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MCF-7aro/ERE, a Novel Cell Line for Rapid Screening of Aromatase Inhibitors, ER α Ligands and ERR α Ligands

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Abstract

We have previously generated a breast cancer cell line, MCF-7aro, which over-expresses aromatase and is also ER-positive. Recently, this MCF-7aro cell line was stably transfected with a promoter reporter plasmid, pGL3-Luc, containing three repeats of ERE (estrogen responsive element). Experiments using MCF-7aro/ERE have demonstrated that it is a novel, non-radioactive screening system for aromatase inhibitors (AIs), ER α ligands and ERR α ligands. The screening is carried out in a 96-well plate format. To evaluate this system, the cells were cultured overnight in charcoal-dextran stripped FBS medium supplemented with 0.1 nM testosterone or 17 β -estradiol, and various concentrations of antiestrogens or AIs. We found that the luciferase activity was induced when the cells were cultured either in the presence of testosterone or 17 β -estradiol. Furthermore, a 50% decrease in luciferase activity could be achieved when the cells were cultured in the presence of testosterone together with letrozole, anastrozole, tamoxifen or fulvestrant (concentrations being 75 nM, 290 nM, 100 nM, and 5 nM, respectively), compared to the testosterone-only cultured cells. Using this assay system, we confirmed that 3 (2'-chlorophenyl)-7-methoxy-4-phenylcoumarin is an agonist of ER. Furthermore, genestein has been shown to be a ligand of ERR α because its binding could be blocked by an ERR α inverse agonist, XCT790. These results indicate that MCF-7aro/ERE is a novel cell line for rapid screening of AIs, ER α ligands and ERR α ligands.

Keywords

Aromatase; Aromatase inhibitor; ER agonist; ERR α ligands; MCF-7aro/ERE

1. Introduction

Aromatase is the key enzyme in catalyzing the conversion of androgen to estrogen. Estrogen plays a crucial role in breast cancer development and progression. In postmenopausal women, while the blood estrogen levels are very low, an abnormally elevated expression of aromatase is frequently found in breast cancer tissues [1–4]. A high level of aromatase activity sustains the endogenous conversion of estrogen in breast cancer cells, which maintains the survival and progression of tumor growth. To suppress the growth and progression of hormone-dependent cancers, antiestrogens and aromatase inhibitors (AIs) are used as first line treatments or as an adjuvant hormonal therapy immediately after surgery.

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Tamoxifen (Nolvadex) is a well-known antiestrogen. It binds to the estrogen receptor (ER) and blocks the effect of estrogen. Beside its antagonistic effect on breast tissue, tamoxifen shows an agonistic effect predominantly in the bone and uterus. Therefore, prolonged use of tamoxifen increases the risk for developing endometrial cancer [5]. Another antiestrogen, fulvestrant ("Faslodex", ICI 182, 780 or ICI) was developed as a true antagonist, and showed no differential agonistic effects in different organs/tissues. ICI also has an ER-deregulatory property in that it binds to the ER, thereby causing it to degrade [6].

AIs inhibit the production of estrogen by suppressing aromatase activity. The FDA has approved three third-generation AIs for use as first-line agents against estrogen-dependent cancers [7]. The third-generation AIs include both non-steroidal (letrozole and anastrozole), and steroidal (exemestane) agents. They have recently been proven to be more effective than tamoxifen as a first-line therapy, effective for second-line therapy (e.g. against tamoxifen-resistant disease), and useful for extended adjuvant therapy after tamoxifen [8]. Among these three third-generation AIs, exemestane has been found to be a mechanism-based inhibitor and an aromatase destabilizer [9]. Although these antiestrogens and AIs are powerful drugs to treat hormone-dependent breast cancer, there is a need to develop additional antiestrogens and AIs for breast cancer patients who become resistant to the currently available antiestrogens/AIs.

