

Selective estrogen receptor modulators: tissue specificity and clinical utility

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Abstract: Selective estrogen receptor modulators (SERMs) are a diverse group of nonsteroidal compounds that function as agonists or antagonists for estrogen receptors (ERs) in a target gene-specific and tissue-specific fashion. SERM specificity involves tissue-specific expression of ER subtypes, differential expression of co-regulatory proteins in various tissues, and varying ER conformational changes induced by ligand binding. To date, the major clinical applications of SERMs are their use in the prevention and treatment of breast cancer, the prevention of osteoporosis, and the maintenance of beneficial serum lipid profiles in postmenopausal women. However, SERMs have also been found to promote adverse effects, including thromboembolic events and, in some cases, carcinogenesis, that have proven to be obstacles in their clinical utility. In this review, we discuss the mechanisms of SERM tissue specificity and highlight the therapeutic application of well-known and emergent SERMs.

Keywords: selective estrogen receptor modulators, SERMs, estrogen receptors

Introduction

Estrogens play critical roles in development, reproduction, cognition, and growth and maintenance of the skeleton. Research suggests that estrogens and their intracellular receptors (ERs), ER α and ER β , may also influence pathophysiologic states of multiple tissues by contributing to diseases including cancer (eg, breast, endometrial, prostate, and colorectal), cardiovascular and metabolic diseases, cognitive diseases such as Alzheimer's, and osteoporosis.^{1,2} Estrogens have been effective in their clinical use as oral contraception, fertility treatment, and hormone therapy in menopause. However, they are contraindicated in some patients due to their multiple effects on various target tissues and implication as a risk factor in the development of breast and uterine cancers.

Selective estrogen receptor modulators (SERMs) are a diverse group of nonsteroidal compounds that function as ligands for ERs. However, unlike estrogens that function as ER agonists differing primarily in potency, SERMs have the unique ability to selectively act as agonists or antagonists in a target gene and in a tissue-specific fashion.³⁻⁵ Thus, the pharmacological advantage of SERMs lies in this mixed agonism/antagonism profile that affords the beneficial estrogenic actions in target tissues and avoids adverse, off-target effects. This mixed agonism/antagonism profile is the result of differentially expressed ER, ligand-dependent receptor conformational changes, and various interactions with co-activators and co-repressors expressed or recruited in a tissue-specific fashion.⁶ Thus each SERM has a distinctive biological effect on its target tissue and since no class effect is associated with their function, each must be evaluated individually.⁷ The development of SERMs has extended the application of estrogen-related therapy and positively impacted how estrogen-related diseases are

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treated. To date, the major clinical applications of SERMs are their use in the prevention and treatment of breast cancer, the prevention of osteoporosis, and maintenance of beneficial serum lipid profiles in postmenopausal women. However, SERMs have also been found to promote adverse effects, mainly thromboembolic events and, in some cases, carcinogenesis. These adverse effects present a major obstacle in treatment with SERMs, especially in cases where long-term treatment (ie, osteoporosis) would be most desirable. This has led to the production of new-generation SERMs that are structurally distinct from their predecessors. The ideal SERM would have strong anti-estrogenic effects on breast cancer and endometrial cancers while stimulating the formation of bone in an estrogenic manner. The ability to design a drug that encompasses all of these qualities has proven difficult, as each drug produced seems to lack one of these important characteristics. Therefore, a clearer understanding of SERM's tissue dependent mechanism and signaling are needed to provide insight into developing SERMs with fewer negative side effects. This review will present the mechanistic

approaches utilized by SERMs to achieve tissue specificity and highlight the therapeutic application of well-known and emergent SERMs.

SERMs: mechanism of tissue specificity

SERM tissue specificity is the culmination of several factors, mainly tissue-specific expression of ER subtypes (ie, ER α and ER β), differential expression of co-regulatory proteins in various tissues (ie, co-activators and co-repressors), and varying ER conformational changes induced by ligand binding⁶ (Figure 1). These factors result in the ability of the therapeutic application of SERMs that can have discrete effects depending on the target tissue. However, these factors also complicate the understanding of individual SERM mechanisms of action and add to the challenge of designing new SERMs.

