Cancer Chemother Pharmacol (2001) 47: 437–443
DOI 10.1007/s002800000259

ORIGINAL ARTICLE

**Mark E. Nuttall · Israil Pendrak · John G. Emery
Daniel P. Nadeau · Paul W. Fisher · Thema A. Nicholson
Yuan Zhu · Larry J. Suva · William D. Kingsbury
Maxine Gowen**

Antagonism of oestrogen action in human breast and endometrial cells in vitro: potential novel antitumour agents

Received: 5 July 2000 / Accepted: 16 November 2000 / Published online: 6 February 2001

Abstract *Purpose:* There is a need to find novel oestrogen receptor (ER) ligands that antagonize oestrogen action in the reproductive tissues and would therefore have therapeutic potential in oestrogen-dependent tumours. We tested novel ER ligands in both breast and endometrial cells to profile agonism/antagonism in these oestrogen target reproductive tissues. *Methods:* Novel analogues of the ER antagonist ICI 182,780 were synthesized and tested for their ability to inhibit gene expression dependent on oestrogen response elements (ERE) in human breast (MCF-7) and endometrial (Ishikawa) cell lines. This activity was correlated with inhibition of oestrogen-induced cell proliferation and ER binding. *Results:* The sulphide analogue (compound 1) and sulphone analogue (compound 2) had no intrinsic ERE-dependent agonism in either breast cancer or endometrial cells in culture. All three compounds dose-dependently inhibited ERE-mediated oestrogen agonism. Moreover, these ER ligands inhibited oestrogen-stimulated proliferation of breast cancer and endometrial cells. ICI 182,780, compound 1 and compound 2 were all able to bind both isoforms of the ER (ERα and ERβ). In endometrial cells, the relative binding to ERβ correlated with the ERE-dependent antioestrogenic effect of these ligands, suggesting that in this tissue this receptor is the predominant isoform that determines antioestrogenic activity. *Conclusions:* The ability of these analogues of ICI 182,780 to inhibit oestrogen-stimulated transcriptional activity and cell proliferation suggests that these agents, in particular the sulphone analogue, have therapeutic potential in the treatment of breast cancer without exhibiting the unwanted oestrogenic effects in the endometrium.

Key words Oestrogen · Steroid · Breast · Antioestrogen · Hormone

M. E. Nuttall (✉) · J. G. Emery · D. P. Nadeau · P. W. Fisher
T. A. Nicholson · L. J. Suva · M. Gowen
Department of Bone and Cartilage Biology,
SmithKline Beecham Pharmaceuticals, 709 Swedeland Road,
King of Prussia, Pennsylvania 19406, USA
E-mail: Mark_E_Nuttall@sbphrd.com
Tel.: +1-610-2704998
Fax: +1-610-2705598

I. Pendrak · W. D. Kingsbury
Department of Medicinal Chemistry,
SmithKline Beecham Pharmaceuticals, 709 Swedeland Road,
King of Prussia, Pennsylvania 19406, USA

Y. Zhu
Department of Molecular Biology,
SmithKline Beecham Pharmaceuticals, 709 Swedeland Road,
King of Prussia, Pennsylvania 19406, USA

Introduction

Although oestrogen is clinically useful in preventing bone loss, it exhibits tissue-specific side effects including endometrial hyperplasia which results in uterine cancer, and proliferative effects in mammary tissue which result in an increased risk of breast cancer [1, 3, 4]. The ideal postmenopausal 'oestrogen' would reproduce the beneficial effects of oestrogen on vasomotor symptoms, skeletal tissue and the cardiovascular system without producing the adverse effects of oestrogen on reproductive tissues. This idea has led to the development of selective oestrogen receptor (ER) modulators (SERMs), which are defined as compounds that have oestrogen agonism in one or more of the desired target tissues, such as bone or liver, and antagonism and/or minimal agonism (i.e. clinically insignificant) in reproductive tissue such as the breast or uterus [14, 16, 32]. The first of these, tamoxifen, is the only antioestrogen used widely for the treatment of breast cancer in women and behaves as a mixed agonist/antagonist of oestrogen action, thus potentially limiting its therapeutic potential for the treatment of breast cancer [16]. Attempts to improve on the pharmacological profile of tamoxifen have resulted in compounds which differ in their oestrogen agonist/antagonist characteristics [14, 23, 29].

The next generation of antioestrogens include the oestrogen antagonist ICI 182,780 [17, 28, 29]. ICI

182,780 is a C7-substituted analogue of oestrogen that has been shown to fully antagonize the trophic effects of endogenous oestrogen in the uterus in intact rats and to prevent bone loss following ovariectomy [26, 29]. In an effort to investigate the comparative antagonism of ICI 182,780, as well as other potential SERMs, we synthesized analogues of this pure oestrogen antagonist [28] and tested them for their activity in vitro. We rationalized that chemical modification of the sulphide group may enhance the antioestrogenic effect of ICI 182,780 in in vitro cell models, and this was indeed the case with enhanced ER binding correlating with antioestrogenic activity. We profile the oestrogen antagonism of two of these potentially novel SERMs in both breast and endometrial cells. The in vitro data reported here suggest that these analogues, particularly the sulphone derivative of ICI 182,780, may have therapeutic potential in the treatment of breast cancer.

