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# Selective Oestrogen Receptor Modulation: Molecular Pharmacology for the Millennium

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**Knowledge of the mechanism of action and pharmacology of tamoxifen and raloxifene, for the prevention of breast cancer and osteoporosis respectively, has opened the door for the discovery of multifunctional medicines. There is now the potential to prevent osteoporosis, coronary heart disease, breast and endometrial cancer in postmenopausal women with elevated risk factors.** © **1999 Elsevier Science Ltd. All rights reserved.**

**Key words: tamoxifen, raloxifene, breast cancer, prevention**
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## INTRODUCTION

THE LINK between oestrogen and the growth and development of breast cancer has been established during the twentieth century [1, 2]. However, the identification and elucidation of the mechanism of oestrogen action through the oestrogen receptor (ER) [3–8] has proved invaluable to target breast cancer treatment and prevention [9, 10]. Non-steroidal anti-oestrogens block the binding of oestradiol to the ER [11, 12] and, as a result, are antagonists of oestrogen action. Tamoxifen, the prototype anti-oestrogen for the treatment of breast cancer, was first shown to have efficacy in the palliative treatment of advanced breast cancer nearly 30 years ago [13, 14]. Although no increased response rate was observed with tamoxifen compared with other available endocrine therapies, it was the low incidence of side-effects that facilitated the strategic use of long-term adjuvant therapy for node-positive and node-negative breast cancer [15–17]. Tamoxifen had been tested extensively in clinical trials for 20 years, therefore the drug became the agent of choice to prove [18] the worth of an anti-oestrogen for the prevention of breast cancer in high-risk women.

This concept of identifying a target (the ER) and developing an agent (tamoxifen) to prevent breast cancer [19] is an example of translational research that has opened the door to broad ranging opportunities in therapeutics for the next century [9, 10]. We are poised to develop a menu of medicines that are designed to influence the natural history of breast cancer, endometrial cancer, osteoporosis and coronary heart disease [20, 21]. It is, therefore, appropriate to review what we know and what we need to learn to understand selective ER modulation (SERM).

## SELECTIVE OESTROGEN RECEPTOR MODULATION

Throughout the 1960s and 1970s, anti-oestrogenic activity was correlated with antitumour activity. However, the finding that triphenylethylene-type anti-oestrogens expressed increased oestrogenic properties, that is vaginal cornification and increased uterine weight in the mouse [22, 23] raised questions about the reasons for the species specificity. One obvious possibility was species-specific metabolism that is, the mouse converts anti-oestrogens to oestrogens via novel metabolic pathways. However, no species-specific metabolic routes to known oestrogens have been identified [24–26], but knowledge of the mouse model created a new dimension for study, which ultimately led to the recognition of the target site-specific actions of triphenylethylene-type anti-oestrogens. This concept is now referred to as selective ER modulation and the class of drugs, known as SERMs, has a prominent place in medical practice. Tamoxifen is used for breast cancer prevention and raloxifene, paradoxically, is used to prevent osteoporosis.

The ER positive breast cancer cell line MCF-7 [27] can be heterotransplanted into immune-deficient athymic mice but the cells can only grow into tumours with oestrogen support. Paradoxically, tamoxifen, an oestrogen in the mouse [22, 23] does not support tumour growth [28] but stimulates mouse uterine growth with the same spectrum of tamoxifen metabolites present in both the mouse uterus and implanted human tumour [29]. To explain the selective actions of tamoxifen in different targets of the same host, it was suggested that the tamoxifen–ER complex could be interpreted as a stimulatory or inhibitory signal at different sites [29]. A similar conclusion can be drawn from the observation that long-term tamoxifen treatment prevents mouse mammary carcinogenesis in high incidence strains [30]. In contrast, tamoxifen initially causes a strong oestrogen-like effect in the uterus that ultimately becomes refractory to oestrogen stimulation within 8 weeks [31].

The concept of the target site-specificity of anti-oestrogens was consolidated with experimental evidence from two further models that translated into the clinic. First, tamoxifen and raloxifene both maintain bone density in the ovariectomised rat but both compounds inhibit oestradiol-stimulated uterine weight [32] and prevent carcinogen-induced mammary tumorigenesis [33]. The laboratory studies on bone density and remodelling have been adequately confirmed and translated to the clinic [34, 35]. Tamoxifen reduces hip and wrist fractures in postmenopausal women and raloxifene prevents fractures of the spine. Tamoxifen [18] and raloxifene [36] also reduce the incidence of breast cancer. Second, the finding that tamoxifen would partially stimulate the growth of a human endometrial carcinoma transplanted into athymic mice [37] allowed the question to be asked: if a human endometrial and breast tumour were transplanted into the same athymic mouse would tamoxifen exhibit differential pharmacology at two human target sites? Tamoxifen demonstrated target site-specificity; breast tumour growth was blocked, but endometrial tumours continued to grow [37]. Again the range of tamoxifen metabolites was the same in the tumours despite the opposite responses. Thus, the tamoxifen–ER complexes must be interpreted differently in the breast and uterus. These data also suggested the possibility of an increased risk of endometrial cancer in women taking long-term tamoxifen therapy. Indeed, after a decade of investigation, there is known to be a 3- to 4-fold increase in the incidence of endometrial cancer in postmenopausal women treated with tamoxifen [18, 38].

It is axiomatic that pharmacology provides a clearer understanding of the subtleties of physiology. An investigation of the action of drugs has resulted in the description of α- and β-adrenergic receptors, the classification of muscarinic and nicotinic receptors and the discovery of specific antagonists to block H1 and H2 receptors for histamine. The challenge is to understand selective oestrogen and anti-oestrogen action and to discover new compounds that can exploit the SERM concept.

The place to start is with the emerging appreciation of the complexities of oestrogen action.

## THE OESTROGEN RECEPTORS (ERS) α AND β

The biological effects of oestrogen are now known to be mediated by two receptors referred to as oestrogen receptor-α (ERα) and ERβ. ERα was first identified and isolated in the mid 1960s [3–7]. The cloning of ERα in 1986 [39, 40] focused research efforts on the existence of only one ER protein (ERα) identical in all target tissues. Almost three decades later after the initial discovery of the ERα, ERβ was identified in the rat, human and mouse [41–43]. The discovery of ERβ has already advanced our understanding of oestrogen signalling and may explain the 'mysterious' responses to oestrogen in tissues in which ERα was not detectable [44]. Thus, the existence of ER α and β subtypes provides a possible explanation for the tissue-selectivity of SERMs.

The two ERs share a functionally conserved structure (domains A–F) (Figure 1) consisting of a variable amino-

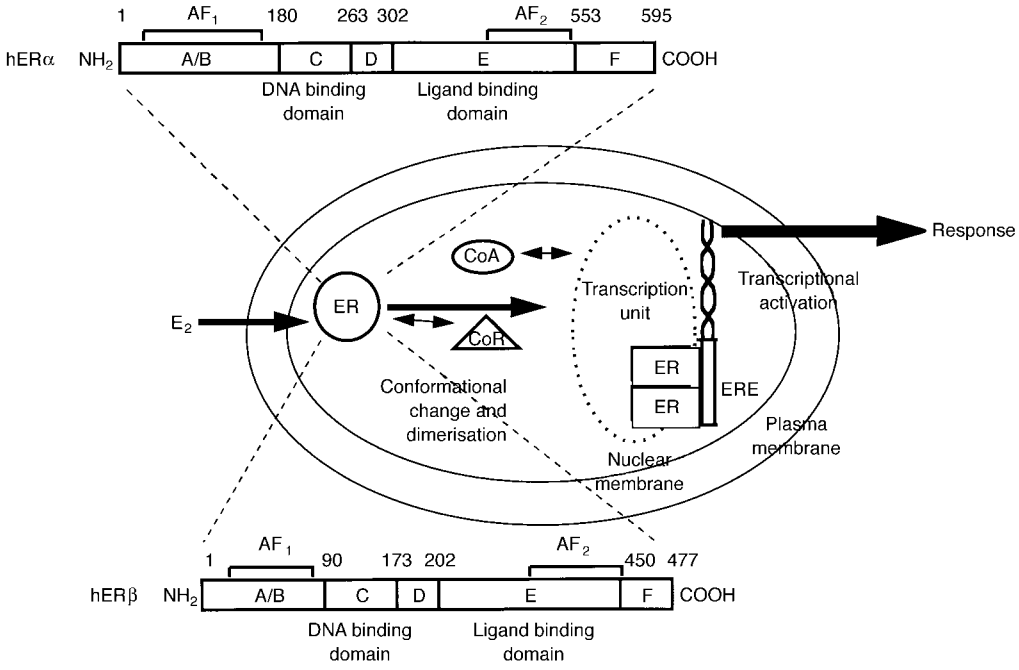


**Figure 1. The regulation of oestradiol ($E_2$) action through the oestrogen receptor (ER) signal transduction pathway. The respective ERα or ERβ proteins are activated and dimerise (homo- or hetero-) to form a transcription unit. This is located appropriately at an oestrogen response element (ERE) in the promoter region of an oestrogen responsive gene. The transcription unit is formed by co-activator (CoA) molecules but can be neutralised if anti-oestrogens are bound to the ER. The anti-oestrogen–ER complex does not bind CoA molecules but attracts co-repressor molecules (CoR). SERM action might depend on the ratio of ERα and β or the ratio of CoA and CoR molecules in different target sites.**

terminal region which is involved in transactivation (A/B), a centrally located, well conserved DNA-binding domain (C), and carboxy-terminal region involved in dimerisation and in binding to Hsp90 (D), a ligand binding domain (LBD) (E) synergise with transactivation functions and an F region [42, 45] which appears to play a role in modulating transcriptional activation by ERα [46]. ERβ is homologous to ERα at the ligand binding (58%) and DNA binding (96%) domains, whereas the A/B region, hinge domain and F region are not well conserved [42].