For decades it was generally accepted that there was only one ER gene coding for ER α which bound estrogen with high affinity. However, with the discovery of ER β ,⁸ the

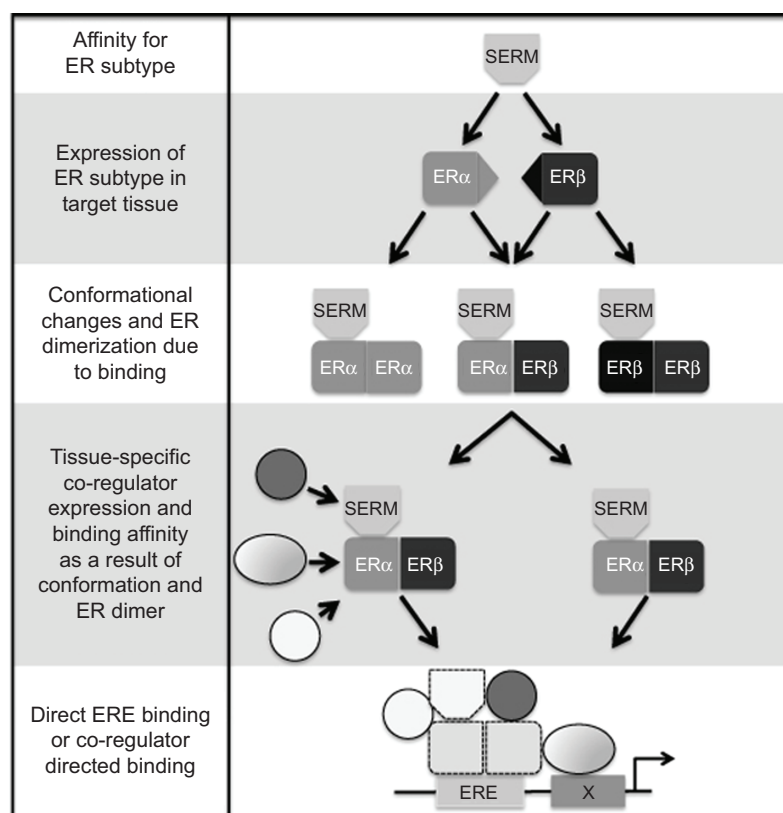


Figure 1 Model for SERM tissue specificity.

Notes: SERM tissue specificity depends on numerous factors: 1) SERMs have differential and specific affinity for ER subtypes; 2) ER subtypes are differentially expressed in target tissues and can be heterogeneously expressed in a particular tissue; 3) SERM binding induces specific conformational changes in ER that influence dimerization and binding to various co-factors that can determine resultant target gene (X) activation or repression; 4) Co-factors (ie, activators and repressors) are differentially expressed in target tissues; and 5) ER-SERM complexes can bind directly to an ERE or be directed to bind other transcriptional motifs as a result of binding to various co-factors.

Abbreviations: ER, estrogen receptor; ERE, estrogen response element; SERM, selective estrogen receptor modulators.

possibility that any selective effect of estrogen could be due to the differential expression of these two ER genes caused a paradigm shift in our understanding of the estrogen signaling system, and made understanding the differential expression of these genes in estrogen-sensitive tissues a major focus of research.^{8–12} The relative tissue expression of ER α and ER β are important determinants of a tissue's response to estrogen. ER α and ER β exert differential effects on growth and differentiation in tissues, including bone, colon, uterus, liver, brain, and mammary gland.^{13–19} For example, ER α is believed to have a proliferative role, whereas ER β has an anti-proliferative role in transfected breast cancer cells.²⁰ Certain tissues, such as hepatocytes in the liver and neural cells in the hippocampus, express high levels of ER α , while in tissues like prostate, ovary, and lung, ER β is expressed at high levels and ER α at low levels.^{21,22} Yet, in mammary gland, bone, uterus, the central nervous system, and the cardiovascular system, both ER α and ER β show equally significant levels of expression and, additionally, can influence each other's functions.^{23–25} In certain tissues, such as testes and mammary glands, both the ER α and ER β are expressed, but their cellular distribution is distinct. For example, in mammary glands, ER β is mostly present in epithelial cell nuclei. In testis, ER α is reported to be localized in the nuclei of the Leydig cells, while ER β is found in germ cells, Sertoli cells, and fetal Leydig cells.²⁶ Thus the mechanism of action of estrogens and SERMs due to the contributions of either ER α and/or ER β receptor has been difficult to determine.