Materials and methods

Cell culture and materials

All chemicals were purchased from Sigma (St. Louis, Mo.) unless otherwise stated. ICI 182,780 and analogues 1 and 2 were prepared according to published procedures [2]. Endometrial Ishikawa cells (kindly provided by Dr. V.C. Jordan, Northwestern, Chicago) [10], human breast cancer cells (MCF-7; ATCC, HTB-22) and human cervical carcinoma cells (Hela; ATCC, CCL-2) were grown routinely in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, Utah), penicillin (10 U/ml) and streptomycin (50 ng/ml) (Gibco BRL Technologies, Grand Island, N.Y.). All experiments were performed in phenol red-free Eagle's modified minimal essential medium (EMEM) containing 10% heat-inactivated charcoal dextran-stripped fetal bovine serum (Hyclone) and supplemented as above. Cells were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO_2. At confluence, cells were subcultured after exposure to trypsin-EDTA (Gibco).

Plasmid construction

The ERα and ERβ mammalian expression constructs were prepared by PCR cloning cDNAs encoding human ERα or ERβ open reading frames into pCR3.1 (Invitrogen, San Diego, Calif.). The template for the ERα cDNA was pRST7-ERα, a generous gift from Donald McDonnell (Duke University) [30]. This cDNA contained a point mutation changing valine 400 to glycine, which was corrected by site-directed mutagenesis prior to cloning in pCR3.1. The ERβ cDNA was generated by RT-PCR from brain tissue and corresponds to the sequence in GenBank accession number X99101 [20].

Transient transfections

Cells were seeded either in six-well plates at 1.5×10^5 cells/well or in 24-well plates at 1.5×10^4 cells/well in phenol red-free medium. DNA was introduced into the breast cancer MCF-7 cell line by the lipofectin method (Life Technologies, Gaithersburg, Md.) and into the endometrial Ishikawa cell line by the calcium phosphate method (Invitrogen, San Diego, Calif.). Briefly, cells were co-transfected with 1 μg per well in six-well plates and 833 ng per well in 24-well plates of either MMTV-ERE(5)-*Luc* or C3-ERE(3)-*Luc* and 25 ng of the control renilla-Luciferase vector (pRL-CMV). MMTV-ERE(5)-*Luc* is a DNA reporter construct comprising the mouse mammary tumour virus promoter, in which the glucocorticoid response elements have been replaced with five copies of a 33-bp vitellogenin ERE cloned upstream of the luciferase reporter gene (provided by D. McDonnell, Duke University) [30]. C3-ERE(3)-*Luc* is the natural complement 3 containing three nonconsensus EREs cloned upstream of the luciferase reporter gene (provided by D. McDonnell) [22]. Transfection efficiency was corrected by cotransfection with a renilla-luciferase vector (Promega, Madison Wis.). Cells were incubated overnight. The transfection medium was then removed and cells were incubated for 48 h with or without hormones as indicated in the figure legends.

Cell lysates were prepared as described in the manufacturer's protocol for the dual luciferase reporter assay to assess transfection efficiency (Promega). Briefly, cells were washed in PBS and then lysed with 1 × passive lysis buffer (500 μl/well for six-well plates or 100 μl/well for 24-well plates) for 15 min while rocking the sample on a rocking platform. Lysates were centrifuged for 30 s at 14,000 *g* and the clear lysate was transferred to a tube prior to reporter enzyme analysis. Samples (20 μl) were transferred to a 96-well luminescence detection plate and reacted with 100 μl of each assay reagent (Promega). Each assay reagent was injected by a microlumat LB96P luminometer (Wallac, Gaithersburg, Md.), which measured luciferase activity. The results are expressed as relative light units (RLU).

Cell proliferation

MCF-7 or Ishikawa cells were seeded in 48-well plates at $5{\times}10^4$ cells/well in phenol red-free medium containing 10% heat-inactivated charcoal-stripped fetal bovine serum, and left overnight. The following day, the cells were washed with PBS and the medium was changed to phenol red-free medium containing 1% serum, and incubated for an additional 24 h. Cells were then treated with ligands for 48 h. Following treatment, 1 μCi ^{3}H-thymidine was added to each well of the 48-well plate and the cells were incubated for 6 h. The cells were then washed with ice-cold PBS and cell lysates were prepared by adding 100 μl 0.1 *N* NaOH for 15 min. The samples were then transferred to scintillation vials containing 10 ml scintillation cocktail for analysis.

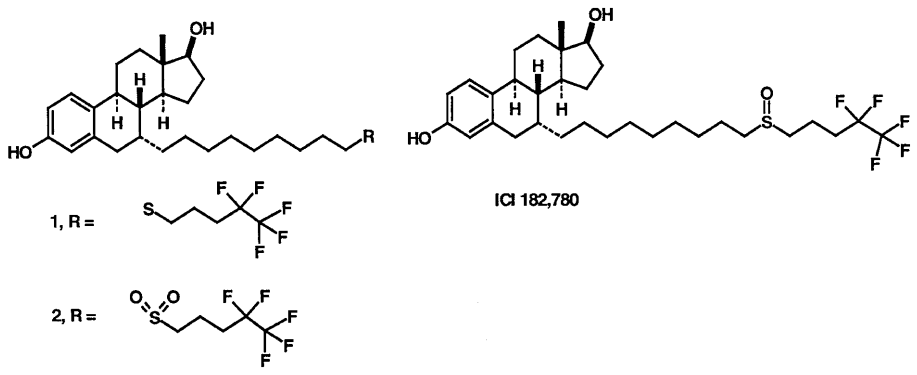
ER competition binding assays

Hela cells were used for oestrogen binding studies based on the fact that they do not express either isoform of the receptor endogenously. Hela cells were transfected with pCR3.1 ERα or pCR3.1 ERβ using Lipofectamine Plus (Life Technologies) according to the manufacturer's instructions. After 24 h the cells were removed from the plate with 200 m*M* EDTA and washed three times in PBS. Cytoplasmic extracts were prepared as described previously [18], except that the cells were resuspended in buffer A (25 m*M* Tris-HCl, 1.5 m*M* EDTA, 10 m*M* α-monothioglycerol, 10% glycerol, 10 m*M* sodium molybdate, pH 7.4) containing 0.2 U/ml aprotinin, 1 m*M* PMSF, and 10 μg/ml leupeptin at a concentration of 1 ml per 100 mg wet cell pellet [18]. Lysis was performed by dounce instead of polytron homogenization.