High-throughput screening (HTS) assays have become one of the major approaches for drug discovery in the modern pharmaceutical industry. So far, the aromatase assay can be carried out in both non-cellular and cell-based formats [10,11]. In both types of assays, aromatase activity is determined by measuring the production of tritiated water from [1β - ^3H] androstenedione following a 20-minute or one-hour incubation, respectively. Typically, the *in-vitro* assay is performed using human placental microsomes that contain a high level of aromatase, and the *in-cell* assay is performed using MCF-7aro cells or other aromatase-positive cells. These assays require the use of radioactive substrate and are not high throughput methods. Also, human placental microsomes, which are required for this method, may be limited in availability. In consideration of these drawbacks, we have developed a new and reliable *in-cell* system for aromatase inhibitor screening, which is a non-radioactive, 96-well high-throughput format. In addition, this MCF-7aro/ERE cell line can also be used to screen ligands of ER α and ER β . Therefore, this is a triple screening system.

This paper describes the construction of pGL3-(ERE) $_3$ -Luc expression plasmid which consists of a hygromycin resistant gene, as well as the generation of this MCF-7aro/ERE cell line by transfection. Experiments have been performed to demonstrate that MCF-7aro/ERE is a novel HTS system to screen AI, ER and ER ligands.

2. Materials and Methods

2.1. Chemicals

Testosterone (T), 17β -estradiol (E_2), tamoxifen, and XCT790 were purchased from Sigma (Sigma chemical, St. Louis, MO), while ICI was purchased from Tocris (Ellisville, MO). Letrozole was provided by Novartis (Basel, Switzerland) and anastrozole was provided by AstraZeneca Pharmaceuticals (Macclesfield, UK). [$2, 4, 6, 7$ - ^3H] estradiol (Specific Activity, 95 Ci/mmol) was purchased from PerkinElmer Life Sciences, Inc. (Boston, MA). Genistein and 3 (2'-chlorophenyl)-7-methoxy-4-phenylcoumarin (Cou) were purchased from Indofine (Somerville, NJ).

2.2. Construction of plasmids

The luciferase reporter plasmid, pGL3-(ERE) $_3$ -Luciferase, was constructed by inserting a DNA fragment which consisted of three repeats of estrogen responsive element (ERE), 5'-

GGTCAGAGTGACC-3', into a pGL3-promotor vector. This DNA fragment was restricted using *NheI* and *XhoI*, and was ligated to linearized pGL3 promotor vector with T4 DNA ligase. After that, the plasmid was cut by *Sal I* and ligated with a hygromycin resistant gene which was generated from a pTRE2hyg vector (Clontech, Mountain View, US).

2.3. Cell Culture

A stable aromatase-expressing, ER positive MCF-7 cell line, named MCF-7aro, was previously generated by aromatase cDNA transfection and G-418 selection in this laboratory [11,12]. The pGL3(ERE)₃-Luc plasmid, which contains a hygromycin resistant gene, was transfected into MCF-7aro cells using Lipofectin, following the manufacturer's protocol (Invitrogen, Carlsbad, US). The stably transfected cells, MCF-7aro/ERE, were grown in regular MEM medium containing 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate and Penicillin/Streptomycin with hygromycin and G418 selection at 37 °C in humidified incubators at 5% CO₂.

2.4. Luciferase assay

The levels of ERE-mediated luciferase expression were determined through luciferase activity measurements. Approximately 1×10^4 MCF-7aro/ERE cells (per well) were transferred into 96-well plates containing 200 µl of phenol red-free MEM/well, supplemented with 10% charcoal/dextran treated FBS. On the following day, the cells were washed once with PBS. Hormones, genistein, antiestrogens and/or AIs dissolved in DMSO were added to the culture medium, and the cells were further incubated for 24 hours. Cells were then lysed using 100 µl of reporter lysis buffer (Promega, Madison, WI). Fifty µl of luciferase substrate (Promega, Madison, WI) was mixed with 40 µl of the cell lysate by a robotic injector and the luciferase assay was carried out using the Victor² V plate reader (Perkin Elmer, Waltham, MA). The luciferase activity was standardized based on the protein concentration of the cell lysates. The protein concentration was determined by using Bio-Rad Protein Assay reagent (Bio-Rad, Hercules, CA) in the Bradford method.