The ligand-dependent molecular mechanism of SERMs is similar to that of 17 β -estradiol and involves binding to its ligand-dependent nuclear ER. ERs have a large and flexible binding pocket that allows for multiple ligands to bind, including steroids, phytoestrogens, and xenobiotics.²⁷ Ligand-bound ERs undergo a conformational change in their hormone binding domain that promotes dimerization and activation of the receptor which can then exert its effects either by binding directly to the estrogen response element (EREs), usually located in the promoter region of target genes, or by binding to co-regulator proteins at their respective promoter sites.^{28–31} The association of the ER with DNA can either positively or negatively regulate target gene transcription. ER α and ER β , including its various isoforms (β 1, β 2, β 4, and β 5), are homologous members of the nuclear receptor superfamily that possess a modular structure and are composed of three major functional domains – the N-terminal domain (NTD), the DNA binding domain (DBD), and the ligand binding domain (LBD) – that serve specific roles.³² In terms of sequence homology, ER β shows a high homology

to ER α in the DBD and in the LBD;^{11,12} however, the NTD of ER β is shorter than that of ER α with very poor sequence homology to that of ER α . It is believed that full transcriptional activity of the ERs occurs through synergism between the two activation function (AF) domains, AF1 and AF2, located in the NTD and LBD respectively,³³ and activity of the AFs occurs in a tissue and promoter specific fashion.^{34,35} AF1 functions in a hormone-independent manner, whereas AF2 function requires the presence of a ligand.³⁶ In addition to the NTD, DBD, and LBD, the ERs consist of “hinge and F” regions. The “hinge” region contains a nuclear localization signal and serves as a flexible region connecting the DBD and LBD. The “F” region, which contains 42 amino acids, is located towards the C-terminal end of the LBD and possesses specific modulation capabilities of gene transcription in a ligand-, promoter-, and tissue-specific manner.^{37–39}

The differences in ligand-induced transcriptional activation of target genes by ER α and ER β may be a result of independent activation of the AF1 and AF2 (LBD) domains.³¹ AF1 and AF2, working together or independently, are able to recruit transcriptional co-regulator proteins following receptor stimulation.³¹ Co-regulator proteins are able to physically interact with the liganded or unliganded ER and modulate transcription of a gene. The recruitment of co-activator proteins such as SRC1 and SRC3, or co-repressor proteins such as nuclear receptor co-repressor (NCoR) or thyroid hormone receptor (SMRT), can either activate or repress transcriptional activity, respectively.^{31,40–43} Furthermore, it appears that regulatory proteins are selectively activated depending on both ER subtype and ligand binding activity. Using phage display, it was determined that ligand binding appears to place the ER in a unique position that alters the biological activity of co-regulatory proteins.⁴⁴ For example, co-crystallization studies with various ligands determined that ERs that are bound to antagonists undergo a three-dimensional repositioning of helix 12 that can interfere with the receptor-co-regulatory protein interaction.^{45,46} There have currently been over 300 regulator proteins identified, and their interactions within ER transcriptional regulation are complex.^{47,48} Furthermore, the recruitment of co-regulator proteins is dependent on the specific ER ligand eliciting a unique receptor confirmation.⁴⁹ This recruitment is thought to occur through an ordered, cyclical, and combinatorial process, and therefore, transcriptional activation by ERs can occur through cooperation between both AFs or through each AF independently.⁵⁰ The activity of co-regulator proteins is also influenced by post-translational modification, such as phosphorylation, sumoylation, ubiquitination, and acetylation, which affect

their ability to influence ER signaling.^{51–53} Therefore, the differential actions seen between ER α and ER β may partially be a result of the structural difference between AF1 and AF2, and tissue dependent activities may be dependent on the expression of co-regulator proteins.⁴⁰

The high degree of sequence homology within the DBD of ER α and ER β results in the ability for both receptors to bind EREs with high affinity^{40,54,55} as either an ER α /ER α homodimer, ER β /ER β homodimer, or an ER α /ER β heterodimer.^{55–57} Each composition of dimer that is formed is believed to mediate distinct hormonal responses, and different tissues express varying degrees of ER subtypes. Therefore, the ability for SERMs to display a selective affinity for ER subtypes based on affinity for a particular AF2 (LBD) may partially explain the tissue specificity of SERMs. Furthermore, it also appears that a single ligand bound receptor is capable of forming a homo- or heterodimer.⁵⁸ This indicates that a SERM having an affinity for one of the ER subtypes may still be able to form an ER α /ER β heterodimer, and as a result, alter response to ligand binding; although, the exact impact of heterodimerization is still unclear.⁵⁸ In some situations, response to estrogen stimulation depended on the

co-presence of both ER α and ER β , suggesting that receptor heterodimerization may alter receptor signaling, possibly by allowing access to new chromatin regions.⁵⁶ Moreover, the discovery of multiple ER β various isoforms (β 1, β 2, β 4, and β 5) with differing tissue distribution and function further adds to the alternative results of ER stimulation.⁵⁸