Unlabelled competitor diluted in 25 μl buffer A with 0.1% charcoal-stripped bovine serum albumin (BSA) was combined with 25 μl 24 n*M* [2,4,6,7-^{3}H]oestradiol (Amersham-Pharmacia) in 96-well U-bottomed microtitre dishes. Cytoplasmic extract (50 μl at 0.7 mg/ml) from Hela cell transfectants was added, and the plates were incubated at 4°C for 18 h. Unbound tritiated oestrogen was removed by adding 100 μl 0.5% dextran-coated charcoal (Sigma) in buffer A with 0.1% BSA, agitating for 15 min at 4°C, and centrifuging at 500 *g* for 5 min. A 100-μl aliquot of the supernatant was counted in 10 ml Ready Safe scintillation fluid (Beckman) in a Beckman Model LS5801 scintillation counter.

Specific binding was determined by subtracting the counts per minute bound in the presence of a 100-fold molar excess of dieth-

Fig. 1 Structures of ICI 182,780, and compounds 1 and 2



ylstilboestrol. The percentage of specific binding is expressed as specifically bound counts per minute in the presence of the indicated concentration of competitor divided by the specifically bound counts per minute in the absence of competitor × 100. The averages of triplicate data points are presented.

Statistical analysis

Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. All statistical analyses were carried out using Statistica for Windows version 5.1 (Statsoft, Tulsa, Okla.).

Results

ICI 182,780, as well as compounds 1 and 2 (Fig. 1), had no intrinsic ERE-dependent agonist transcriptional activity in endometrial cells (Fig. 2). ICI 182,780, and compounds 1 and 2 inhibited ERE-dependent agonism by oestrogen in human endometrial cells in vitro (Fig. 2). ICI 182,780 had an IC_{50} of 1.1 ± 3 n*M* in inhibiting the agonist response of oestrogen (1 n*M*) in Ishikawa endometrial cells, compound 1 an IC_{50} of 67 ± 8 n*M* and compound 2 an IC_{50} of 0.3 ± 3 n*M* (Fig. 2). This indicates similar potency for ICI 182,780 and compound 2 in the inhibition of ERE-dependent agonism of oestrogen in human endometrial cells in vitro. Compound 1 was the least potent compound tested (antagonism of estrogenic action: ICI 182,780 ≈ compound 2 > compound 1).

We next sought to determine whether the effects of these compounds on ERE-dependent gene transcription translated into a measurable cellular endpoint by investigating proliferation of Ishikawa endometrial cells in the presence of these ER ligands. As expected, based on the lack of ERE-dependent agonism in these cells, ICI 182,780 and compounds 1 and 2 did not stimulate but significantly inhibited cell proliferation compared with vehicle control (Fig. 3). In contrast oestrogen was a potent stimulator of endometrial cell proliferation (Fig. 3). In competition studies all three compounds dose-dependently inhibited oestrogen-stimulated cell proliferation (Fig. 3). The rank order of potency in the proliferation assay was similar to the ability of the compounds to inhibit oestrogen-induced ERE-dependent reporter gene activation (ICI 182,780 ≈ compound 2 > compound 1; Figs. 2 and 3).

We next extended these studies to examine the effect of ICI 182,780 and compounds 1 and 2 on both ERE-dependent reporter gene activation and the proliferation of human breast cancer (MCF-7) cells. Similar to the findings in human endometrial cells in culture, ICI 182,780 and compounds 1 and 2 had no agonist activity on ERE-dependent reporter gene activation. All three compounds inhibited oestrogen-stimulated reporter gene expression

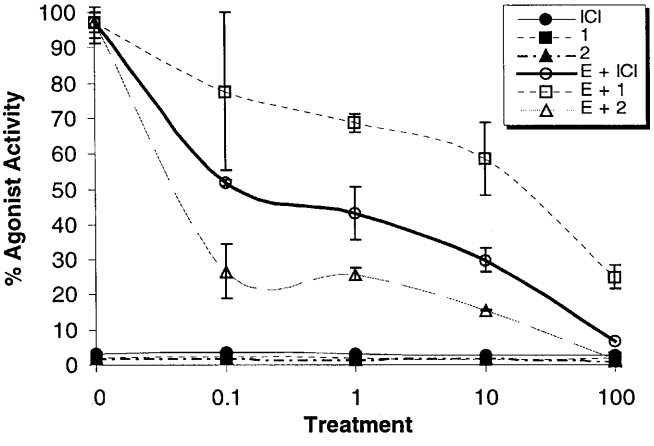


Fig. 2 The effects of oestrogen, ICI 182,780, and compounds 1 and 2 on transcription through the ERE in human endometrial cells (Ishikawa). Human endometrial adenocarcinoma (Ishikawa) cells were transfected with the MMTV ERE(5)-*Luc* reporter plasmid as described in Materials and methods. After transfection, cells were treated with ICI 182,780, compound 1 or compound 2 in the presence or absence of 1 n*M* oestrogen for 48 h. ERE-dependent luciferase reporter activation was measured in relative light units (RLU) as described in Materials and methods. Oestrogen produced a significant increase in luciferase reporter activation. In contrast, ICI 182,780, compound 1 and compound 2 showed no significant agonist activity. The maximal agonist response is considered to be 100% (ordinate) with 1 n*M* oestrogen treatment. Significantly, when compounds were added to the cells in the presence of oestrogen, ICI 182,780 and compounds 1 and 2 all antagonized oestrogen-induced gene transcription through the ERE. The results are presented as means ± SD ($n = 3$); $^*P < 0.05$ compared with agonism of oestrogen alone (the levels of significance of individual compound treatments were determined in relation to the vehicle control)