Both ERα and ERβ contain two activation functions (AFs) which contribute to the ER's transcriptional activity. AF-1 is located in the amino-terminal region within the A/B region and is believed to be constitutively active and ligand-independent [45, 47, 48]. It has been demonstrated that ligand-independent activation of ER via the AF-1 domain is closely related to the phosphorylation status of the receptor [49–51]. In particular, Ser-118 in the AB region of ERα is important for the activation through the Ras-MAP kinase (MAPK) signalling cascade [51, 52] and Ser-106 and Ser-124 are two phosphorylation sites in the A/B region of ERβ which are essential for ligand-independent activation of the ERβ via the MAPK cascade [53, 54]. In addition, both receptors contain a second activation domain, AF-2, which is present at the carboxy-terminus and is ligand-dependent [55, 56]. Mutational analyses demonstrate the importance of this region for ER transactivation [57–59] because AF-2 can interact with a number of putative transcriptional co-activators in a ligand-dependent manner [60–63].

AF-1 and AF-2 can activate transcription independently but in most cases they synergise with one another in a promoter- and cell context-specific manner [47, 59]. It is believed that ER activates gene expression by binding to oestrogen response elements (EREs) in responsive genes through the synergistic action of AF-1 and AF-2 [64]. ER β was also shown to activate transcription of target genes through EREs [42, 65]. However, it has been demonstrated recently that while oestrogen can induce an AP-1 site in a reporter construct through ERα, it is inactive via ERβ [66]. Interestingly, ER antagonists activate ERβ to induce activity through an AP-1 site [66] and through the human retinoic acid receptor α-1 promoter [67].

The two receptors ERα and ERβ may form functional heterodimers on DNA [53, 65, 68] that could bind the coactivator, SRC-1, and stimulate transcription of a target gene [69]. The ability of ERα and ERβ to form heterodimers suggests that ER may function through different dimeric states, and it is possible that the dimers could be activated by selective ligands [70].

## A MOLECULAR MECHANISM FOR OESTROGEN ACTION

The existence of two rather than one ER, indicates that the mechanism of action of oestrogen and anti-oestrogens is even more complex than previously thought. Oestrogen, upon binding to its high-affinity receptor (or receptors), triggers the expression of multiple genes involved in the regulation of cell proliferation and differentiation. This process may sound simple, but is actually very complex. The binding to the agonist allows the ER to dissociate from heat shock protein, dimerise, bind to specific DNA sequences and stimulate transcription of responsive genes. Now it is well known that ER itself is far from being the direct controller of transcription: it requires an interaction with a complex of co-regulatory proteins (co-activators or co-repressors), which act as signalling intermediates between the ER and the general transcriptional

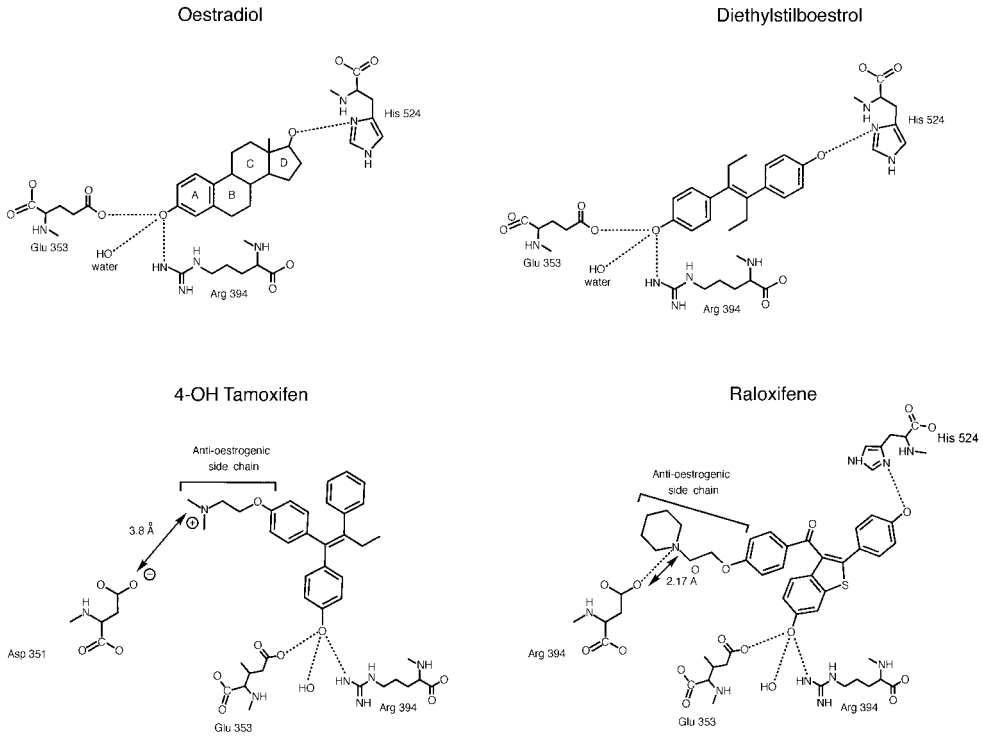


**Figure 2. The interaction of oestrogenic and anti-oestrogenic ligands with specific amino acids in the ligand binding domain of ERα.**

machinery [71] (Figure 1). Recently, the crystal structure of the ligand binding domain (LBD) of ERα was determined with oestradiol [72] and synthetic non-steroidal oestrogen diethylstilboestrol [73]. Both high-affinity ligands interact with the same amino acids to locate the oestrogens correctly (Figure 2). A key feature of the agonist–receptor structure is the ability of steroid to be enveloped in a hydrophobic pocket that is closed by helix 12 (an essential site for AF-2 activation) in the ligand-binding site of ERα (Figure 3a). Apparently, helix 12 is critical for the recruitment of coactivators to the AF-2 site and subsequent initiation of RNA polymerase activity. The repositioning of the helix 12 after ligand binding has been proposed as an important mechanism for full oestrogen action at ERα [72–75].

## CO-ACTIVATORS

There is considerable evidence that nuclear steroid receptors must associate with other nuclear proteins to form a

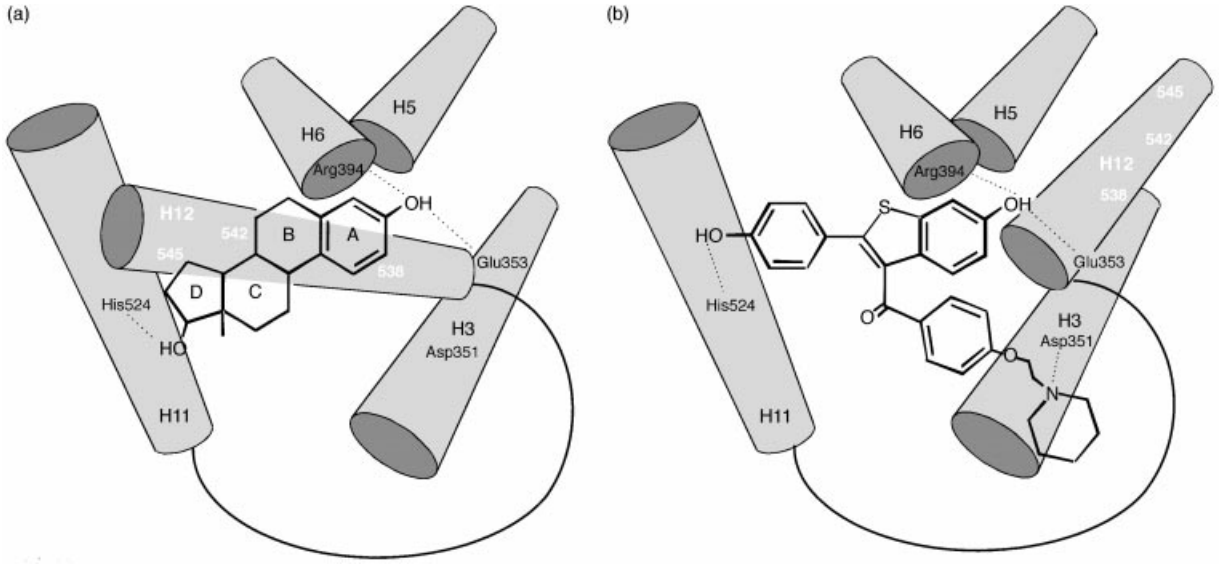


**Figure 3. (a) The locking of oestradiol in the ligand binding domain of ERα. Helix 12 seals the steroid into a hydrophobic pocket and exposes sites on the complex that can bind to co-activators. (b) The wedging of raloxifene in the ligand binding domain of ERα. Helix 12 cannot seal the selective oestrogen receptor (ER) modulator (SERM) into the hydrophobic pocket because the anti-oestrogenic side chain interacts with Asp 351, which acts as a pivot for the helix. Helix 12 blocks coactivator binding. Adapted from [72].**