Triphenylethylene SERMs Tamoxifen

Tamoxifen is a nonsteroidal triphenylethylene compound that was the first SERM to be used successfully to prevent and treat breast cancer. It quickly became the treatment of choice for ER α positive breast cancer and to reduce the risk of breast cancer in high-risk patients.^{59,60} Tamoxifen was first developed as an ER antagonist for breast cancer treatment. However the subsequent discovery of its agonist role in bone and the uterus spawned efforts to create SERMs with precise functions depending on tissue^{61,62} (Figure 2). Tamoxifen is routinely used to treat all stages of breast cancer. Adjuvant breast cancer trials have shown that tamoxifen's use reduced both breast cancer reoccurrence and contralateral breast cancer by 40%–50%.⁶³ Tamoxifen efficacy was further investigated

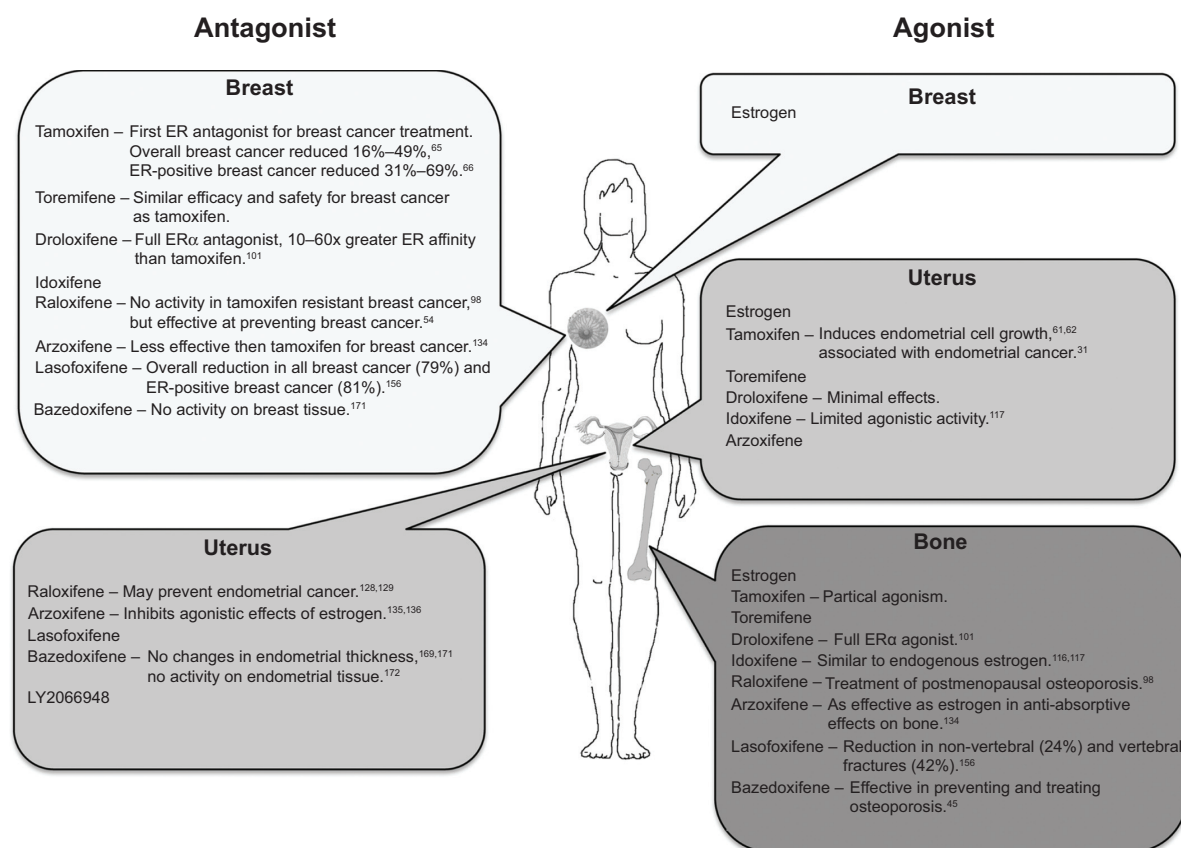


Figure 2 SERM tissue activity and clinical action in breast, uterus, and bone.

