficacy of pure antiestrogens against tumor cell growth *in vitro* we have shown previously for ICI 164,384 (16, 28–30) and here for ICI 182,780 (Table 3) that fewer of the cells remain in the actively proliferating fraction than is the case when partial agonists like tamoxifen, 4′-hydroxytamoxifen, or hydroxyclomiphene are used. This has been attributed to a residual stimulatory estrogenic effect of the partial agonists which, although small (16, 31), is amplified synergistically by the concurrent presence of other breast cell mitogens like insulin (16) and insulin-like growth factor 1 (32). The pure antiestrogens obviate such effects. The corollary of these data in the clinical setting is the possibility that differences of antitumor efficacy between tamoxifen and pure antiestrogens may be greater than otherwise anticipated.

The order of magnitude lower potency between the p.o. and parenteral routes of administration (Fig. 3) suggests strongly that the p.o. bioavailability of ICI 182,780 is relatively low. A common means of circumventing the practical constraints consequent on the poor p.o. bioavailability of steroids is to use parenteral depot formulations with an extended duration of action. The utility of this approach was demonstrated with ICI 182,780 dispersed in arachis oil. Thus, single s.c. injections of ICI 182,780 in ovariectomized, estrogen-treated rats and monkeys (Table 2) provided extended antiestrogenic activity.

The potential efficacy of "oil depot" formulations of ICI 182,780 was demonstrated in nude mouse antitumor studies. The antitumor action of a single parenteral dose of ICI 182,780 on MCF-7 xenografts was similar to that achieved by daily administration of a high dose of tamoxifen over a 4-week period (Fig. 9). Tumor growth ceased in ICI 182,780-treated animals but no significant tumor involution was seen, an effect consistent with previous observations in this (33) and other (34) laboratories where estrogen withdrawal failed to precipitate tumor regression. The absence of superior efficacy of ICI 182,780, compared with that of tamoxifen, is consistent with the fact that tamoxifen lacks significant *in vivo* tumor growth-stimulatory action, and high doses of tamoxifen did not cause tumor regression in short-term studies with this tumor model (34). The effect of ICI 182,780 *in vivo* is therefore consistent with a cytostatic rather than a cytotoxic action. Others, using the same model system, have reported tumor involution during tamoxifen treatment to the extent that tumors almost disappeared after 3–4 weeks (35). This has been attributed to an alteration in cell death rate (35). In the current study (Fig. 9), 3–4 weeks of continuous high dose tamoxifen treatment produced a significant decrease ($P < 0.05$) of mean tumor volume, compared with that at the start of treatment, but not tumor disappearance. A recent study of the effects of estrogen withdrawal on MCF-7 tumors in nude mice has also demonstrated tumor regression associated with both cessation of cell proliferation and the activation of programmed cell death (36). Kyprianou *et al.* (36) attribute interlaboratory differences in apparent response to hormone withdrawal to variations in MCF-7 cell phenotype where some but not all sublines have lost the capacity to initiate apoptosis. Whether differences exist between pure and partial agonist antiestrogens in their capacity to promote apoptosis, as well as the apparent differences in sensitivity between different strains of MCF-7 cells, remains to be determined.

In long-term exposure of MCF-7 xenografts tamoxifen-dependent tumors appear occasionally (37) and it has been shown that the continuing growth of such tumors can be blocked by concurrent administration of a pure antiestrogen (ICI 164,384; Ref. 21). It is possible that the latter studies represent a process occurring in some patients with advanced breast cancer who respond initially but then relapse on tamoxifen therapy. In those cases apparent tumor resistance may in reality represent the development of hypersensitivity to the stimulatory action of tamoxifen (38). Such tumors would be expected to respond well to second-line endocrine treatment with a pure antiestrogen. In first-line endocrine treatment, pure antiestrogens like ICI 182,780 would obviate the induction of such "resistant" tumors and the occurrence of tumor flare at the initiation of treatment (39). Similarly, concerns that the estrogenic action of tamoxifen on the endometrium in breast cancer patients may increase the incidence of endometrial carcinoma (40) would be obviated by the use of a pure antiestrogen. However, because the majority of studies do not show an excess of uterine cancers in patients treated with tamoxifen, this is unlikely to be a real clinical issue, although comparative studies of tamoxifen and the pure antiestrogen ICI 164,384 in nude mice bearing human endometrial carcinoma do support this hypothesis (22).

As an alternative to MCF-7 tumors we also studied the transplantable Br10 human breast cancer (41). This tumor has the advantage over MCF-7-derived tumors of growing in intact female nude mice without exogenous estrogen supplementation (41, 42). The growth of such tumors ceases on ovariectomy or tamoxifen treatment but, as was the case with MCF-7 tumors, tumor stasis rather than regression was seen (41, 43). The sustained attenuation of the growth of Br10 tumors by a single parenteral dose of ICI 182,780 observed here (Fig. 10) is therefore consistent with previous work. In this tumor the pure antiestrogen appeared slightly more effective than continuous high dose tamoxifen treatment. For both *in vivo* human breast cancer models studied a substantial and sustained antitumor effect was produced by a single bolus parenteral treatment with ICI 182,780.

In summary, ICI 182,780 offers significant advantages compared with pure antiestrogens reported previously, particularly with respect to *in vivo* potency. Although p.o. potency appears to be limited, probably as a result of poor absorption, this disadvantage is offset by the sustained antiestrogenic and anti-

tumor activity following parenteral administration of ICI 182,780 in oil suspension. The data available to date for ICI 182,780 presented here and for ICI 164,384 (12, 21, 22, 44) indicate that pure antiestrogens may find a valuable place in the treatment of breast cancer. ICI 182,780 will be used to test this proposition.

Finally, although we have emphasized the potential advantages to be obtained with total hormone ablation by pure antiestrogen treatment, it should be remembered that the agonist activity of tamoxifen may confer certain advantages, particularly in terms of ameliorating the development of osteoporosis and cardiovascular disease in the adjuvant setting (4). Whether the use of pure antiestrogens would sacrifice such advantages must await clinical trials, but studies of effects of pure antiestrogens on bone density in rats have shown that uterine regression can be achieved without bone loss.[3] Such studies emphasize that important differences of sensitivity to estrogen and antiestrogen effects exist between different peripheral sites of action as well as between peripheral and central targets.

ACKNOWLEDGMENTS

We thank B. Curry, L. Green, E. Monk, J. Morrell, E. Newboult, J. Pittam, S. Peters, R. Stevenson, J. Tattershall, and L. Yarwood for their assistance with these studies.

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[3] M. Dukes and A. E. Wakeling, unpublished studies.

A Potent Specific Pure Antiestrogen with Clinical Potential

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Cancer Res 1991;51:3867-3873.