transcription complex. For example, it has been shown that the AF-1 and AF-2 domains of ERα bind to TATA-binding protein (TBP) *in vitro* [76]. Moreover, ER can interact with components of the transcription factor IID (TFIID) complex [77] and TFIIB [78]. Interactions between ER and TFIID-associated factor (TAF), $TAF_{II}30$, have also been described [79]. However, while all these interactions are necessary, they are not sufficient to mediate transcription. The multiprotein complex must not only provide the machinery to transcribe the appropriate gene but also facilitate a mechanism for exposing the quiescent gene. This process must destabilise the DNA histone complex to allow RNA polymerase access to complete transcription.

Several co-activators have been described for ER based upon their ability to interact with agonist-bound receptor but not with an antagonist-bound receptor. ERAP 160 [80] and RIP 140 [81, 82] were identified using the LBD of ER as bait. Interestingly, RIP 140 does not interact with basal factors of the transcription machinery, suggesting a mechanism other than a bridging function for a co-activator [81]. SRC-1, on the other hand, was isolated from a human cDNA library using the LBD of hPgR as bait [83]. However, studies indicate that SRC-1 and ERAP 160 are variants of the same family of proteins.

The majority of receptor-interacting factors have been identified by using LBD of nuclear receptor as a bait this, therefore, explains why the AF-2 site is believed to be the most important site for co-activator recruitment. However, it has been demonstrated that SRC family members are not only a transcriptional mediator for the ligand-dependent AF-2 of ERα [84] but are also involved in ligand-independent interaction with AF-1 [84] and with ERβ through phosphorylation of AF-1 by the MAP-kinase signalling cascade [54]. Functional interaction of ER and SRC-1 occurs in the absence of exogenous ligand through Cyclin D1 bridging [85]. In addition, SRA (steroid receptor RNA activator), which is part of the SRC-1 complex, mediates transactivation via AF-1 [86]. Moreover, ERα/ERβ heterodimers were able to bind the SRC-1 and stimulate transcription of a reporter gene [69].

TIF2 belongs to the family of transcriptional intermediary factors (TIFs) which mediate AF activity to the transcription machinery [71]. The SRC-1-related TIF2 also efficiently stimulates AF-2 activity of ER *in vivo* [87]. Another protein involved in co-activator complex with SRC-1 is p300/CBP, a 300-kDa protein related to the cAMP response element-binding protein, CBP [88–90]. It has been demonstrated that SRC-1 and p300/CBP contain intrinsic acetyltransferase activity and can interact with other histone acetyltransferases (HATs). Acetylation by SRC-1 of histones bound at specific promoters could be a mechanism by which the AFs of ER and associated co-activators activate transcription of specific genes by enhancing formation of a stable pre-initiation complex [91].

## A MOLECULAR MECHANISM FOR ANTI-OESTROGEN ACTION

Anti-oestrogens are competitive inhibitors of oestrogen action. The shape of the ligand, with a strategically placed alkylaminoethoxy side chain, is essential to convert an oestrogenic complex to an anti-oestrogenic complex with a reduced oestrogenic efficacy. Earlier models of anti-oestrogen action (proposed before the ER was cloned and sequenced) suggested that the anti-oestrogenic side chain prevents the receptor from closing so that the activation of the complex would be incomplete [92, 93]. Partial agonism of receptor complexes would occur because of an equilibrium mixture of activated and unactivated receptor complexes [94]. This was an adaptation of Belleau's macromolecular perturbation theory [95].

McDonnell's group extended these observations and presented convincing evidence that the shape of different anti-oestrogen–ER complexes are not all the same [96]. A mutated ER assay methodology in human liver cancer cells, was used to classify new drugs based on their interaction with an engineered $C_3$ promoter target. However, it is the realisation that a third component of the signal transduction system is involved [97] that has provided the most excitement for solving the secrets of SERMs. Co-activators and possibly co-repressors are key components that can modulate gene activation. Clearly, the shape of an anti-oestrogenic ER complex will dictate how or if any other protein in a transcription unit will bind.

The crystallisation of the LBD of the ER with oestrogens and anti-oestrogens has provided enormous insight into the change in protein shape that prevents anti-oestrogens from building a transcription complex at AF-2 [72, 73]. It is known that 4-hydroxytamoxifen silences AF-2, while AF-1 remains constitutively activated [98–101].

Both raloxifene and 4-hydroxytamoxifen fit into the hydrophobic pocket of the LPD but the anti-oestrogenic side chain prevents the re-orientation of helix 12 that must seal the ligand into the receptor before coactivators can bind and produce a transcription complex. The high-affinity ligands both interact, through phenolic hydroxyls with Glu 353 and Arg 394 to locate the ligands correctly in the binding domain (Figure 2) [72, 73]. However, the side chain that is critical for anti-oestrogenic activity interacts with Asp 351. Clearly, this interaction is the key to anti-oestrogenicity as it controls the repositioning of helix 12. This molecular model provides an elegant solution to AF-2 silencing, but it should be noted that there are significant differences in the interaction of the anti-oestrogenic side chains of raloxifene and 4-hydroxytamoxifen with amino acids in helix 3 that ultimately cause minor, but significant differences in the positioning of helix 12 (Figure 3b). These differences have the potential to alter the oestrogenic efficacy of the different anti-oestrogen–ER complexes.

## CO-REPRESSORS

Although the experimental evidence suggests that activating functions can be prevented in the anti-oestrogen–ER complex by stopping co-activators binding, an alternative hypothesis is an increased binding of proteins that only interact with the anti-oestrogen–ER complex. Co-repressors would, therefore, neutralise an anti-oestrogen–ER complex. Although several proteins have been identified that can switch off an antihormone receptor complex [102–104] one would have to argue that the process is serendipity unless natural antihormones are identified. There is no doubt that serendipity is a possibility, as there is every reason to believe that the novel shape of an anti-oestrogen–receptor complex could sequester random molecules in the vicinity of the genome. Clearly, if enough key transcription factors are 'squelched' inappropriately, the machinery for cell replication could fail. Finding the 'mystery molecules' is the challenge.

Although the concept of a repressor molecule is a fundamental of molecular biology, models of anti-oestrogen action

could as easily depend entirely on the dynamics of co-activator binding. For example, studies of transforming growth factor-α (TGFα) gene activation with oestradiol or 4-hydroxytamoxifen in MDA-MB-231 cells stably transfected with the cDNA for ER, show equal gene activation by anti-oestrogen– and oestrogen–receptor complexes [105]. This result could be interpreted as a lack of co-repressors in MDA-MB-231 cells so that anti-oestrogen action cannot occur. However, raloxifene and the pure anti-oestrogen ICI 182,780 block oestradiol and 4-hydroxytamoxifen stimulated TGFα gene activation so that explanation is unlikely [106]. We prefer the hypothesis that MBA-MB-231 cells contain an excess of specific coactivators that, through the law of mass action, can activate the 4-hydroxytamoxifen–ER complex. The altered shape of the raloxifene–ER complex does not allow co-activators to bind so the complex is less promiscuous.