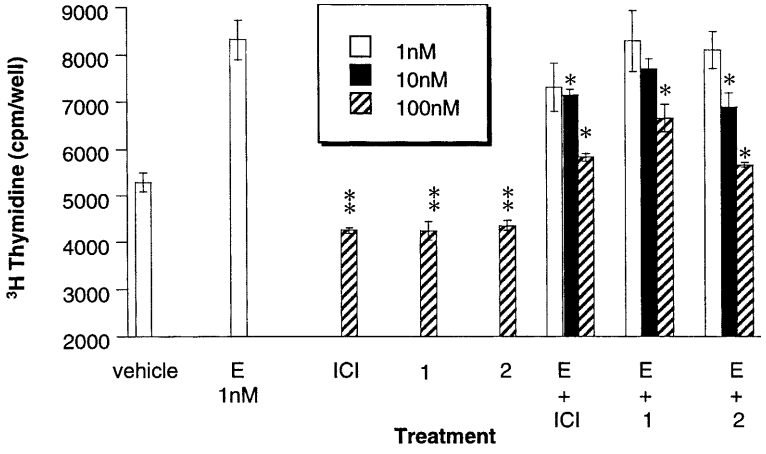


Fig. 3 The effects of ICI 182,780 on proliferation of human endometrial (Ishikawa) cells. Cells were plated and grown as described in Materials and methods with or without ER ligands for 48 h. ICI 182,780, compound 1 and compound 2 all significantly inhibited proliferation compared with vehicle control and also antagonized proliferation stimulated by 1 n*M* oestrogen. The results are presented as means ± SD, $n = 3$ (*$P < 0.05$ vs oestrogen treatment alone, **$P < 0.05$ vs vehicle treatment alone)

in MCF-7 cells (Fig. 4). ICI 182,780 had an IC_{50} of 7 ± 4 n*M* in inhibiting the agonist response of oestrogen (1 n*M*) in MCF-7 breast cancer cells, compound 1 was a poor antagonist of oestrogen action and at the maximum concentration was unable to reach 50% inhibition, and compound 2 had an IC_{50} of 13 ± 7 n*M* (Fig. 4). Similar to the findings in endometrial cells, all three compounds significantly inhibited basal proliferation and, indeed, inhibited oestrogen-induced breast cancer cell proliferation (Fig. 5). The rank order potency of these compounds in MCF-7 cells was similar to that observed in human endometrial Ishikawa cells (ICI 182,780 ≈ compound 2 > compound 1; Figs. 2, 3, 4 and 5).

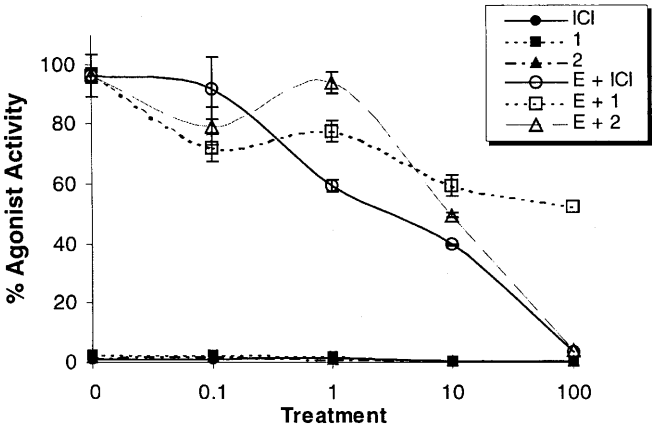


Fig. 4 The effects of oestrogen, ICI 182,780 and compounds 1 and 2 on transcription through the ERE in human breast cells (MCF-7). Human breast (MCF-7) cells were transfected with the MMTV ERE(5)-*Luc* reporter plasmid as described in Materials and methods. Cells were transfected and treated with oestrogen, ICI 182,780, compound 1 or compound 2 for 48 h. ERE-dependent luciferase reporter activation was measured in relative light units (RLU) as described in Materials and methods. Oestrogen (1 n*M*) produced a significant increase in luciferase reporter activation. In contrast, ICI 182,780, compound 1 and compound 2 had no significant agonist activity. The maximal agonist response is considered to be 100% (ordinate) with 1 n*M* oestrogen treatment. Significantly, when compounds were added to the cells in the presence of oestrogen (1 n*M*), ICI 182,780 and compounds 1 and 2 all antagonized oestrogen-induced reporter gene transcription through the ERE. The results are presented as means ± SD, $n = 3$ (*$P < 0.05$ vs agonism of oestrogen alone; the levels of significance of individual compound treatments were determined in relation to the vehicle control)

These ERE-dependent effects seen with five copies of the 33-bp vitellogenin ERE were also observed with the natural human complement (C3) promoter containing three nonconsensus EREs [22] in both Ishikawa (Fig. 6) and MCF-7 (Fig. 7) cells. These findings suggest that the agonistic/antagonistic effects of these ligands observed in these cell systems were not dependent on the number of EREs (Figs. 6 and 7).