The degree of inhibition was measured as a percentage of maximum activity induced by T or E₂ for each concentration of inhibitor and was calculated as {[luciferase activity of inhibitor]-background}/[luciferase activity of T or E₂]- background} × 100%.

2.5. Whole-cell competitive ER binding assay

The procedure was similar to that previously reported by Stoessel and Leclercq [13], with minor modification. Briefly, ER positive MCF-7 cells were suspended in Dulbecco's modified Eagle's medium (phenol red-free) supplemented with 10% charcoal-treated FBS, non-essential amino acids, penicillin (100 U/ml), and streptomycin (100 µg/ml), and were seeded into 24-well plates at a density of 8×10^4 cells/ml per well. Twenty-four hours later, the seeding medium was changed to the same culture medium containing 1 nM of [2, 4, 6, 7-³H] estradiol (E₂) and varying concentrations of either E₂ or the test compounds (1 nM to 1 µM) and incubated at 37 °C for 1 hour. The [³H] E₂ containing medium was removed and the cells were washed three times with PBS, and 250 µl of 100% ethanol was added to each well for 20 min to solubilize the bound [³H] E₂. The radioactivity in 200 µl of the ethanol extract was determined. The amount of bound [³H] E₂ in the presence or absence of the test compounds was calculated after correcting for non-specific binding as measured by the amount of bound [³H] E₂ in the presence of 1000-fold (1.0 µM) excess of E₂. All compounds were tested with triplicates at each concentration in at least three separate experiments. Binding affinity of Cou is approximately 1/40th of the E₂ binding affinity. Using this method, the EC₅₀ values for ICI and tamoxifen are 15 nM and 630 nM, respectively. As a negative control, we did not detect significant amount of [³H] E₂ bound to cells when ER-negative HeLa cells were used.

3. Results

3.1. Construction of a stable MCF-7aro/ERE cell line

The MCF-7aro/ERE cell line was generated by transfecting pGL3-(ERE)₃-promoter luciferase reporter plasmid into MCF-7aro cells (Fig. 1A). Since MCF-7aro cells were already resistant to G418, MCF-7aro/ERE cells were selected using hygromycin. Several clones were picked, and the clone which could be induced by T or E₂ at maximal luciferase activity was chosen to perform the antiestrogen and AI assays.

3.2. Inductive response of the ERE-mediated luciferase activity in MCF-7aro/ERE by T or E₂

To evaluate the utility of MCF-7aro/ERE, we began by assessing the ability of these cells to respond to different concentrations of T and E₂. In these cells, aromatase converts T to E₂, after which E₂ activates ER, triggering luciferase expression through the ERE interaction, as shown in Fig. 1B. The dose dependency of luciferase activity (as an indication of the ERE-mediated luciferase expression) to T or E₂ was assessed. The EC₅₀ values for T and E₂ were estimated to be 1.9 pM and 1.2 pM, respectively (Fig. 2). The activity levels of luciferase induced by T were the same as E₂, and they displayed typical sigmoidal dose-response curves. The luciferase activity remained at the basal level when treated at concentrations below 0.1 pM T or E₂, started to increase at 1 pM, and plateaued at 0.1 nM with no further increase of luciferase activity beyond the concentration of 0.1 nM. The ratio of maximal to basal luciferase expression was about 6.

3.3. Effect of antiestrogens (ICI and tamoxifen) on the ERE-mediated luciferase activity in MCF-7aro/ERE

Fig. 3A and 3B show the inhibitory effects of ICI and tamoxifen on the luciferase activity in the presence of T or E₂. The luciferase activity was at the basal level when MCF7-aro/ERE cells were treated with DMSO, while the luciferase activity reached the maximum at 0.1 nM T or 0.1 nM E₂. The luciferase activity decreased with an increased concentration of antiestrogens (ICI or tamoxifen), both in the presence of 0.1 nM T or 0.1 nM E₂. There was no significant difference between treatment of T+ICI and E₂+ICI, or T+tamoxifen and E₂+tamoxifen. At the concentration of 8 nM ICI or 1 μM tamoxifen, the luciferase activity was almost completely inhibited, both in the presence of T or E₂. The calculated EC₅₀ of ICI and tamoxifen in the presence of T were 5 nM and 100 nM, respectively, while the EC₅₀ of ICI and tamoxifen in the presence of E₂ were 4 nM and 80 nM, respectively (Table 1). As expected, ICI was more effective than tamoxifen in the inhibition of the ERE-mediated luciferase expression. We did not detect any intrinsic ER agonistic activity of ICI or tamoxifen using this assay system.