## WHAT WE DO NOT KNOW ABOUT THE MECHANISMS OF ACTION OF SERMS

Although some progress has been made with our understanding of oestrogen and anti-oestrogen action there is no unifying theory that can explain the target site-specific actions of SERMs. Despite this deficit, there are opportunities to imagine multiple mechanisms. In other words, there may be different mechanisms at different sites or groups of targets. By way of example, it is intriguing that raloxifene expresses less oestrogen-like activity than 4-hydroxytamoxifen in breast and uterine cells. The shapes induced by the ligands in the LBD are similar enough that one could infer that both raloxifene and 4-hydroxytamoxifen silenced AF-2. However, the crystal structures tell us nothing about the relationship between AF-1 and AF-2. The whole protein has not been crystallised. If 4-hydroxytamoxifen is known to silence only AF-2 and AF-1 is constitutively active [47, 98–101] then, based on our results at the TGFα gene where the raloxifene–receptor complex is without activity [107], one would have to conclude that raloxifene is less oestrogenic because it silences AF-1 and AF-2. This conclusion would be a reasonable explanation, based on the shape of ERα for the reduced oestrogenicity in the rodent [108] and human uterus [109]. The shape of the complex would dictate whether excess coactivators in a target tissue would bind. The hypothesis would also apply to drug resistance to tamoxifen. One could imagine that breast cancer cells with wild-type receptor would be cloned out if an excess of oestrogenic co-activators could facilitate gene transcription through the tamoxifen–ER complex. Less oestrogenic anti-oestrogens would, therefore, be valuable second-line agents to treat tamoxifen-resistant breast cancer [110] because the new shape of the complex, or destruction of the receptor would prevent gene transcription [111]. Furthermore, the hypothesis suggests that raloxifene-like compounds may not be completely cross-resistant with tamoxifen.

These interpretations of differential ER action by anti-oestrogens at a breast cancer target present a dilemma for a unifying theory of SERM action. If the shape of the raloxifene and 4-hydroxytamoxifen–ERα complexes dictate the silencing of AFs selectively, then ERα shape cannot explain the similarity of the oestrogen-like properties of tamoxifen and raloxifene on bone [34, 35] and circulating cholesterol [112, 113].

An alternative idea is that the anti-oestrogen–ERα complex interacts with another sequence of DNA that could be referred to as ''an anti-oestrogen response element'', in contrast to an oestrogen response element. Such an idea has been suggested by McDonnell and coworkers by screening cDNA libraries [114] and Yang and coworkers have proposed a raloxifene response element [115]. The latter authors now suggest not a receptor–DNA interaction but a receptor–protein interaction that enhances the oestrogen-like properties of raloxifene at a transforming growth factor β promoter [116]. This could explain the bone effects of raloxifene but 4-hydroxytamoxifen was not tested in the system so a unifying mechanism cannot be proposed.

Nevertheless, the idea of anti-ER complexes providing gene activation through a protein interaction at the genome is appealing in light of the observation that 4-hydroxytamoxifen–ERβ complexes can initiate gene transcription at AP-1 (*fos* and *jun*) sites [66]. The effect of raloxifene at ERβ is not related to the affinity of the ligand for the receptor but rather the ability to activate a reporter at high concentrations of ligand i.e.: 1–10 μM. Unexpectedly, however, the pure anti-oestrogen ICI 164, 384 can also activate AP-1 sites through ERβ but oestradiol and diethylstilbestrol block gene activation. Clearly, these data are inconsistent with the known effects of oestrogen and pure anti-oestrogens on bone remodelling.

Another idea is that the ratio of ERα and ERβ is important to express oestrogen-like effects of anti-oestrogens; the higher the proportion of ERβ the greater the likelihood of an oestrogen-like effect. At present, the hypothesis is being tested in breast tumours [117, 118] but the difficulty appears to be the lack of a useful antibody for ERβ. All results on the distribution of ERβ are based on the use of RT–PCR and extrapolation of RNA levels to deduce the presence of a pharmacological target.

The most compelling solution to describing a role for ERβ in SERM action would be to identify an ERβ specific agonist or antagonist. As yet, none have been reported but since the cDNAs for both ERα and ERβ are known there is no reason why an automated compound screen cannot come up with a molecule with differential actions (see 'note added in proof' at the end of the text).

## NEW MOLECULES

The successful development of tamoxifen as a breast cancer preventive, and the introduction of raloxifene to prevent osteoporosis, has encouraged the investigation of 'new' molecules that may have unforeseen advantages. Toremifene and idoxifene are similar molecules to tamoxifen, which, although they potentially have multiple applications as SERMs, are only targeted for the treatment of advanced breast cancer (Figure 4). Similarly, droloxifene (3-hydroxytamoxifen) is known to have efficacy as a breast cancer drug [119] but is also a SERM at bone sites [120].

The compounds listed in Figures 5 and 6 (with the exception of ICI 182,780) are potentially able to be applied either as breast cancer therapies or as agents for osteoporosis or both. The drugs GW 5638 and CP 336,156 are both particularly interesting because they maintain bone density in animals [121, 122] and could find an application as preventives for osteoporosis but with the potential to prevent breast cancer because of their structural similarity to tamoxifen and nafoxidine, respectively. Furthermore, the new generation SERM LY353381 is designed to be more bioavailable than raloxifene so that a more sustained blood level can be maintained [123]. However, the new drug EM 652 currently being developed by Schering Plough is a surprise. The compound

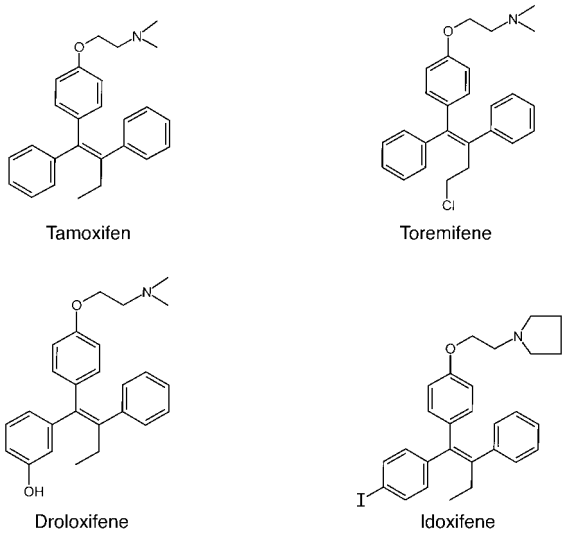


**Figure 4. Selective oestrogen receptor modulators (SERMs) related to tamoxifen.**

has been evaluated rigorously in animal models and is classified as an orally active pure anti-oestrogen because the molecule silences both AF-1 and AF-2 in ERα [54]. The formula of the drug is drawn in the publications to show a structural similarity to the pure anti-oestrogen ICI 182, 780 [124].

We have developed an assay system *in vitro* that can distinguish between tamoxifen-like compounds, raloxifene-like compounds and pure anti-oestrogens (Figure 7). The assay depends on the differential regulation of the TGFα via wild-type and Asp 351Tyr mutant ER [105–107]. EM 652 was reclassified as a raloxifene-like compound [125] that may not only have use in its original application as a second-line drug after tamoxifen failure, but also as a drug with potential to prevent coronary heart disease and/or osteoporosis. New SERMs can now be rapidly classified based on functional assays and only select agents need to be crystallised, if necessary, to confirm the biological data.

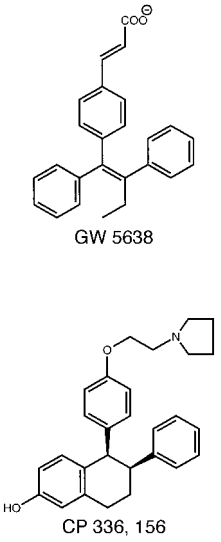


**Figure 5. Selective oestrogen receptor (ER) modulators (SERMs) that express less oestrogen-like actions in the rodent uterus than tamoxifen.**

## CHEMOTHERAPY TO CHEMOPREVENTION

At the beginning of the twentieth century, Paul Ehrlich proposed and developed the concept of chemotherapy [126]. His hypothesis was that a synthetic compound could be discovered to cure patients by identifying and targeting disease caused by invading bacteria. He based this hypothesis on the selective ability of synthetic dyes to stain bacteria but to leave the surrounding tissues unaffected. Ehrlich also devised a scientific method for the discovery of compounds with selective toxicity. A toxic moiety, in this case arsenic, was incorporated into an organic carrier molecule, tested in the laboratory for selective toxicity against animal disease and the successful compound tested in clinical trials. Salvarsan, or the 606th compound to be tested against spirochaetes, became the prototype chemotherapy for syphilis and changed the strategic approach to medicine in modern times. The sulphonamides, antibiotics and antimalarials have all resulted from the commercialisation of the chemotherapy concept. However, despite the synthesis and testing of huge numbers of chemotheraputic agents for cancer, a cure for the disease has remained elusive. Part of the problem has been the difficulty in identifying a specific cancer target. If cancer is derived from self, then it is difficult to implement a selective method of finally killing the last rogue cancer cell without killing the patient. Although there have been notable exceptions, such as testicular cancer and childhood leukaemia, the track record for successfully attacking the major solid tumours remains disappointing. Even Paul Ehrlich turned his attention to cancer chemotherapy in the first decade of the twentieth century, but eventually he was to declare that he