To determine whether the relative antioestrogenic effects of ICI 182,780 and the related compounds were due to their affinity for the ERs (α and β), we determined the relative affinity of each ligand for ERα and ERβ in competition binding assays with radiolabelled oestrogen. The affinity of ICI 182,780 for ERα was 17 times lower than that of 17β-oestradiol, with a relative binding affinity (RBA) of 5.9 (Fig. 8A, Table 1). Compounds 1 and 2 bound ERα with slightly lower affinities, with RBAs of 2.7 and 3.5, respectively. Compound 2 reproducibly bound with greater affinity than compound 1 (Fig. 8A, Table 1). Similar to ERα, all three compounds bound ERβ weakly compared to 17β-oestradiol. Compound 2 had the highest affinity, with an RBA of 4.5 (Fig. 8B, Table 1). ICI 182,780 and compound 1 bound ERβ with slightly lower affinities, with RBAs of 3.0 and 2.0, respectively.

Discussion

ICI 182,780 and compounds 1 and 2 were all able to antagonize both oestrogen-induced ERE-dependent gene activation and oestrogen-induced proliferation in both human breast cancer cells and endometrial cells in culture. The effects of ER ligands on cell proliferation have been reported as the mechanistic explanation for the unwanted side effects seen in the reproductive tissues, such as endometrial hyperplasia and breast tumour proliferation [7, 8, 11, 12]. Compound 2 had the highest affinity for ERβ (Fig. 8B, Table 1) and this correlated with antagonism of ERE-dependent oestrogen agonism and proliferation in endometrial and breast cancer cells,

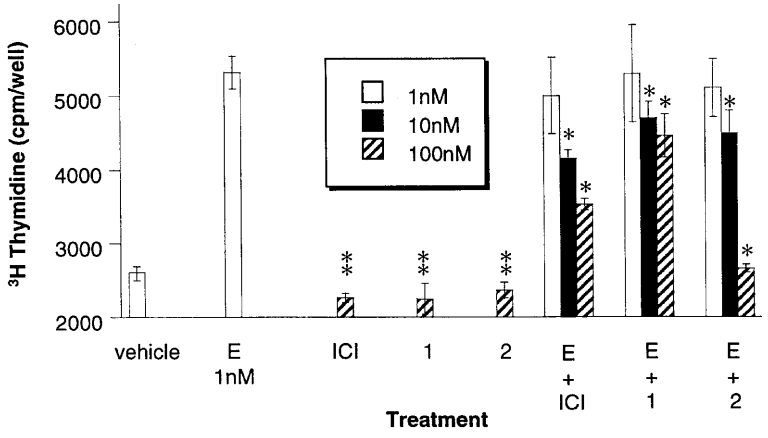


Fig. 5 The effects of ICI 182,780 on proliferation of human breast (MCF-7) cells. Cells were plated and grown as described in Materials and methods with or without ER ligands for 48 h. ICI 182,780, compound 1 and compound 2 all significantly inhibited proliferation in the presence and absence of oestrogen (1 n*M*). Results are expressed as means ± SD, $n = 3$ (*$P < 0.05$ vs oestrogen treatment alone, **$P < 0.05$ vs vehicle treatment, unpaired Student's *t*-test)

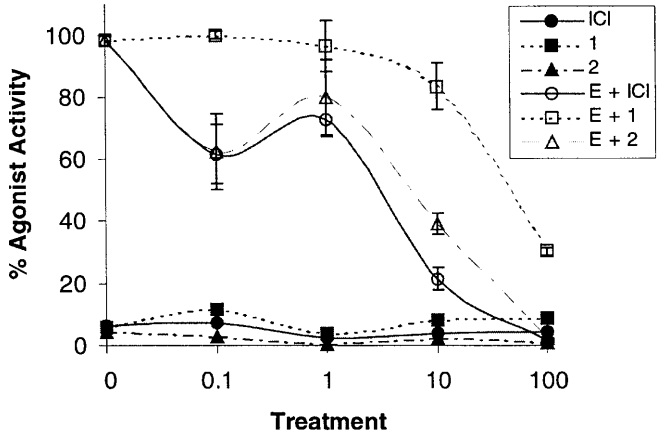


Fig. 6 The effects of oestrogen, ICI 182,780 and compounds 1 and 2 on transcription through the complement 3 ERE in human endometrial cells (Ishikawa). Human endometrial adenocarcinoma (Ishikawa) cells were transfected with the C3-ERE(3)-*Luc* reporter plasmid as described in Materials and methods. After transfection, cells were treated with ICI 182,780, compound 1 or compound 2 in the presence or absence of 1 n*M* oestrogen for 48 h. ERE-dependent luciferase reporter activation was measured in relative light units (RLU) as described in Materials and methods. Oestrogen produced a significant increase in luciferase reporter activation. In contrast, ICI 182,780, compound 1 and compound 2 had no significant agonist activity. The maximal agonist response is considered to be 100% (ordinate) with 1 n*M* oestrogen treatment. Significantly, when compounds were added to the cells in the presence of oestrogen, ICI 182,780 and compounds 1 and 2 all antagonized oestrogen-induced gene transcription through the ERE. The results are presented as means ± SD, $n = 3$ (*$P < 0.05$ vs agonism of oestrogen alone; the levels of significance of individual compound treatments were determined in relation to the vehicle control)

