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ICI 182,780, A NEW ANTIOESTROGEN WITH CLINICAL POTENTIAL

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Summary—Previous studies in this laboratory identified a series of 7α-alkylamide analogues of 17β-oestradiol which are pure antioestrogens. Among this initial lead series of compounds, exemplified by ICI 164,384, none was of sufficient *in vivo* potency to merit serious consideration as a candidate for clinical evaluation. Further structure–activity studies identified a new compound, ICI 182,780, 7α-[9-(4,4,5,5,5-pentafluoro-pentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β-diol, with significantly increased antioestrogenic potency. The antiuterotrophic potency of ICI 182,780 is more than 10-fold greater than that of ICI 164,384. ICI 182,780 has no oestrogen-like trophic activity and, like ICI 164,384 is peripherally selective in its antioestrogenic effects. The increased *in vivo* potency of ICI 182,780 was also reflected, in part, by intrinsic activity at the oestrogen receptor and in the growth inhibitory potency of ICI 182,780 in MCF-7 human breast cancer cells. 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. Sustained antioestrogenic effects of ICI 182,780, following a single parenteral dose of ICI 182,780 in oil suspension, were apparent in both rats and pigtail monkeys. *In vivo*, the antitumour activity of ICI 182,780 was demonstrated with xenografts of MCF-7 and Br10 human breast cancers in athymic mice where, over a 1 month period, a single injection of ICI 182,780 in oil suspension achieved effects comparable with those of daily tamoxifen treatment. Thus, ICI 182,780 provides the opportunity to evaluate clinically the potential therapeutic benefits of complete blockade of oestrogen effects in endocrine-responsive human breast cancer.

INTRODUCTION

Nonsteroidal partial-agonist antioestrogens like tamoxifen (ICI 46,474: Nolvadex†) provide excellent palliative treatment for breast cancer [1, 2]. However, the diversity of the biological actions of such compounds which range between full agonist, oestrogen-like trophic effects, through partial agonism to complete blockade of oestrogen action [3], raises the issue of whether their clinical efficacy is in any way limited, compared with that which might be achieved by complete oestrogen ablation. None of the endocrine treatments currently available for breast cancer can remove completely the trophic influences of endogenous or exogenous (e.g. of dietary origin) oestrogens. Potentially, antagonist molecules which bind to oestrogen receptors (ER) with high affinity without activating any of the normal transcriptional hormone responses, would offer the chance of achieving complete blockade of oestrogen action, whatever the source of the oestrogenic stimulus. Such pure antioestrogen molecules would be distinctively different from tamoxifen-like ligands. The first examples of such compounds have been described elsewhere [4–6]. The prototype pure antioestrogen, ICI 164,384, *N*-*n*-butyl-*N*-methyl-11-(3, 17β-dihydroxyoestra-1,3,5(10)-triene-7α-yl)undecanamide (Fig. 1), is devoid of stimulatory activity and completely blocks the trophic actions of oestrogens, and of the partial-agonist antioestrogens, in all oestrogen-responsive cell and animal models examined to date (see Ref. [7] for a review).

Here, we describe a new pure antioestrogen, ICI 182,780, 7α-[9-(4,4,5,5,5-pentafluoro-pentylsulphinyl)nonyl]oestra-1,3,5(10)-triene-3,17β diol (Fig. 1), with a profile of activity which makes it a prime candidate for clinical efficacy studies in oestrogen-responsive breast cancer.

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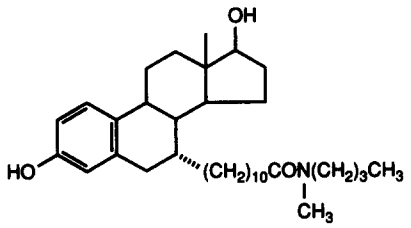
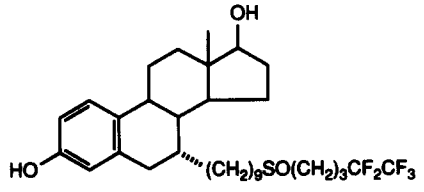
†Nolvadex is a Trade Mark, the property of Imperial Chemical Industries Plc.



ICI 164,384



ICI 182,780

Fig. 1. Structure of pure antioestrogens.

OESTROGENIC AND ANTIOESTROGENIC ACTIVITY

In rats and mice, ICI 182,780 was devoid of uterotrophic activity and, when co-administered with oestradiol, completely blocked the uterotrophic action of oestradiol in a dose-dependent manner (see [8] for rat and Fig. 2 for mouse data). The order of magnitude potency advantage of ICI 182,780, compared with ICI 164,384 is shown in Fig. 3. Complete blockade of oestrogen action was achieved with a dose of 0.5 mg ICI 182,780/kg/day s.c. The uterotrophic action of tamoxifen was also blocked in a dose-dependent manner by co-adminstration of ICI 182,780 [8].