3.4. Effect of AIs (letrozole and anastrozole) on the ERE-mediated luciferase activity in MCF-7aro/ERE

Fig. 3C and 3D show the inhibitory effects of letrozole and anastrozole on the luciferase activity in MCF-7aro/ERE cells. Again, the luciferase activity reached the maximum level when induced by 0.1 nM T or 0.1 nM E₂. The luciferase activity decreased at increased concentrations of letrozole and anastrozole, and in the presence of 0.1 nM T, but there was no reduction of luciferase activity when treated with E₂+letrozole or E₂+anastrozole. At the concentration of 400 nM letrozole or 1 μM anastrozole, luciferase activity was completely inhibited in the presence of T, but not in the presence of E₂. The EC₅₀ of letrozole and anastrozole in the presence of T were 75 nM and 290 nM respectively (Table 1), indicating that letrozole was more effective than anastrozole in the inhibition of the ERE-mediated luciferase expression. These results agreed with previously published data [14]. No ER agonistic activity of letrozole or anastrozole was detected using this assay system.

3.5. Effect of genistein on the ERE-mediated luciferase activity in MCF-7aro/ERE

Fig. 4 shows that genistein could induce luciferase activity significantly at concentrations of 1 μ M and 10 μ M. While genistein has been suggested to be an agonist of ER, the luciferase activity could only be slightly suppressed by ICI, but effectively by an ERR α inverse agonist, XCT790 [15,16]. These results confirm our previous findings that genistein is an effective ERR α ligand [17] and demonstrate that MCF-7aro/ERE is also useful for the screening of ERR α ligands.

3.6. Identification of 3 (2'-chlorophenyl)-7-methoxy-4-phenylcoumarin (Cou) as an agonist of ER

Using a whole-cell competitive ER binding assay, we have found that this coumarin derivative is a ligand of ER (Fig. 5). The binding affinity of this compound is approximately one fortieth that of E₂. As a proof-of-principle experiment, we evaluated the effect of Cou on the ERE-mediated luciferase activity in MCF-7aro/ERE. This compound could induce luciferase activity significantly with an EC₅₀ value of 0.24 nM (Fig. 6). The induced luciferase activity could be suppressed by ICI, but not by XCT790 (Fig. 7). These results confirm that Cou is a ligand and an agonist of ER. This compound cannot suppress T-induced luciferase (results not shown), indicating that this compound is not an inhibitor of aromatase.

4. Discussion

The utility of MCF-7aro/ERE as a tool for screening AIs and ER α ligands was carefully evaluated. The dose-response curves induced by T and E₂ overlap (Fig. 2), suggesting that aromatase converts T to E₂ efficiently inside MCF-7aro/ERE cells. The pGL3-(ERE)₃-Luc activity was increased more than 6-fold with 0.1 nM of T and E₂, compared to the DMSO control. This fold difference was significant enough to allow us to evaluate the potencies of different chemicals. The maximum concentrations of T and E₂ (i.e., 0.1 nM) used in this assay system were 10 times less than that of the traditional protocol [14]. The MCF-7aro/ERE screening system has the advantage over ligand binding assays in that this is a functional assay, i.e., the assay allows us to determine whether the detected chemicals are inhibitors of aromatase, agonists or antagonists of ER α and ERR α . Since it is not a ligand binding assay, K_i or K_d values of the ligands cannot be determined. The screen system is a very sensitive one, as indicated by very low EC₅₀ values for T and E₂ (Fig. 2). Due to the fact that we could perform analysis using 0.1 nM T or E₂, the EC₅₀ values of tested ligands were typically significantly lower than those determined by ligand binding assays. For example, EC₅₀ of Cou determined by the MCF-7aro/ERE screening system (0.24 nM) was significantly lower than that determined by whole-cell competitive ER binding assay (i.e., 45 nM). This feature makes the MCF-7aro/ERE screening system a very sensitive method to screen ligands for ER α and ERR α . However, the high sensitivity of ER α to E₂ requires a total suppression of aromatase activity for aromatase inhibitor screening. Therefore, EC₅₀ values of aromatase inhibitors determined by the MCF-7aro/ERE screening system could be slightly higher than those determined by direct enzyme assay.