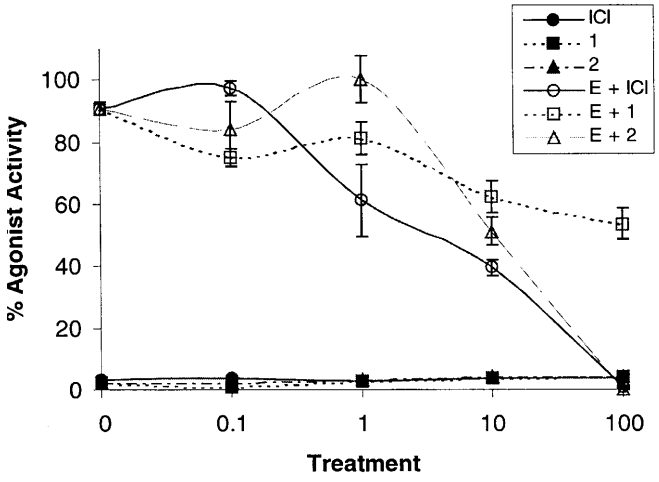


Fig. 7 The effects of oestrogen, ICI 182,780 and compounds 1 and 2 on transcription through the complement 3 ERE in human breast cells (MCF-7). Human breast (MCF-7) cells were transfected with the C3-ERE(3)-*Luc* reporter plasmid as described in Materials and methods. Cells were transfected and treated with oestrogen, ICI 182,780, compound 1 or compound 2 for 48 h. ERE-dependent luciferase reporter activation was measured in relative light units (RLU) as described in Materials and methods. Oestrogen (1 n*M*) produced a significant increase in luciferase reporter activation. In contrast, ICI 182,780, compound 1 and compound 2 had no significant agonist activity. The maximal agonist response is considered to be 100% (ordinate) with 1 n*M* oestrogen treatment. Significantly, when compounds were added to the cells in the presence of oestrogen (1nM), ICI 182,780 and compounds 1 and 2 all antagonized oestrogen-induced reporter gene transcription through the ERE. The results are presented as means ± SD, $n = 3$ (*$P < 0.05$ vs oestrogen agonism alone; the levels of significance of individual compound treatments were determined in relation to the vehicle control)

respectively (Figs. 2, 4, 6 and 7). These findings suggest that in the context of this tissue it may be the ability of the antagonist to bind to this ER isoform which determines the potency of oestrogen antagonism. These findings agree with a previous report of a lack of any uterotrophic effect of ICI 182,780 in the oestrogen-deplete condition after ovariectomy in vivo [28].

These results indicate that this in vitro model may be predictive of the effect of these ER ligands in the uterus. This does suggest that potency could be related to affinity of binding to the ER, although this has been previously reported not to be the case in all tissues [13]. It has been reported previously that ICI 182,780 may also inhibit cellular growth through downregulation of insulin receptor substrate 1 in breast cancer cell lines [25] and it remains to be determined whether this is a pathway used by compounds 1 and 2 in their ability to inhibit MCF-7 cell proliferation. We have shown previously that in order for oestrogen to elicit a response via this reporter, it is essential to have a functional ERE [23].

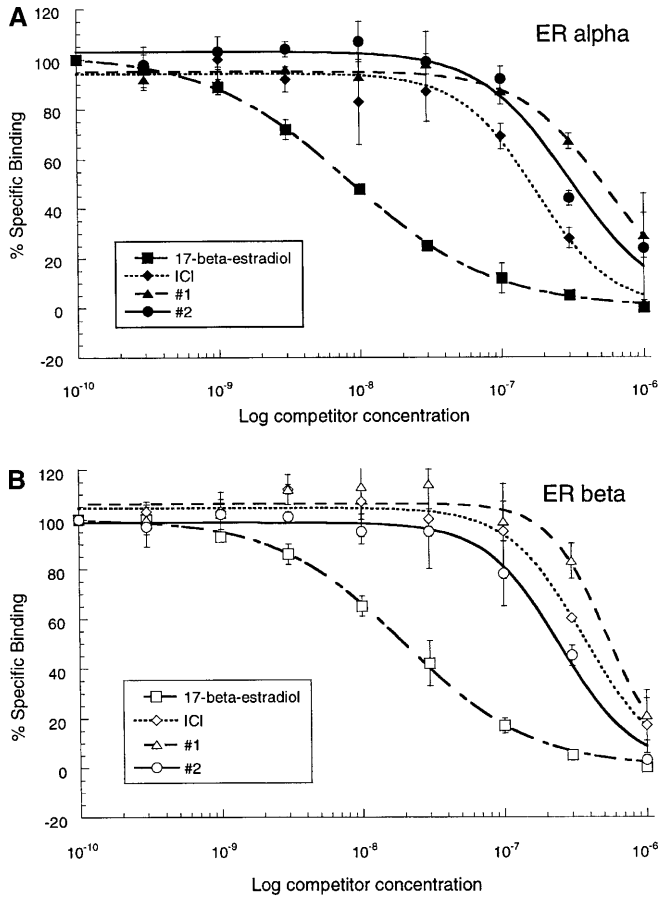


Fig. 8A, B The binding of ER ligands to the ERs. ER binding studies were performed as described in Materials and methods with ERα (**A**) and ERβ (**B**). The results are presented as means ± SD, $n = 3$

Table 1 Relative binding affinity (RBA) of ICI 182,780 and related compounds to ERα and ERβ (RBA is calculated by dividing the IC_{50} value of competitor by that of 17-β-oestradiol and multiplying by 100)

Compound	RBA ERα	RBA ERβ
17-β-estradiol	100	100
ICI 182780	5.9	3.0
Compound 1	2.7	2.0
Compound 2	3.5	4.5

These studies extend classical ER binding since it has been suggested previously that receptor binding does not necessarily correlate with the modulation of gene expression in a given tissue [13]. These assays provide a functional readout of oestrogen agonism, which is downstream of ligand binding.