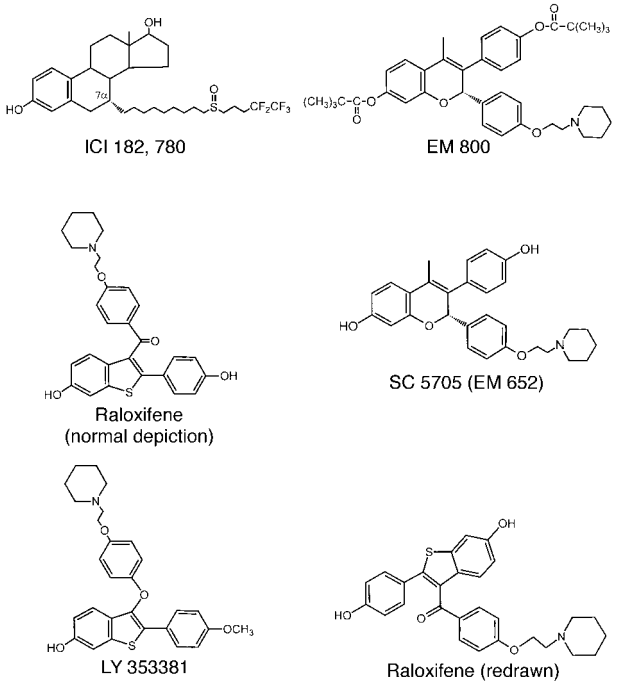


**Figure 6. A comparison of selective oestrogen receptor (ER) modulators (SERMs) with the structure of the pure anti-oestrogen ICI 182,780. EM 800 is metabolically activated to EM 652 and the metabolite has now been renamed SC 5705. Although the drug has a passing structural resemblance to ICI 182,780 the compound is related pharmacologically to raloxifene and the new SERM LY 353,381. The structure of raloxifene is redrawn to show the close structural similarity with SC 5705.**

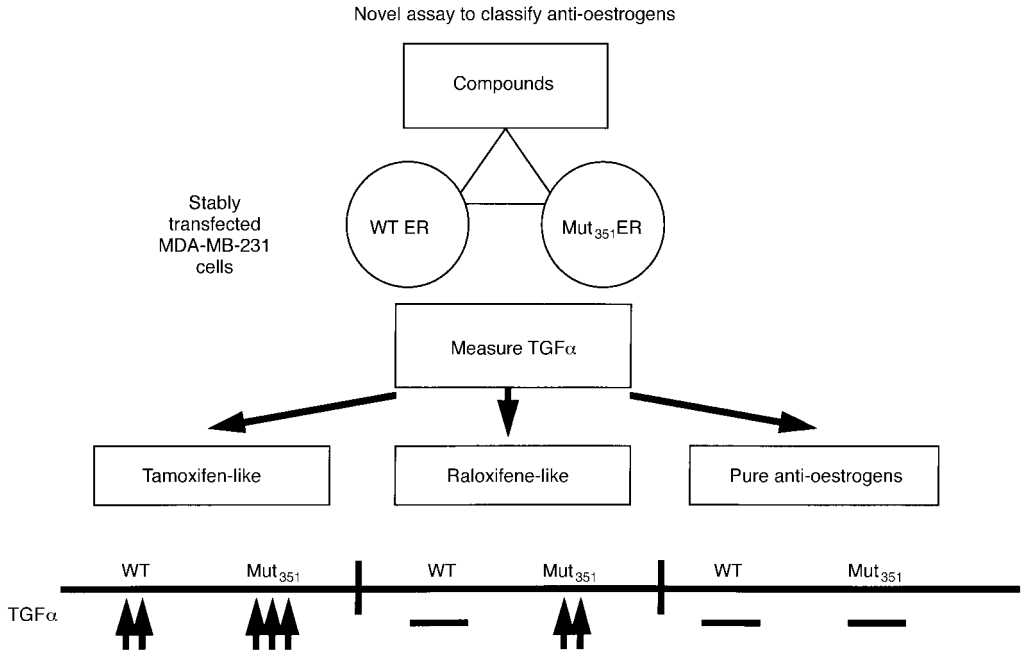


**Figure 7. Anti-oestrogens can be classified by their interaction with the oestrogen receptor (ER). Tamoxifen analogues with a similar side chain are promiscuous and readily activate the transforming growth factor-α (TGFα) gene in MDA-MB-231 cells stably transfected with cDNAs for ER. Raloxifene analogues have increased oestrogenicity with a mutated ER (Asp351Tyr) because this changes the protein conformation to increase activating functions. Pure anti-oestrogens are not affected by the ER mutation at 351 [105–107].**

had wasted the last 15 years of his life [127]. However, others took another path.

In a lecture in 1935 at the annual meeting of the American Association for Cancer Research in Boston, U.S.A. Professor Antoine Lacassagne of Paris' Institute du Radium reviewed the evidence for a hormonal contribution to the pathogenesis of mammary carcinoma. He suggested that one might one day be able to identify women at specific risk and antagonise the actions of oestrogenic hormones to reduce that risk. He wrote at the conclusion of his paper:

> If one accepts the consideration of adenocarcinoma of the breast as the consequence of a special heredity sensitivity to the proliferative actions of oestrone, one is led to imagine a therapeutic preventive for subjects predisposed by their heredity to this cancer. It would consist—perhaps in the very near future when the knowledge and use of hormones will be better understood—in the suitable use of a hormone, antagonistic or excretory to prevent the stagnation of oestrone in the ducts of the breast. [19]

Forty years ago Jensen proposed the ER as a mechanism of oestrogen action specific to target tissues [3] and Lerner [128, 129] described the first non-steroidal anti-oestrogen that blocked oestrogen action with no oestrogen-like activity in any other species or target tissue. However, the development of a pure anti-oestrogen would not have allowed the implementation of Lacassagne's vision. If oestrogen is essential for a woman, to maintain bone density and protect against coronary heart disease, then the long-term administration of an anti-oestrogen would provide no overall health benefit, despite the prevention of breast cancer. Ehrlich's method of screening and translational research was used to discover tamoxifen that Arthur Walpole at Astra Zeneca believed would be a safe and effective chemotherapy for the palliative treatment of advanced breast cancer [130]. Serendipitously, tamoxifen was discovered to possess the properties of a SERM and has become the lead compound for drug discovery in the first century of the next millennium.

With an ageing population there are demands for a disease-free life, as cures remain elusive. To achieve this goal Sporn first used the term 'chemoprevention' and described a strategy to arrest or prevent the process of carcinogenesis [131, 132]. Tamoxifen is the first chemopreventive for breast cancer [18], but the demonstration of proof of principle [9, 10] has opened the door to prevent more than cancer. The recognition of SERM action [29–33, 37] resulted in a paradigm shift in drug discovery in 1990 that is currently having an important impact on general medicine [133].

> We have obtained valuable information about this group of drugs that can be applied to other disease states. Research does not travel in straight lines and observations in one field of science often become major discoveries in another. Important clues have been garnered about the effects of tamoxifen on bones and lipids so it is possible that derivatives could find targeted applications to retard osteoporosis or atherosclerosis. The ubiquitous application of novel compounds to prevent diseases associated with the progressive changes after menopause may, as a side-effect, significantly retard the development of breast cancer. The targeted population would be postmenopausal women in general, thereby overcoming the requirement to select a high-risk group to prevent breast cancer [133].

Raloxifene is the first SERM that holds the promise of multiple applications [134]. The drug is used for the prevention of osteoporosis and is being tested in the STAR trial against tamoxifen for the prevention of breast cancer in high-risk postmenopausal women. Additionally, raloxifene reduces circulating cholesterol so it is being tested in women at risk for coronary heart disease to determine its worth as a preventive. Finally, there are hopes that raloxifene will reduce the risk of endometrial cancer.

The challenge of the twenty-first century will be to refine the target site-specificity of SERMs and to amplify the

required properties. Clearly, a drug that is oestrogen-like in the brain could protect against Alzheimer's disease and hot flushes but which can simultaneously modulate the appropriate peripheral targets to prevent osteoporosis, coronary heart disease, breast and endometrial cancer would be a major discovery and have enormous clinical potential. The key to therapeutic success, however, is that an individual must want to take a medicine because it makes her feel better. A public health strategy for disease prevention can only work if there is a minimum of side-effects and the quality of life is improved.

It would be naïve to believe that preventive medicines could be marketed adequately to make a profound impact on the epidemiology of breast cancer. Nevertheless, it is wise to prepare for a change in the practice of oncology if the proposed use of SERMs becomes widespread. If SERMs can reduce breast cancer incidence by 50% then another target must be identified to prevent the disease completely. The increase in the age of the 'baby boom' generation over the next two decades will result in a sharp increase in the numbers of breast cancers. The judicious use of SERMs in high-risk populations could reduce the incidence of breast cancer by 50% but the absolute numbers will still rise within a decade. Additionally, there will be a need to devise new treatment modalities, as hormone-responsive disease may become increasingly rare in some sections of society. Pure anti-oestrogens and aromatase inhibitors can be used to treat some of the SERM-resistant disease but, this in effect, will be second-line treatment. A vigorous re-examination of breast cancer treatment options must be made now to prepare for the challenges of the next millennium.