It was predicted that inhibiting the conversion of T to E₂ by AIs as well as reducing ER α availability with antiestrogens would result in the suppression of ERE-mediated luciferase activity (Fig. 1B). Through this assay system, as a proof-of-principle study, we confirmed tamoxifen and ICI to be antiestrogens and letrozole and anastrozole to be AIs. Both T- and E₂-induced luciferase activities could be blocked by antiestrogens at a concentration of 8 nM of ICI or 1 μ M tamoxifen (Fig. 3A, 3B). As expected, only T-induced luciferase activity could be blocked by letrozole or anastrozole.

There was an induction of luciferase activity when the MCF-7aro/ERE cells were treated with genistein in the absence of T or E₂. The higher the concentration of genistein, the higher the activity of luciferase could be induced. However, with the combination of genistein and ICI, the luciferase activity was only slightly suppressed. These results suggest that ER is not the major receptor that genistein binds to. Genistein is an isoflavone phytoestrogen which has shown agonistic activity to ER and ERRs [17]. ERRs are able to bind to the ERE. To confirm our previous findings, the genistein-induced luciferase expression was effectively suppressed by XCT790, an inverse agonist of ERR α . Our microarray analysis revealed that there is minimal expression of ER β in our MCF-7aro cells [18]. This is confirmed by our results indicating that the genistein-induced activity is completely suppressed by XCT790, while genistein has been reported to be a better ligand of ER β than ER α [19].

To demonstrate the utility of this screening system, an ER ligand (identified by competitive ER binding assay), Cou, was found to be an agonist of ER, probably ER α because this cell line has a minimal expression of ER β . Using the same system, this compound was found not to be a ligand of ERR α nor an inhibitor of aromatase.

In summary, we have developed a new assay system which is able to screen for ER α ligands and AIs at the same time. The assay is carried out in a 96-well plate, and it facilitates a high throughput rapid screening. It is sensitive, easy to handle, and reproducible at low cost. Also, the MCF-7aro/EREs can possibly be used to screen ERR agonists and antagonists. This triple screening system will be valuable in searching for chemicals, such as environmental chemicals, that interact with ER α , ERR α and/or aromatase at the same time.

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References

1. Killinger DW, Pere IE, Daiilescu D, Kharlip L, Blackstein ME. Aromatase activity in the breast and other peripheral tissues and its therapeutic regulation. *Steroids* 1987;50:523–535. [PubMed: 3332939]
2. Miller WR, O'Neill J. The importance of local synthesis of estrogen within the breast. *Steroids* 1987;50:537–548. [PubMed: 3504615]
3. Reed MJ, Owen AM, Lai LC, Coldham NG, Ghilchik MW, Shaikh N, et al. In situ oestrone synthesis in normal breast and breast tumour tissues: effect of treatment with 4-hydroxyandrostenedione. *Int J Cancer* 1989;44:233–237. [PubMed: 2759729]
4. Silva MC, Rowlands MG, Dowsett M, Gusterson B, Mckinna JA, Fryatt I, et al. Intratumoral aromatase as a prognostic factor in human carcinoma. *Cancer Res* 1989;49:2588–2591. [PubMed: 2713843]
5. Fisher B, Constantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. *J. Natl. Cancer Inst* 1994;86:527–537. [PubMed: 8133536]
6. Dauvois S, Danielian PS, White R, Parker MG. Antiestrogen ICI 164,384 reduces cellular estrogen receptor content by increasing its turnover. *Proc. Natl. Acad. Sci. USA* 1992;89:4037–4041. [PubMed: 1570330]
7. Winer EP, Hudis C, Burstein HJ. American Society of Clinical Oncology technology assessment on the use of aromatase inhibitors as adjuvant therapy for women with hormone receptor-positive breast cancer, status report. *J. Clin. Oncol* 2002;20:3317–3327. [PubMed: 12149306]
8. Ariazi EA, Ariazi JL, Cordera F, Jordan VC. Estrogen receptors as therapeutic targets in breast cancer. *Curr Top Med Chem* 2006;6:195–216.
9. Wang X, Chen S. Aromatase destabilizer: novel action of exemestane, a food and drug administration-approved aromatase inhibitor. *Cancer Res* 2006;66:10281–10286. [PubMed: 17079446]
10. Hall PF, Chen S, Nakajin S, Shinoda M, Shively JE. Purification and characterization of aromatase from human placenta. *Steroids* 1987;50:37–50. [PubMed: 3504067]