It has been shown that ER ligands induce unique conformational changes to the ERα that are distinct from oestrogen [9, 19]. These specific ligand-receptor complexes are differentially recognized by the cellular transcriptional machinery in a cell-specific context [13]. ERα contains two transactivation domains, AF-1 and AF-2, and the relative contributions of these domains to receptor function differ from cell to cell. Tamoxifen functions as an AF-2 antagonist, inhibiting ER activity in cells where AF-2 is dominant. In contrast, it functions as an agonist in cells where AF-1 alone is required for activity. These observations provide a mechanistic basis for the cell-specific effects of ER modulators. However, Willson et al. [31] have suggested that this does not strictly correlate in all tissues because in the context of bone, in which ER ligands can be osteoprotective, different degrees of AF-1 and AF-2 agonist activity protect against bone loss. The observation that ICI 182,780 and compounds 1 and 2 are complete antagonists of ERα in endometrial and breast cell lines suggests that these tissues could represent either an AF-1 or AF-2 dominant environment, as ICI 182,780 is known to block both these functions. However, reports of tamoxifen being a mixed agonist/antagonist in the uterus suggest this is not an AF-2-dominant environment.

This mechanistic explanation has become more complex with the discovery of a new ER isoform (ERβ) which has different ligand specificities and tissue distribution [20]. The observation that the isoforms of the ER can heterodimerize has important implications for the tissue-specific effects of ER ligands [5]. Both MCF-7 cells and Ishikawa cells express both ER isoforms. We have estimated that MCF-7 cells express approximately 1.5×10^4 copies of ERα and 6×10^4 copies of ERβ per microgram of RNA, whereas Ishikawa cells express 4×10^6 copies of ERα and 4×10^4 copies of ERβ per microgram of RNA (not shown). Paech et al. [24] have shown that antioestrogens such as tamoxifen, raloxifene and ICI 164,384 can act as agonists with ERβ at AP1 sites. There is approximately 100-fold more ERα than ERβ in Ishikawa cells, but the responses of these two cell lines to compounds 1 and 2 and ICI 182,780 were very similar, suggesting that it is not purely binding to a predominant form of the receptor that determines mechanism of action when comparing these two cell lines directly. In addition to the existence of multiple ERs, the recently discovered nuclear hormone coactivators and corepressors clearly contribute to the tissue selectivity of ER modulators [27].

Recently, distinctions have been made between ICI 18,780 and tamoxifen. For example, Dudley et al. [6] have shown that, unlike tamoxifen which binds through the hormone binding site, the pure antioestrogen ICI 182,780 may cause ERα activation through an allosteric binding site. In addition, unlike tamoxifen, ICI 182,780 inhibits progesterone-induced gene transcription [21] and accelerates the growth of tamoxifen-resistant KPL-1 human breast cancer cells in vivo, but not in vitro [15]. This suggests that the distinct binding (hormone binding site versus allosteric binding sites) have important mechanistic outcomes in the action of these 'antioestrogens'. It remains to be determined where compounds 1 and 2 bind to both isoforms of the ER. However, based on structure one would predict an allosteric-based mechanism of action.

The in vitro data also suggest that ICI 182,780 and closely related analogues, in particular compound 2, may be therapeutic alternatives to the available antioestrogens. However, the relative potency of these compounds in vivo will be determined ultimately by oral bioavailability and pharmacokinetics. The application of ICI 182,780, or one of these analogues as a chemotherapeutic agent in breast cancer will depend on the overall tissue-specific agonist/antagonist activity in vivo. The encouraging data reported with ICI 182,780 (Faslodex) [17, 26] suggest that this compound, and maybe derivatives of this compound, will have a favourable therapeutic profile in vivo.

The full profile of agonism/antagonism of ICI 182,780 and analogues 1 and 2 remain to be determined in vivo. However, in human endometrial and breast cells in vitro they are potent antagonists and thus, in particular the sulphone derivative of ICI 182,780 (compound 2), may provide a favourable alternative to current antioestrogen therapies.