## NOTE ADDED IN PROOF

The first report of agents that can discriminate between ERα and ERβ has appeared [135]. An aryl-substituted pyrazole is an ERα potency-selective agonist. The compound has 120-fold higher potency to stimulate ERα versus ERβ. The R,R-enantiomer of a tetrahydrochrysene derivative is a complete antagonist at ERβ. The S,S-enantiomer is a partial agonist at ERβ and both enantiomers are agonists at ERα.


1. Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma: suggestions for a new method of treatment with illustrative cases. *Lancet* 1896, **ii**, 104–107.
2. Boyd S. On oophorectomy in cancer of the breast. *Br Med J* 1900, **ii**, 1161–1167.
3. Jensen EV, Jacobson HI. Basic guides to the mechanism of estrogen action. *Recent Prog Horm Res* 1962, **18**, 387–414.
4. Toft D, Gorski J. A receptor molecule for estrogens: isolation from the rat uterus and preliminary characterization. *Proc Natl Acad Sci USA* 1966, **55**, 1574–1581.
5. Toft D, Shyamala G, Gorski J. A receptor molecule for estrogen: studies using a cell-free system. *Proc Natl Acad Sci USA* 1967, **57**, 1740–1743.
6. Gorski J, Toft D, Shyamala G, Smith D, Notides A. Hormone receptors: studies on the interaction of estrogen with the uterus. *Recent Prog Horm Res* 1968, **24**, 45–80.
7. Jensen EV, Suzuki T, Karashima T, Stumpf WE, Jungblut PW, DeSombre ER. A two step mechanism for the interaction of estradiol with rat uterus. *Proc Natl Acad Sci USA* 1968, **59**, 632–638.
8. Gorski J, Welshons WV, Sakai D. Remodeling the estrogen receptor model. *Mol Cell Endocrinol* 1984, **36**, 11–15.
9. Jordan VC, William L. McGuire Memorial Lecture. "Studies on the estrogen receptor in breast cancer"; 20 years as a target for the treatment and prevention of breast cancer. *Breast Cancer Res Treat* 1995, **36**, 267–285.
10. Jordan VC. B. J. Kennedy Lecture. Estrogen receptor as a target for the prevention of breast cancer. *J Lab Clin Med* 1999, **133**, 408–414.
11. Jensen EV, Jacobson HI, Smith S, Jungblut PW, DeSombre ER. The use of estrogen antagonists in hormone receptor studies. *Gynecol Invest* 1972, **3**, 108–123.
12. Jordan VC, Koerner S. Tamoxifen (ICI 46, 474) and the human carcinoma 8S estrogen receptor. *Eur J Cancer* 1975, **11A**, 205–206.
13. Cole MP, Jones CTA, Todd IDH. A new antiestrogenic agent in late breast cancer. An early appraisal of ICI 46, 474. *Br J Cancer* 1971, **25**, 270–275.
14. Ward HWC. Antiestrogenic therapy for breast cancer: a trial of tamoxifen at two dose levels. *Br Med J* 1973, **1**, 13–14.
15. Jordan VC. Laboratory studies to develop general principles for the adjuvant treatment of breast cancer with antiestrogens: problems and potential for future clinical applications. *Breast Cancer Res Treat* 1983, **3**(Suppl.), 73–86.
16. Swedish Breast Cancer Cooperative Group. Randomized trial of two versus five years of adjuvant tamoxifen for postmenopausal early stage breast cancer. *J Natl Cancer Inst* 1996, **88**, 1543–1549.
17. Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomized trials. *Lancet* 1998, **351**, 1451–1467.
18. Fisher B, Costantino JP, Wickerham DL, *et al*. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst* 1998, **90**, 1371–1388.
19. Lacassagne A. Hormonal pathogenesis of adenocarcinoma of the breast. *Am J Cancer* 1936, **27**, 217–225.
20. Jordan VC. Tamoxifen: the herald of a new era of preventive therapeutics. *J Natl Cancer Inst* 1997, **89**, 747–749.
21. Jordan VC. Designer estrogens. *Scientific Am* 1998, **279**, 60–67.
22. Harper MJK, Walpole AL. A new derivative of triphenylethylene: effect on implantation and mode of action in rats. *J Reprod Fertil* 1967, **130**, 101–119.
23. Terenius L. Structure-activity relationships of antiestrogens with regard to interaction with 17β oestradiol in the mouse uterus and vagina. *Acta Endocrinol (Copenh)* 1971, **66**(Suppl.), 431–447.
24. Lyman SD, Jordan VC. Metabolism of nonsteroidal antiestrogens. In Jordan VC, ed. *Estrogen/antiestrogen action and breast cancer therapy*. Madison, Wisconsin, USA, University of Wisconsin Press, 1986, 191–219.
25. Robinson SP, Langan-Fahey SM, Jordan VC. Implications of tamoxifen metabolism in the athymic mouse for the study of antitumor effects upon human breast cancer xenografts. *Eur J Cancer Clin Oncol* 1989, **25**, 1769–1776.
26. Robinson SP, Langan-Fahey SM, Johnson DA, Jordan VC. Metabolites, pharmacodynamics and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. *Drug Metat Disp* 1991, **19**, 36–43.
27. Levenson AS, Jordan VC. MCF-7: the first hormone responsive breast cancer cell line. (Perspectives in Cancer Research). *Cancer Res* 1997, **57**, 3071–3078.
28. Osborne CK, Hobbs K, Clark GM. Effects of estrogens and antiestrogens on growth of human breast cancer cells in athymic nude mice. *Cancer Res* 1985, **45**, 584–590.
29. Jordan VC, Robinson SP. Species specific pharmacology of antiestrogens: role of metabolism. *Fed Proc* 1987, **46**, 1870–1874.
30. Jordan VC, Lababidi MK, Langan-Fahey S. Suppression of mouse mammary tumorigenesis by long-term tamoxifen therapy. *J Natl Cancer Inst* 1991, **83**, 492–496.
31. Jordan VC, Lababidi MK, Mirecki DM. The anti-oestrogenic and anti-tumour properties of prolonged tamoxifen therapy in C3H/OUJ mice. *Eur J Cancer* 1990, **26A**, 718–721.
32. Jordan VC, Phelps E, Lindgren JU. Effects of antiestrogens on bone in castrated and intact female rats. *Breast Cancer Res Treat* 1987, **10**, 31–35.
33. Gottardis MM, Jordan VC. The antitumor actions of keoxifene (raloxifene) and tamoxifen in the *N*-nitroso-methyl urea—induced rat mammary carcinoma model. *Cancer Res* 1987, **47**, 4020–4024.
34. Love RR, Mazess RB, Barden HS, *et al*. Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. *N Engl J Med* 1992, **326**, 852–856.