11. Zhou D, Pompon D, Chen S. Stable expression of human aromatase complementary DNA in mammalian cells: a useful system for aromatase inhibitor screening. *Cancer Res* 1990;50:6949–6954. [PubMed: 2208160]
12. Sun XZ, Zhou D, Chen S. Autocrine and paracrine actions of breast tumor aromatase. A three-dimensional cell culture study involving aromatase transfected MCF-7 and T-47D cells. *J. Steroid. Biochem. Mol. Biol* 1997;63:29–36. [PubMed: 9449203]
13. Stoessel S, Leclercq G. Competitive binding assay for estrogen receptor in monolayer culture: measure of receptor activation potency. *J. Steroid. Biochem* 1986;25(5A):677–682. [PubMed: 2432356]
14. Kijima I, Itoh T, Chen S. Growth inhibition of estrogen receptor-positive and aromatase-positive human breast cancer cells in monolayer and spheroid cultures by letrozole, anastrozole, and tamoxifen. *J. Steroid. Biochem. Mol. Biol* 2005;97:360–368. [PubMed: 16263272]
15. Busch BB, Stevens WC Jr, Matrin R, Ordentlich P, Zhou S, Sapp DW, et al. Identification of a selective inverse agonist for the orphan nuclear receptor estrogen-related receptor α . *J Med Chem* 2004;47:5593–5596. [PubMed: 15509154]
16. Willy PJ, Murray IR, Qian J, Busch BB, Stevens WC Jr, Martin R, et al. Regulation of PPAR γ coactivator 1 α (PGC-1 α) signaling by an estrogen-related receptor α (ERR α) ligand. *Proc Natl Acad Sci USA* 2004;101:8912–8917. [PubMed: 15184675]
17. Suetsugi M, Su L, Karlsberg K, Yuan YC, Chen S. Flavone and isoflavone phytoestrogens are agonists of estrogen-related receptors. *Mol. Cancer Res* 2003;1:981–991. [PubMed: 14638870]
18. Wang X, Masri S, Pheng S, Chen S. The role of amphiregulin in exemestane-resistant breast cancer cells: evidence of an autocrine loop. *Cancer Res.* 2008in press.
19. Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β . *Endocrinology* 1997;138:863–870. [PubMed: 9048584]

List of abbreviations

AI, aromatase inhibitor; Cou, 3 (2'-chlorophenyl)-7-methoxy-4-phenylcoumarin; E₂, 17 β -estradiol; ER, estrogen receptor; ERE, estrogen responsive element; ERR, estrogen-related receptor; HTS, high-throughput screening; ICI, ICI182,780 or Faslodex; T, testosterone.

Fig. 1.

A. Restriction map of pLG3-ERE-Luciferase hygromycin plasmid. Three repeats of ERE were inserted into *NheI* and *XhoI* restriction sites while the hygromycin gene was inserted into *XhoI* site of the pGL3 promotor plasmid. **B.** A schematic diagram to show the working principles of MCF7-aro/ERE cells. T is converted into E₂ by aromatase in MCF7-aro/ERE cells. Then E₂ activates ER. The E₂-ER complex binds to the ERE and induces luciferase expression. Aromatase inhibitor blocks the conversion of T to E₂. Without the formation of the E₂-ER complex, luciferase activity is eliminated. Anti-estrogen binds to ER to prevent ER from binding to E₂, therefore inhibiting luciferase activity. An inverse agonist of ERRs leads to deletion of its luciferase activity.