In adult female rats increasing daily doses of ICI 182,780 reduced the weight of the uterus in a dose-dependent fashion (Table 1). At the highest dose in this study, 1 mg/kg/day, involution of the uterus after 14 days was comparable with 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, serum LH (Table 1), FSH and prolactin concentrations [8] were largely unaffected, indicating a peripherally selective action of ICI 182,780.

A comparison of the oral and parenteral antiuterotrophic potency of ICI 182,780 indicated that the oral bioavailability of the compound is relatively poor [8]. It is known that many steroids administered orally are subject to rapid metabolism by the liver and subsequent excretion. A well-established procedure to mitigate such effects is to administer steroids parenterally in oil. Such formulations often have a sustained duration of action. This procedure was effective in the case of ICI 182,780 where a bolus dose in arachis oil sustained antioestrogenic activity for in excess of 1 month in both rats and monkeys [8].

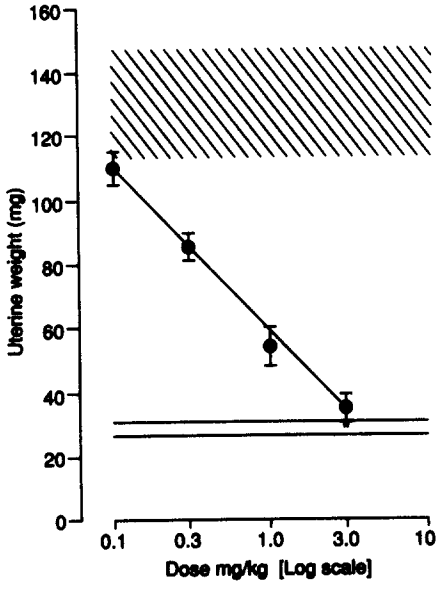


Fig. 2. Effects of ICI 182,780 on uterine weight of ovariectomized mice. Groups of 5 adult mice ovariectomized 2 weeks before treatment received daily, a single dose of arachis oil vehicle alone (open bar), 0.5 μg 17β-oestradiol benzoate s.c. alone (hatched bar), or the indicated doses of ICI 182,780 s.c. together with oestradiol (continuous line), for 3 days. The effect of 3 mg/kg ICI 182,780 alone is indicated by *.

ER INTERACTION

The competitive inhibition, by ICI 182,780, of oestrogen and tamoxifen uterotrophic effects is

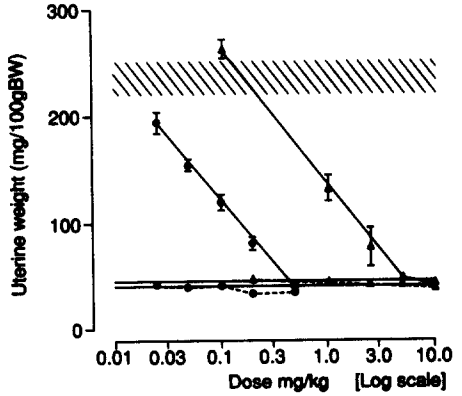


Fig. 3. Comparative antiuterotrophic activity of ICI 164,384 (▲) and ICI 182,780 (●). Experimental procedure as described for Fig. 2 except that immature rats were used.

Table 1. Effect of ovariectomy or 14 days' treatment with ICI 182,780 on uterine and body weight and plasma LH of adult female rats

Parameter	Intact control	OVX control	182,780 mg/kg/day, s.c. 0.03	0.1	0.3	1.0
Uterine weight (g)	292.2 ± 33.7	75.6 ± 4.2*	253.4 ± 21.2	180.4 ± 21 1*	132.2 ± 10.8*	98.0 ± 6.1*
Body weight (g)	40.0 ± 2.5	64.8 ± 1.9*	43 6 ± 2.5	44.6 ± 1 7	45.8 ± 2.0	42.6 ± 2.1
LH (ng/ml)	2.4 ± 0.6	19.7 ± 2.2*	2.1 ± 0.2	1.2 ± 0.1	1.0 ± 0.1	2.3 ± 0.3

Values are mean ± SEM, $n = 5$ *$P < 0.001$ cf intact control.

consistent with each class of ligand acting through an ER-mediated pathway. Formal proof of this is provided by the capacity of ICI 182,780 to compete with [^{3}H]oestradiol for binding to rat uterine ER in a concentration-dependent manner [8]. IC_{50} values of 0.83, 0.94 and 4.8×10^{-8}M were recorded for oestradiol, ICI 182,780 and ICI 164,384, respectively with equivalent relative binding affinities of 0.89 and 0.19 for ICI 182,780 and ICI 164,384, respectively, compared with oestradiol = 1.