References

1. Barrett-Connor E (1992) Hormone replacement and cancer. Br Med Bull 48:345–355
2. Bowler J, Lilley TJ, Pittman JD, Wakeling AE (1989) Novel steroidal pure antiestrogens. Steroids 54:71–99
3. Colditz GA, Hankinson SE, Hunter DJ, Willett WC, Manson JE, Stampfer MJ, Hennekens C, Rosner B, Speizer FE (1995) The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. N Engl J Med 332:1589–1593
4. Compston JE (1992) HRT and osteoporosis. Brit Med Bull 48:309–314
5. Cowley SM, Hoare S, Mosselman S, Parker MG (1997) Oestrogen receptors α and β form heterodimers on DNA. J Biol Chem 272:19858–19862
6. Dudley MW, Sheeler CQ, Wang H, Khan S (2000) Activation of the human estrogen receptor by the antiestrogens ICI 182,780 and tamoxifen in yeast genetic systems: implications for their mechanism of action. Proc Natl Acad Sci U S A 97:3696–3701
7. Furr BJA, Jordan VC (1984) The pharmacology and clinical uses of tamoxifen. Pharmacol Ther 25:127–205
8. Gottardis MM, Robinson SP, Satyaswaroop PS, Jordan VC (1988) Contrasting actions of tamoxifen on endometrial and breast tumour growth. Cancer Res 48:812–815
9. Grese TA, Sluka JP, Bryant HU, Cullinan GJ, Glasebrook AL, Jones CD, Matsumoto K, Palkowitz AD, Sato M, Termine JD, Winter MA, Yang NN, Dodge JA (1997) Molecular determinants of tissue selectivity in oestrogen receptor modulators. Proc Natl Acad Sci U S A 94:14105–14110
10. Holinka CG, Hata H, Kuramoto H, Gurpide E (1986) Effects of steroid hormones and antisteroids on alkaline phosphatase activity in human endometrial cancer cells (Ishikawa line). Cancer Res 46:2771–2774
11. Jensen EV, DeSombre ER (1972) Mechanism of action of the female sex hormones. Annu Rev Biochem 41:203–230
12. Jordan VC (1992) Overview from the international conference on long-term tamoxifen therapy for breast cancer. J Natl Cancer Inst 84:231–234
13. Katzenellenbogen JA, O'Malley BW, Katzenellenbogen S (1996) Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. Mol Endocrinol 10:119–131
14. Kauffman RF, Bryant HU (1995) Selective oestrogen receptor modulators. Drug News Perspect 8:531–538
15. Kurebayashi J, Otsuki T, Yamamoto S, Kurosumi M, Nakata T, Akinaga S, Sonoo H (1998) A pure antiestrogen, ICI 182,780, stimulates the growth of tamoxifen-resistant KPL-1 human breast cancer cells in vivo but not in vitro. Oncology 55 [Suppl 1]:23–34
16. Love RR, Cameron L, Connell BL, Leventhal H (1991) Symptoms associated with tamoxifen treatment in postmenopausal women. Arch Int Med 151:1842–1847
17. Martel C, Labrie C, Belanger A, Gauthier A, Merand Y, Li X, Provencher L, Candas B, Labrie F (1998) Comparison of the effects of the new orally active antiestrogen EM-800 with ICI 182,780 and toremifine on oestrogen-sensitive parameters in the ovariectomized mouse. Endocrinology 139:2486–2492
18. Martel C, Provencher L, Li X, St Pierre A, Leblanc G, Gauthier S, Merand Y, Labrie F (1998) Binding characteristics of novel nonsteroidal antiestrogens to the rat uterine oestrogen receptors. J Steroid Biochem Mol Biol 64(3-4):199–205
19. McDonnell DP, Clemm DL, Herman T, Goldman ME, Pike JW (1995) Analysis of oestrogen receptor function in vitro reveals three distinct classes of antiestrogens. Mol Endocrinol 9:659–669
20. Mosselman S, Polman J, Dijkema R (1996) ER beta: identification and characterization of a novel human oestrogen receptor. FEBS Lett 392(1):49–53
21. Nawaz Z, Stancel GM, Hyder SM (1999) The pure antiestrogen ICI 182,780 inhibits progestin-induced transcription. Cancer Res 59:372–376
22. Norris JD, Fan D, Wagner BL, McDonnell DP (1996) Identification of the sequences within the human complement 3 promoter required for oestrogen responsiveness provides insight into the mechanism of tamoxifen mixed agonist activity. Mol Endocrinol 10:1605–1616
23. Nuttall ME, Bradbeer JN, Stroup GB, Nadeau DP, Hoffman SJ, Zhao H, Rehm S, Gowen M (1998) Idoxifene: a novel selective oestrogen receptor modulator prevents bone loss and lowers cholesterol levels in ovariectomized rats and decreases uterine weight in intact rats. Endocrinology 139:5224–5234
24. Paech K, Webb P, Kuiper GGJM, Nilsson S, Gustafsson JA, Kushner PJ, Scanlan TS (1997) Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. Science 277:1508–1510
25. Salerno M, Sisci D, Mauro L, Guvakova MA, Ando S, Surmacz E (1999) Insulin receptor substrate 1 is a target for the pure antiestrogen ICI 182,780 in breast cancer cells. Int J Cancer 81:299–304
26. Sibonga JD, Dobnig H, Harden RM, Turner RT (1998) Effect of the high-affinity oestrogen receptor ligand ICI 182,780 on the rat tibia. Endocrinology 139:3736–3742
27. Smith CL, Nawaz Z, O'Malley BW (1997) Coactivators and corepressors regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. Mol Endocrinol 11:657–666
28. Wakeling AE (1993) The future of new pure antiestrogens in clinical breast cancer. Breast Cancer Res Treat 25:1–9
29. Wakeling AE, Dukes M, Bowler J (1991) A potent specific pure antiestrogen with clinical potential. Cancer Res 51:3867–3873
30. Wen DX, Xu Y-F, Goldman MK, McDonnell DP (1994) The A and B isoforms of the human progesterone receptor operate through distinct signalling pathways within target cells. Mol Cell Biol 14:8356–8364
31. Willson TM, Norris J D, Wagner BL, Asplin P B, Brown HR, Jones SS, Henke B, Sauls H, Wolfe S, Morris DC, McDonnell DP (1997) Dissection of the molecular mechanism of action of GW5638, a novel oestrogen receptor ligand, provides insights into the role of oestrogen receptor in bone. Endocrinology 138:3901–3911
32. Yamashita S, Takayanagi A, Shimizu N (1996) Temporal and cell-type specific expression of c-fos and c-jun protooncogenes in the mouse uterus after oestrogen stimulation. Endocrinology 137:5468–5475