Abbreviations: ER, estrogen receptor; SERM, selective estrogen receptor modulators.

in four major Phase III trials where it reduced overall breast cancer incidence between 16% and 49% and ER-positive breast cancer incidence between 31% and 69%.^{64,65} Tamoxifen has also been determined to have a luteotropic effect if used by women in mid luteal phase and therefore is effective in reducing premenstrual mastalgia.^{66,67}

The ability of tamoxifen to inhibit ER α positive breast cancer cell proliferation may be due to interaction with co-repressors. Tamoxifen, similar to endogenous estrogens, requires metabolic activation by cytochrome P450 enzymes to form its active metabolites, 4-hydroxytamoxifen (4-OHT) and endoxifen.⁶⁸ NCoR and SMRT are co-repressor proteins that 4-OHT, a potent anti-estrogen, is known to recruit.⁴⁹ Furthermore, *in vivo* inhibition of NCoR or SMRT and *in vitro* studies using fibroblasts from NCoR knockout mice, demonstrated an increase in antagonistic activity of 4-OHT.^{69,70} Additionally, the ability of 4-OHT to inhibit ER α breast cancer cell proliferation is impaired in NCoR and SMRT deficient cells.⁷¹ It is important to note that cells deficient in NCoR and SMRT do not show an activation of all estrogen dependent response genes, indicating that there are factors influencing SERM-induced repression that are important in this complex signaling. The mechanism of co-repression due to NCoR and SMRT is still being fully determined, but it appears that histone de-acetylase activity may be involved in this transcriptional repression.^{72,73} Repressor of estrogen action is another co-repressor potentiated by 4-OHT. When stimulated, resveratrol competes with the co-activator for binding to the estrogen bound ER and therefore potentiates 4-OHT as an estrogen antagonist.^{74,75}

In uterine cells, tamoxifen acts as an estrogen agonist and induces growth of endometrial cells.^{61,62} In addition, tamoxifen can display beneficial partial agonistic effects on bones and the cardiovascular system in postmenopausal women.⁷⁶ The difference in tamoxifen activity in each tissue is thought to be due to the expression of the co-regulator proteins such as SRC1, NCoR, or SMRT. SRC1, a co-activator promoted by tamoxifen, is highly expressed in uterine cells, but has low expression in breast cancer cells.⁷² This suggests that the stimulatory effect tamoxifen has on uterine cells may partially be explained by the distribution of co-activator proteins such as SRC1. This mechanism may also be contingent on the association of intrinsic histone acetyl transferase activity associated with SRC1 which helps activate transcriptional activity.⁷⁷

Despite clinical success with tamoxifen, long-term tamoxifen use has been associated with an increased risk of endometrial cancer, as well as other side effects such as hot flashes, ocular changes, an increased risk of

stroke, and pulmonary embolism.^{29,78–82} In endometrial tissue, tamoxifen has partial estrogen-agonist properties and unopposed exogenous estrogens are known to be carcinogenic in the endometrium.^{83,84} Some of the adverse effects associated with tamoxifen have been attributed to its genotoxic metabolites. The increased incidence of endometrial cancer observed during tamoxifen administration are believed to be due to the formation of major DNA adducts. Tamoxifen may also mediate hepatocarcinogenesis by the formation of these DNA adducts.^{85–87} Tamoxifen resistance is also possible and can be achieved through either intrinsic resistance, where ER-positive breast cancer either never responds to treatment, or ER-positive breast cancer initially responds to treatment but subsequently acquires resistance.²⁹

Toremifene (chlorotamoxifen)

Toremifene (TOR) is a nonsteroidal triphenylethylene SERM that is similar to tamoxifen in both structure and function. TOR differs from tamoxifen's structure in only a single chloride atom on its side group; a chloride atom substituted for a hydrogen atom in the ethyl group attached to part of the ethylene bond.⁸⁸ TOR functions in a similar way to other Type I SERMs, displaying a higher affinity for ER α (about 5% of that of estradiol) than ER β .⁸⁹ It is an effective SERM for the treatment of breast cancer in postmenopausal women and may be therapeutically applicable in preventing fracture risk during hormone therapy. TOR may be a suitable alternative treatment for tamoxifen in hormone receptor-positive breast cancer in premenopausal women. A retrospective study comparing the efficacy and safety of TOR and tamoxifen in the treatment of operable hormone receptor-positive breast cancer in premenopausal women (n=1,847) found that the TOR group (n=396) and the tamoxifen group (n=1,451) were similar in efficacy and safety.⁹⁰ Furthermore, TOR lacks the DNA adduct-forming ability of tamoxifen and the genotoxicity of tamoxifen, suggesting that TOR has a lesser uterotrophic effect than tamoxifen, and it appears that TOR is not associated with an increased risk of endometrial cancer.^{91–95} Overall, TOR has been found to have similar efficacy to tamoxifen in treating advanced ER α positive breast cancer, with a similar side effect profile. TOR has also been determined to have a luteotropic effect if used by women mid-luteal phase, and therefore is effective in reducing cyclic breast pain compared to placebo.^{67,96} Not surprisingly, due to the similarities between TOR and tamoxifen in structure and function, the two drugs are cross-reactive. In a double-blind, cross over trial, 66 postmenopausal women were