35. Delmas PD, Bjarnason NH, Mitlak BH, *et al*. Effects of raloxifene on bone mineral density, serum cholesterol concentrations and uterine endometrium in postmenopausal women. *N Engl J Med* 1997, **337**, 1641–1647.
36. Jordan VC, Glusman JE, Eckert S, et al. Raloxifene reduces incident primary breast cancers. Integrated data from multicenter double blind, placebo controlled randomized trials in postmenopausal women. *Breast Cancer Res Treat* 1998, **50**, 227 (Abstract 2).
37. Gottardis MM, Robinson SP, Satyaswwoop PG, Jordan VC. Contrasting actions of tamoxifen on endometrial and breast tumor growth in the athymic mouse. *Cancer Res* 1988, **48**, 812–815.
38. Assikis VJ, Neven P, Jordan VC, Vergote I. A realistic clinical perspective of tamoxifen and endometrial carcinogenesis. *Eur J Cancer* 1996, **32A**, 1464–1476.
39. Green S, Walter P, Kumar V, *et al*. Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. *Nature* 1986, **320**, 134–139.
40. Greene GL, Gilna P, Waterfield M, Baker A, Hort Y, Shine J. Sequence and expression of human estrogen receptor complementary DNA. *Science* 1986, **231**, 1150–1154.
41. Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J-Å. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA* 1996, **93**, 5925–5930.
42. Mosselman S, Polman J, Dijkema R. ERβ: identification and characterization of a novel human estrogen receptor. *FEBS Lett* 1996, **392**, 49–53.
43. Tremblay GB, Tremblay A, Copeland NG, *et al*. Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor β. *Mol Endocrinol* 1997, **11**, 353–365.
44. Couse JF, Korach KS. Exploring the role of sex steroids through studies of receptor deficient mice. *J Mol Med* 1998, **76**, 497–511.
45. Kumar V, Green S, Stack G, Berry M, Jin JR, Chambon P. Functional domains of the human estrogen receptor. *Cell* 1987, **51**, 941–951.
46. Montano MM, Muller V, Trobaugh A, Katzenellenbogen BS. The carboxy-terminal F domain of the human estrogen receptor: role in the transcriptional activity of the receptor and the effectiveness of antiestrogens as estrogen antagonists. *Mol Endocrinol* 1995, **9**, 814–825.
47. Berry M, Metzger D, Chambon P. Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. *EMBO J* 1990, **9**, 2811–2818.
48. Metzger D, Ali S, Bornert JM, Chambon P. Characterization of the amino-terminal transcriptional activation function of the human estrogen receptor in animal and yeast cells. *J Biol Chem* 1995, **270**, 9535–9542.
49. Weigel NL, Zhang Y. Ligand-independent activation of steroid hormone receptors. *J Mol Med* 1998, **76**, 469–479.
50. Aronica SM, Katzenellenbogen BS. Stimulation of estrogen-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-1. *Mol Endocrinol* 1993, **7**, 743–752.
51. Bunone G, Briand P-A, Miksicek RJ, Picard D. Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO J* 1996, **15**, 2174–2183.
52. Kato S, Endoh H, Masuhiro Y, *et al*. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. *Science* 1995, **270**, 1491–1494.
53. Tremblay GB, Tremblay A, Labrie F, Giguere V. Dominant activity of activation function 1 (AF-1) and differential stoichiometric requirements for AF-1 and -2 in the estrogen receptor α–β heterodimeric complex. *Mol Cell Biol* 1999, **19**, 1919–1927.
54. Tremblay A, Tremblay GB, Labrie F, Giquere V. Ligand-independent recruitment of SRC-1 to estrogen receptor β through phosphorylation of activation function AF-1. *Mol Cell* 1999, **3**, 513–519.
55. Tora L, White J, Brou C, *et al*. The human estrogen receptor has two independent nonacidic transcriptional activation functions. *Cell* 1989, **59**, 477–487.
56. Lees JA, Fawell SE, Parker MG. Identification of two transactivation domains in the mouse oestrogen receptor. *Nucleic Acid Res* 1989, **17**, 5477–5487.
57. Wrenn CK, Katzenellenbogen BS. Structure–function analysis of the hormone binding domain of the estrogen receptor by region-specific mutagenesis and phenotypic screening in yeast. *J Biol Chem* 1993, **268**, 24089–24098.
58. Danielian PS, White R, Lees JA, Parker MG. Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. *EMBO J* 1992, **11**, 1025–1033.
59. Tzukerman MT, Esty A, Santiso-Mere D, *et al*. Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. *Mol Endocrinol* 1994, **8**, 21–30.
60. vom Baur E, Zechel C, Heery D, *et al*. Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. *EMBO J* 1996, **15**, 110–124.
61. L'Horset F, Dauvois S, Heery DM, Cavailles V, Parker MG. RIP-140 interacts with multiple nuclear receptors by means of two distinct sites. *Mol Cell Biol* 1996, **16**, 6029–6036.
62. Cavailles V, Dauvois S, Danielian PS, Parker MG. Interaction of proteins with transcriptionally active ectrogen receptors. *Proc Natl Acad Sci USA* 1994, **91**, 10009–10013.
63. Beato M, Sanchez-Pacheco A. Interaction of steroid hormone receptors with the transcription initiation complex. *Endocrine Rev* 1996, **17**, 587–609.
64. Kraus WL, McInerney EM, Katzenellenbogen BS. Ligand-dependent, transcriptionally productive association of the amino- and carboxyl-terminal regions of a steroid hormone nuclear receptor. *Proc Natl Acad Sci USA* 1995, **92**, 12314–12318.
65. Pace P, Tayler J, Suntharalingam S, Coombes RC, Ali S. Human estrogen receptor β binds DNA in a manner similar to and dimerizes with estrogen receptor α. *J Biol Chem* 1997, **272**, 25832–25838.
66. Paech K, Webb P, Kuiper GGJM, *et al*. Differential ligand activation of estrogen receptors ERα and ERβ at AP-1 sites. *Science* 1997, **277**, 1508–1510.
67. Zou A, Marschke KB, Arnold KE, *et al*. Estrogen receptor β activates the human retinoic acid receptor α-1 promoter in response to tamoxifen and other estrogen receptor antagonists, but not in response to estrogen. *Mol Endocrinol* 1999, **13**, 418–430.
68. Pettersson K, Grandien K, Kuiper GGJM, Gustafsson J-Å. Mouse estrogen receptor β forms estrogen response element-binding heterodimers with estrogen receptor α. *Mol Endocrinol* 1997, **11**, 1486–1496.
69. Cowley SM, Hoare S, Mosselman S, Parker MG. Estrogen receptors α and β form heterodimers on DNA. *J Biol Chem* 1997, **272**, 19858–19862.
70. Mitlak BH, Cohen FJ. Selective estrogen receptor modulators. *Drugs* 1999, **57**, 653–663.
71. Horwitz KB, Jackson TA, Bain DL, Richer JK, Takimoto GS, Tung L. Nuclear receptor coactivators and corepressors. *Mol Endocrinol* 1996, **10**, 1167–1177.
72. Brzozowski AM, Pike ACW, Dauter Z, *et al*. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* 1997, **389**, 753–758.
73. Shiau AK, Barstad D, Loria PM, *et al*. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* 1998, **95**, 927–937.
74. Feng W, Ribeiro RC, Wagner RL, *et al*. Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors. *Science* 1998, **280**, 1747–1749.
75. Moras D, Gronemeyer H. The nuclear receptor ligand-binding domain: structure and function. *Curr Opin Cell Biol* 1998, **10**, 384–391.
76. Sadovsky Y, Webb P, Lopez G, *et al*. Transcriptional activators differ in their responses to overexpression of TATA-box-binding protein. *Mol Cell Biol* 1995, **15**, 1554–1563.
77. Brou C, Wu J, Ali S, *et al*. Different TBP-associated factors are required for mediating the stimulation of transcription *in vitro* by the acidic transactivator GAL-VP16 and the two nonacidic activation functions of the estrogen receptor. *Nucleic Acids Res* 1993, **21**, 5–12.