BREAST CANCER CELL GROWTH INHIBITION

ICI 182,780 inhibited 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. The growth inhibitory action of ICI 182,780 on MCF-7 cells was reversed in a competitive manner by oestradiol [8]. A comparison of the effect of ICI 182,780 with that of other antioestrogens on the growth of MCF-7 cells (Fig. 4) 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 (approx. 80% cf 50%, Fig. 4).

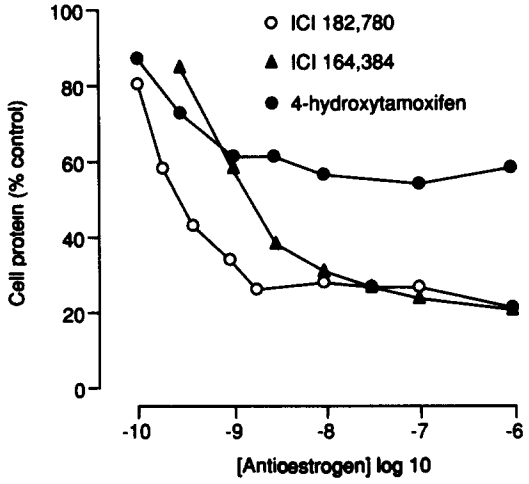


Fig. 4. Effects of ICI 164,384, ICI 182,780 and 4′-hydroxytamoxifen on the proliferation of MCF-7 human breast cancer cells. Cells were plated in 24-well dishes (4×10^4/well) and cultured for 2 days in MEM with 5% charcoal stripped foetal calf serum containing phenol red and insulin but no additional oestrogen. 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 antioestrogens added in ethanol (1 μl/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 5. Points are the mean of quadruplicate observations where SEM was <5%.

Flow cytometric analysis of cell cycle and population distribution of MCF-7 cells treated with tamoxifen or ICI 182,780 showed that both antioestrogens caused accumulation of cells in G_0/G_1 and also reduced the proportion of cells capable of continued DNA synthesis. However, the maximal efficacy of ICI 182,780 compared with that of tamoxifen, when both compounds were used at optimum antioestrogenic (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 [8].

ANTITUMOUR EFFICACY

The growth of xenografts of MCF-7 human breast cancer cells, supported by continuous treatment with ethynyl oestradiol, was blocked completely for at least 4 weeks by a single s.c. injection of 5 mg ICI 182,780 in oil suspension. The magnitude of this effect was comparable with that in animals treated continuously with a high dose of tamoxifen. Similarly, the growth of transplants of the Br10 solid human breast tumour was also suppressed effectively by ICI 182,780 [8].

CONCLUSIONS

In comparison with the first reported pure antioestrogen ICI 164,384, ICI 182,780 demonstrates substantially increased potency. This is clearly manifest *in vivo* where, in anti-uterotrophic assays, ICI 182,780 was at least an order of magnitude more potent than ICI 164,384 (ED_{50}s = 0.06 and 0.9 mg/kg, respectively). *In vitro*, the intrinsic potency difference appears somewhat less, for example there is

only a 4- to 5-fold advantage for ICI 182,780 in terms of affinity for ER. The apparent 2-fold difference in potency ratio improvement between *in vitro* and *in vivo* assays for the two compounds is probably a reflection of differences in their distribution and metabolism. The order of magnitude lower potency between the oral and parenteral routes of administration suggests strongly that the oral bioavailability of ICI 182,780 is relatively low. A common means of circumventing the practical constraints consequent on the poor oral 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, tumour growth ceased for at least 1 month after a single injection of ICI 182,780 [8].

Of particular relevance to the therapeutic potential of ICI 182,780 are the enhanced efficacy compared with 4′-hydroxytamoxifen (or tamoxifen) on breast tumour cells and the excellent antiuterotrophic action achieved without affecting body weight and gonadotrophin secretion. 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 oestrogen action, a property shared with ICI 164,384 [9]. If translated to the clinical setting, this peripheral selectivity of action would obviate blockade of central negative oestrogen feedback and consequent increases of oestrogen production in the premenopausal patient. Also, such selectivity would be particularly advantageous in the treatment of benign uterine and breast pathologies in premenopausal patients.

In respect of the enhanced efficacy of pure antioestrogens against tumour cell growth *in vitro*, 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 oestrogenic effect of the partial agonists which, although small [10, 11], is amplified synergistically by the concurrent presence of other breast cell mitogens like insulin [11] and IGF-1 [12]. The pure antioestrogens obviate such effects. The corollary of these data in the clinical setting is the possibility that differences of antitumour efficacy between tamoxifen and pure antioestrogens may be greater than otherwise anticipated.

In summary, ICI 182,780 offers significant advantages compared with pure antioestrogens reported previously, particularly with respect to *in vivo* potency. Although oral potency appears to be limited, probably as a result of poor absorption, this disadvantage is offset by the sustained antioestrogenic and antitumour 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 [7, 13–15] indicate that pure antioestrogens may find a valuable place in the treatment of breast cancer. ICI 182,780 will be used to test this proposition.

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