started on either TOR (140 mg/day) or tamoxifen (40 mg/day). Forty-four of the women were then crossed over to the alternative treatment, and no response was achieved from either of the second line treatments.⁹⁷ Additionally, in an international Phase III study evaluating TOR use in preventing vertebral fractures in men with non-metastatic prostate cancer being treated with androgen deprivation therapy, it was determined that TOR was associated with a reduced risk of vertebral fractures compared to placebo. However, in this investigation, TOR was also associated with an increased rate of venous thromboembolic events and therefore has not been approved for fracture prevention in men receiving androgen deprivation therapy.⁹⁸ There have been reports that the differences in the actions of tamoxifen compared to TOR may be due to a lower estrogenic to antiestrogenic effect.⁹⁹

Droloxifene

The goal in the production of droloxifene (3-[1-[4-(2-Dimethylaminoethoxy)phenyl]-2-phenyl-but-1-enyl]phenol) was to make a version of tamoxifen that was more potent but had fewer side effects, such as liver tumors or the formation of DNA adducts.¹⁰⁰ The differences between droloxifene and tamoxifen are the removal of methyl on the amino group and the addition of an alcohol at the third position. As a result of these changes, the drug has a 10–60× greater affinity for the ER and a shorter serum half-life than its parent molecule tamoxifen. With the greater level of affinity for the ER α receptor, it was thought that dosing periods could be shortened with droloxifene.¹⁰⁰ Droloxifene is a full ER α antagonist in the breast and a full ER α agonist in bone. Similar to tamoxifen, droloxifene blocks cells in the G1 phase of the cell cycle thereby inhibiting their growth.¹⁰¹ Also similar to tamoxifen, it was shown to cause an increase in TGF- β which in bone causes an increase in pre-osteoclast apoptosis and MCF-7 breast cancer cell apoptosis.¹⁰² Based on this finding, it can be hypothesized that droloxifene also causes an inhibition of insulin-like growth factor (IGF)-1 stimulated cell growth and prevents expression of estrogen stimulated oncogene c-myc, as these represent the mechanism of tamoxifen. In addition to being active in bone and breast, droloxifene was shown to have increased anti-estrogenic activity and lowered estrogenic activity in the immature rat uterus compared to tamoxifen.¹⁰⁰ Through its preclinical trials, droloxifene showed promise in treating both breast and bone cancers while having minimal activity on uterine and endometrial tissues. In a Phase I trial conducted by Buzdar et al 30 patients were enrolled and received doses of 20, 40, 100, 200, or 300 mg. No patients noted objective responses,

only four showed moderate responses and droloxifene was very well tolerated by the patients, even at the higher doses.¹⁰³ Given that all the patients in the trial had previously failed tamoxifen therapy and had very few negative side effects from treatment, droloxifene was pursued in Phase II trials. In a randomized Phase II trial consisting of 369 postmenopausal women who were either ER positive or had unknown ER status, doses of 20, 40, or 100 mg of droloxifene showed no significant differences. The average objective response across all doses was 39.3%.¹⁰⁴ These positive results led to the Phase III trial for droloxifene. The major side effects noted for droloxifene were hot flashes, nausea, fatigue, headache, backpain, and dyspnea.^{103,105} The Phase III trial of droloxifene consisted of 1,354 patients across multiple countries. The trial once randomized had 673 patients in the tamoxifen group and 681 patients in the droloxifene group. The objective response rate was 18% for droloxifene and 23% for tamoxifen, indicating that droloxifene was not significantly better than tamoxifen in responses. Interestingly, both drugs were more efficacious in postmenopausal than premenopausal woman. In women younger than 65 years, the tumor response rates for droloxifene and tamoxifen were 15% and 23% respectively. In women older than 65 years, the response rate was 38% for both tamoxifen and droloxifene.¹⁰⁶ The possible short comings of droloxifene in this trial for premenopausal women could have been due to the short half-life of droloxifene (1 day) leading to greater fluctuation in serum concentration.^{107,108} It is thought that this could be avoided with higher doses, as shown by several studies that show serum levels of sex hormones and binding proteins are concentration-dependent between 40 and 100 mg.^{107,108} This suggests that at higher doses, the drug is able to reach higher concentrations and even with equivalent excretion rates, maintain therapeutic levels for an extended period of time. In spite of this theory, the Phase III trial of droloxifene was closed at its second interim analysis as it was deemed to be ineffective in comparison to tamoxifen.