78. Ing NH, Beekman JM, Tsai SY, Tsai M-J, O'Malley BW. Members of the steroid receptor superfamily interact with TFIIB (S3000-II). *J Biol Chem* 1992, **267**, 17617–17623.
79. Jacq X, Brou C, Lutz Y, Davidson I, Chambon P, Tora L. Human $TAF_{II}30$ is present in a distinct TFIID complex and is required for transcriptional activation by the estrogen receptor. *Cell* 1994, **70**, 107–117.
80. Halachmi S, Marden E, Martin G, MacKay H, Abbondanza C, Brown M. Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription. *Science* 1994, **264**, 1455–1458.
81. Cavailles V, Dauvois S, L'Horset F, *et al*. Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *EMBO J* 1995, **14**, 3741–3751.
82. Cavailles V, Dauvois S, Danielian PS, Parker MG. Interaction of proteins with transcriptionally active estrogen receptors. *Proc Natl Acad Sci USA* 1994, **91**, 10009–10013.
83. Onate SA, Tsai SY, Tsai M-J, O'Malley BW. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* 1995, **270**, 1354–1357.
84. Onate SA, Boonyaratanakornkit V, Spencer TE, *et al*. The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors. *J Biol Chem* 1998, **273**, 12101–12108.
85. Zwiisen RML, Buckle RS, Hijmans EM, Loomans CJM, Bernards R. Ligand-independent recruitment of steroid receptor coactivators to estrogen receptor by cyclin D1. *Genes Devel* 1998, **12**, 3488–3498.
86. Lanz RB, McKenna NJ, Onate SA, *et al*. A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. *Cell* 1999, **97**, 17–27.
87. Voegel JJ, Heine MJS, Zechel C, Chambon P, Gronemeyer H. TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO J* 1996, **15**, 3667–3675.
88. Kamei Y, Xu L, Heinzel T, *et al*. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* 1996, **85**, 403–414.
89. Hanstein B, Eckner R, DiRenzo J, *et al*. P300 is a component of an estrogen receptor coactivator complex. *Proc Natl Acad Sci USA* 1996, **93**, 11540–11545.
90. Chakravarti D, LaMorte VJ, Nelson MC, *et al*. Role of CBP/p300 in nuclear receptor signalling. *Nature* 1996, **383**, 99–103.
91. Spencer TE, Jenster G, Burcin MM, *et al*. Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature* 1997, **389**, 194–198.
92. Lieberman ME, Gorski J, Jordan VC. An estrogen receptor model to describe the regulation of prolactin synthesis by antiestrogens *in vitro*. *J Biol Chem* 1983, **258**, 4741–4745.
93. Tate AC, Greene GL, DeSombre ER, Jensen EV, Jordan VC. Differences between estrogen and antiestrogen–estrogen receptor complexes identified with an antibody raised against the estrogen receptor. *Cancer Res* 1984, **44**, 1012–1018.
94. Jordan VC, Lieberman ME. Estrogen-stimulated prolactin synthesis *in vitro*: classification of agonists, partial agonist and antagonist actions based on structure. *Mol Pharmacol* 1984, **26**, 274–285.
95. Belleau B. A molecular theory of drug action based on induced conformational perturbations of receptors. *J Med Chem* 1964, **7**, 776–778.
96. McDonnell DP, Clemm DL, Herman T, Goldman ME, Pike JW. Analysis of estrogen receptor function reveals three distinct classes of antiestrogens. *Mol Endocrinol* 1995, **9**, 659–669.
97. Katzenellenbogen JA, O'Malley BW, Katzenellenbogen BS. Tripartite steroid receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. *Mol Endocrinol* 1996, **10**, 119–131.
98. Webster NJ, Green S, Jin JR, Chambon P. The hormone-binding domains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function. *Cell* 1988, **54**, 199–207.
99. Gronemeyer H, Benhamou B, Berry M, *et al*. Mechanisms of antihormone action. *J Steroid Biochem Mol Biol* 1992, **41**, 217–221.
100. McInerney EM, Katzenellenbogen BS. Different regions in activation function-1 of human estrogen receptor required for antiestrogen- and estradiol-dependent transcriptional activation. *J Biol Chem* 1996, **271**, 24172–24178.
101. Metzger D, Berry M, Ali S, Chambon P. Effect of antagonists on DNA binding properties of the human estrogen receptor *in vitro* and *in vivo*. *Mol Endocrinol* 1995, **9**, 579–591.
102. Horlein AJ, Naar AM, Heinzel T, *et al*. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* 1995, **377**, 397–403.
103. Kurokawa R, Soderstrom M, Horlein A, *et al*. Polarity-specific activities of retinoic acid receptors determined by a co-repressor. *Nature* 1995, **377**, 451–454.
104. Chen JD, Evans RM. A transcriptional corepressor that interacts with nuclear hormone receptors. *Nature* 1995, **377**, 454–457.
105. Levenson AS, Tonetti DA, Jordan VC. The estrogen-like effect of 4-hydroxytamoxifen on induction of transforming growth factor α mRNA in MDA-MB-231 breast cancer cells stably expressing the estrogen receptor. *Br J Cancer* 1998, **77**, 1812–1819.
106. Levenson AS, Catherino WH, Jordan VC. Estrogenic activity is increased for an antiestrogen by a natural mutation of the estrogen receptor. *J Steroid Biochem Mol Biol* 1997, **60**, 261–268.
107. Levenson AS, Jordan VC. The key to the antiestrogenic mechanism of raloxifene is amino acid 351 (aspartate) in the estrogen receptor. *Cancer Res* 1998, **58**, 1872–1875.
108. Black LJ, Sato M, Rowley ER, *et al*. Raloxifene (LY139481 HCI) prevents bone loss and reduces serum cholesterol without causing uterine hypotrophy in ovariectomized rats. *J Clin Invest* 1994, **93**, 63–69.
109. Boss SM, Huster WJ, Neild JA, Glant MD, Eisenhut CC, Draper MW. Effect of raloxifene hydrochloride on the endometrium of postmenopausal women. *Am J Obstet Gynecol* 1997, **177**, 1458–1464.
110. Howell A, Defriend D, Robertson J, Blarney R, Walton P. Response to a specific antioestrogen (ICI 182,780) in tamoxifen-resistant breast cancer. *Lancet* 1995, **345**, 29–30.
111. Dauvois S, White R, Parker MG. The antiestrogen ICI 182,780 disrupts estrogen receptor nucleocytoplasmic shuttling. *J Cell Sci* 1993, **106**, 1377–1388.
112. Love RR, Wiebe DA, Newcomb PA, *et al*. Effects of tamoxifen on cardiovascular risk factors in postmenopausal women. *Ann Intern Med* 1991, **115**, 860–864.
113. Walsh BW, Kuller LH, Wild RA, *et al*. Effects of raloxifene on serum lipids and coagulation factors in healthy postmenopausal women. *JAMA* 1998, **279**, 1445–1451.
114. Dana SL, Hoener PA, Wheeler DA, Lawerence CB, McDonnell DP. Novel estrogen response elements identified by genetic selection in yeast are differentially responsive to estrogens and antiestrogens in mammalian cells. *Mol Endocrinol* 1994, **8**, 1193–1207.
115. Yang NN, Venugopalan M, Hardikar S, Glasebrook A. Identification of an estrogen response element activated by metobolite of 17β estradiol and raloxifene. *Science* 1996, **273**, 1222–1225.
116. Yang NN, Venugopalan M, Hardikar S, Glasebrook A. Correction. Raloxifene response needs more than an element. *Science* 1997, **275**, 1249.
117. Speirs V, Parkes AT, Kerin MJ, *et al*. Coexpression of estrogen receptor α and β: poor prognostic factors in human breast cancer. *Cancer Res* 1999, **59**, 525–528.
118. Dotzlaw H, Leygue E, Watson PH, Murphy LC. Estrogen receptor-β messenger RNA expression in human breast tumor biopsies: relationship to steroid receptor status and regulation by progestins. *Cancer Res* 1999, **59**, 529–532.
119. Rauschning W, Pritchard KI. Droloxifene, a new antiestrogen: its role in metastatic breast cancer. *Breast Cancer Res Treat* 1994, **31**, 83–94.
120. Ke HZ, Simmons HA, Pirie CM, Crawford DT, Thompson DD. Droloxifene, a new estrogen antagonist/agonist, prevents bone loss in ovariectomized rats. *Endocrinology* 1995b, **136**, 2435–2440.
121. Willson TM, Norris JD, Wagner BL, *et al*. Dissection of the molecular mechanism of action of GW5638, a novel estrogen receptor ligand, provides insights into the role of estrogen receptor in bone. *Endocrinology* 1997, **138**, 3901–3911.

122. Ke HZ, Paralkar VM, Grasser WA, *et al*. Effects of CP-336,156, a new, nonsteroidal estrogen agonist/antagonist, on bone, serum cholesterol, uterus and body composition in rat models. *Endocrinology* 1998, **139**, 2068–2076.
123. Sato M, Turner CH, Wang T, Adrian MD, Rowley E, Bryant HU. LY353381.HCl: a novel raloxifene analog with improved SERM potency and efficacy *in vivo*. *J Pharmacol Exp Ther* 1998, **287**, 1–7.
124. Gauthier S, Caron B, Cloutier J, *et al*. (S)-(+)-[4-[7-(2,2-dimethyl-1-oxopropoxy)-4-methyl-2-[4-[2-(1-piperidinyl) ethoxy]phenyl]-2H-1-benzopyran-3-yl]phenyl]-2,2-dimethyl-propanoate (EM-800): a highly potent, specific and orally active non-steroidal antiestrogen. *J Med Chem* 1997, **40**, 2117–2122.
125. MacGregor JI, Liu H, Tonetti DA, Jordan VC. The interaction of raloxifene and the active metabolite of the antiestrogen EM-800 with the human estrogen receptor (ER). *Cancer Res* 1999, **59**, 4308–4313.
126. Baumler E. *Paul Ehrlich—Scientist for Life*. New York, Holmes and Meier, 1984.
127. Schrek R. Fashions in cancer research. In Wartman WB, ed. *Year Book of Pathology and Clinical Pathology*. Chicago, Year Book Publishers, 1960, 26–39.
128. Lerner LJ, Holthaus JF, Thompson CR. A nonsteroidal estrogen antagonist 1-(p-2 diethylaminoethoxyphenyl)-1-phenyl-2-p-methoxyphenyl-ethanol. *Endocrinology* 1958, **63**, 295–318.
129. Lerner LJ. The first non-steroidal antiestrogen- MER 25. In Sutherland RL, Jordan VC, eds. *Nonsteroidal Antioestrogens: Molecular Pharmacology and Antitumour Activity*. Sydney, Australia, Sydney Academic Press, 1981, 1–6.
130. Jordan VC. The development of tamoxifen for breast cancer: a tribute to the late Arthur L. Walpole. *Breast Cancer Res Treat* 1988, **11**, 197–209.
131. Sporn MB, Dunlop NM, Newton DL, Smith JM. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). *Fed Proc* 1976, **35**, 1332–1338.
132. Sporn MB. Approaches to prevention of epithelial cancer during the preneoplastic period. *Cancer Res* 1976, **36**, 2699–2702.
133. Lerner LJ, Jordan VC. Development of antiestrogens and their use in breast cancer. B.F. Cain Memorial Lecture. *Cancer Res* 1990, **50**, 4177–4189.
134. Jordan VC, Morrow M. Tamoxifen, raloxifene, and the prevention of breast cancer. *Endocrine Rev* 1999, **20**, 253–278.
135. Sun J, Meyers JM, Bink BE, Rajendran R, Katzenellenbogen JA, Katzenellenbogen BS. Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-α or estrogen receptor-β. *Endocrinol* 1999, **140**, 800–804.

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