Fig. 2.

Dose-response curves of T or E₂ driven ERE-mediated luciferase activities in MCF-7aro/ERE cells. The T or E₂ stock solutions were added at a 1:1000 dilution (v/v) to MEM medium which was supplemented with 10 % of charcoal/dextran treated FBS. The cells were cultured in this medium for 24 hours and the luciferase activity was normalized using protein concentration. The assays were performed in triplicate.

DMSO	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
T (0.1 nM)	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
E ₂ (0.1 nM)	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
ICI (1 nM)	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-
ICI (2 nM)	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
ICI (4 nM)	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-
ICI (8 nM)	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	+

DMSO	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
T (0.1 nM)	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
E ₂ (0.1 nM)	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
Tam (0.1 μM)	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-
Tam (0.2 μM)	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-
Tam (0.5 μM)	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-
Tam (1 μM)	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-

Fig. 3.

The ERE-mediated luciferase activity of MCF-7aro/ERE cells in response to ICI 182, 870 (ICI) (A), tamoxifen (Tam) (B), letrozole (Let) (C) and anastrozole (Ana) (D). The DMSO treated cells served as a negative control, and the cells cultured with 0.1 nM T or 0.1 nM E₂ which showed maximum ER-triggered transactivation served as the positive control. Inhibition of ERE-mediated luciferase activity was carried out by introducing different concentrations of ICI (A), Tam (B), Let (C) or Ana (D), in the presence of 0.1 nM T or 0.1 nM E₂. In addition, effects of ICI only (A), Tam only (B), Let only (C) or Ana only (D) in the absence of T or E₂, were also examined.

Fig. 4.

The ERE-mediated luciferase activity of MCF-7aro/ERE cells in response to genistein (Gen). The DMSO treated MCF-7aro/ERE cells served as a negative control, and cells cultured with T or E₂ as the positive control. The luciferase activities induced by Gen, combination of Gen and ICI, or combination of Gen and XCT790 were examined.

Fig. 5.

Demonstration of 3 (2'-chlorophenyl)-7-methoxy-4-phenylcoumarin as a ligand of ER by whole-cell competitive ER binding assay. The experimental conditions are provided in "Materials and Methods." The binding of 1 nM [2, 4, 6, 7-³H] estradiol was competed with varying concentrations of either E₂ or the test compounds (1 nM to 1 μM). The assays were performed in triplicate.

Fig. 6.

Dose-response curves of the ERE-mediated luciferase activity of MCF-7aro/ERE cells driven by 3 (2'-chlorophenyl)-7-methoxy-4-phenylcoumarin (Cou). The coumarin stock solutions were added at a 1:1000 dilution (v/v) to MEM medium which was supplemented with 10 % of charcoal/dextran treated FBS. The cells were cultured in this medium for 24 hours and the luciferase activity was normalized using protein concentration. The assays were performed in triplicate.

Fig. 7.

The ERE-mediated luciferase activity of MCF-7aro/ERE cells in response to 3 (2'-chlorophenyl)-7-methoxy-4-phenylcoumarin (Cou). The DMSO treated MCF-7aro/ERE cells served as a negative control, and cells cultured with T or E₂ as the positive control. The luciferase activities induced by Cou, combination of Cou and ICI, or combination of Cou and XCT790 were examined.

Table 1

Calculated IC₅₀ values of ICI 182, 870, tamoxifen, letrozole and anastrozole in the presence of either 0.1 nM T or 0.1 nM E₂.

	Potency EC ₅₀ (nM)	
	With T	With E ₂
Inhibition of ERE transcriptional activity		
ICI 182, 780 (ICI)	5	4
Tamoxifen (Tam)	100	80
Letrozole (Let)	75	N.A.
Anastrozole (Ana)	290	N.A.

N.A., not applicable