Idoxifene

Idoxifene ((*E*)-1-(2-(4-(1-(4-Iodophenyl)-2-phenylbut-1-en-1-yl)phenoxy)ethyl)pyrrolidine) was developed as an anti-estrogen with lower estrogenic effects on endometrial growth with greater estrogenic activity than the current standard, tamoxifen.^{109,110} The developers of idoxifene tried to minimize the negative side effects of tamoxifen by substituting the dimethylamino group found on tamoxifen with a pyrrolidino group to avoid the production of a desmethyl metabolite and formaldehyde after metabolism in the liver.¹¹¹ The second

structural difference in idoxifene was the addition of an iodine at the 4th position to reduce the estrogenic activity in addition to inhibiting glucuronidation and 4-hydroxylation allowing for a longer duration of action.^{112,113} In initial trials and testing, the modifications were very successful, as idoxifene was found to have a 2.5× slower rate of metabolism compared to tamoxifen which resulted in a terminal half-life that was about double that of tamoxifen.¹¹⁴ The peak plasma level was achieved after 2–8 hours post administration, and the drug had linear pharmacokinetics. The slower metabolism and longer half-life gave idoxifene a steady state concentration that was 50% higher than that of tamoxifen. Elimination of the drug was in a biphasic manner with linear kinetics over the dose range tested (10–60 mg).¹¹¹

Functionally, idoxifene was determined to work through the estrogen response element.¹¹⁵ Compared to its parent drug, tamoxifen, idoxifene displayed 2–2.5× greater affinity for the ER α receptor^{110,112} and displayed desired effects in breast, bone, uterus, and endometrium.^{115–117} In breast tissue, idoxifene was found to have similar effects to that of tamoxifen indicating that it acted as an ER α antagonist.¹¹⁷ In MCF-7 cells, idoxifene was 1.5× more effective in inhibiting estrogen dependent cell growth compared to tamoxifen. On a gross level, idoxifene caused overall reduction in tumor size which indicated efficacy in breast cancer.¹¹⁷ In the endometrium and uterus, idoxifene was determined to have limited agonistic activity and blocked gene expression in endometrial cells, possibly indicating antagonistic behavior.¹¹⁶ This low level of activity in the uterus is lower than that of tamoxifen and was shown to decrease the risk of uterine cancer development posttreatment, which is typically elevated in tamoxifen, posttherapy.¹¹² In bone, idoxifene functioned as an ER agonist, acting in a similar way to endogenous estrogen in that it promoted the activity of osteoblasts while promoting apoptosis of osteoclasts.^{115,116} The effects of idoxifene on bone included a suppression of urinary pyridium cross link expression and a serum osteocalcin level increase that is typically seen in estrogen withdrawal-based bone turn over. In addition, alkaline phosphatase levels were increased, suggesting increased bone formation.¹¹⁶ The combinations of these promising results led to a Phase I trial for idoxifene. This trial, conducted by Coombes et al treated 14 patients with idoxifene who had previously received tamoxifen therapy and showed that four of the 14 had tumor stabilization for 14, 8, 8, and 1.5 months, while two of the 14 showed a partial response to the new drug.¹¹¹ Endocrine changes for idoxifene were identical to tamoxifen for the luteinizing hormone (LH) and follicle stimulating hormone (FSH), in that patients saw

a decrease in both. However, in contrast to tamoxifen, there was no change in sex hormone binding globulin (SHBG) when the patients were treated with idoxifene. This suggested a slightly different mechanism for idoxifene compared to its parent tamoxifen, as tamoxifen typically causes an increase in SHBG. This study proved promising which led to Phase II trials for idoxifene.

In Phase II trials for idoxifene, clinical data showed that of the 25 patients who received idoxifene, two had a partial response (PR) and two had steady disease for greater than 6 months. The PR duration was for 30 months and 5 months. In the patients who had steady disease, they had demonstrated resistance, as they had a PR or complete response to tamoxifen in prior treatments 2 and 7 years prior. In addition, in the Phase II trial, the endocrine changes were identical to that of tamoxifen, suggesting that the Phase I trial was underpowered. The clinical data for idoxifene proved to be not significantly different than tamoxifen, but the drug was further tested in Phase III trials.¹¹⁸

During Phase III trials for idoxifene, it was determined that patient outcomes for idoxifene were not significantly different than those for tamoxifen. Complete response, PR, and steady disease groups were not significantly different and neither was long-term survival. Due to these findings, Phase III enrollment was cut before the study was completed by the sponsor, SmithKline Beecham, due to limited potential for profit. Some of the side effects reported during the study included nausea, anorexia, vomiting, increased urinary incontinence, increased risk of pelvic organ prolapse, increased endometrial thickness, and increased risk of leucorrhea.^{119,120} It is important to note that deaths due to treatment were not significantly different in treatment with either idoxifene and tamoxifen, indicating that idoxifene is a safe but not significantly safer treatment option than tamoxifen.¹²⁰

Benzothiophene SERMs

Raloxifene

A second generation SERM (formally called keoxifene) is a chemically distinct polyhydroxy phenol benzothiophene series SERM that has different tissue-specific effects compared to tamoxifen. Raloxifene was first developed for breast cancer therapy, however it was determined that raloxifene did not have activity in tamoxifen resistant breast cancer patients. It soon became apparent that raloxifene may prevent bone loss and prevent breast cancer, which led to clinical trials and eventually raloxifene became the first SERM to be approved by the Food and Drug Administration for the treatment and prevention of postmenopausal osteoporosis.^{97,121–124} Although

the exact mechanism of raloxifene's effects has yet to be determined, its metabolism is not reported to proceed through the P450 pathway like tamoxifen.

Raloxifene is extensively metabolized through glucuronidation pathways and may proceed through oxidation by the liver to electrophilic diquinone methide and o-quinones.¹²⁵ Although both of these metabolites may potentially be destructive, either their short half-life or stable minor production decreases their clinical toxicity.

Raloxifene is mostly known for its therapeutic use in osteoporosis, and while it failed as a breast cancer treatment, it is still effective at preventing breast cancer.⁴⁹ Raloxifene's effectiveness as a long-term therapeutic for reducing the occurrence of invasive breast cancer was examined in the MORE trial (multiple outcomes of raloxifene evaluation) where it was determined that it reduced the incidence of osteoporosis and breast cancer in postmenopausal women.^{126,127} Furthermore, unlike tamoxifen, raloxifene is not associated with, and may even be effective at, preventing endometrial cancer.^{127,128} The realization that raloxifene has fewer estrogen-like effects than tamoxifen in laboratory rats resulted in a clinical trial to compare tamoxifen and raloxifene's efficacy as a breast cancer preventive and their effect in the uterus. In the Study of Tamoxifen and Raloxifene (STAR) trial, a Phase II, randomized double-blind evaluation of the efficacy of raloxifene (60 mg oral) compared to tamoxifen (20 mg oral), it was determined that tamoxifen and raloxifene are equally effective at preventing breast cancer progression over a 5-year period. Furthermore, in 81 months, raloxifene is 75% as effective as tamoxifen, and there were less thromboembolic events and fewer cataracts in the raloxifene group.⁶² In trials assessing raloxifene's effect on the endometrium, it has been determined that, compared to placebo, there was no difference, and compared to tamoxifen, raloxifene reduced the incidence of endometrial cancer.^{62,129} The observation that raloxifene does not increase the incidence of endometrial cancer, may be a result of its inability to stimulate the co-activator protein SRC1 in both uterine cells and breast cancer cell lines.⁷² Raloxifene also has no effect on vaginal lubrication,¹³⁰ and similar to tamoxifen, raloxifene is associated with an increase in hot flashes, an increased risk of blood clots, and resistance.²⁹

Arzoxifene

Arzoxifene is a third generation SERM that is a raloxifene analogue with the replacement of the carbonyl functional group with an ether linkage and the addition of a methyl group to the 4' phenolic hydroxyl group.^{131,132} After raloxifene

failed to show effects as a therapeutic in metastatic breast cancer, arzoxifene was developed to show that benzothio-phenes were still a viable drug option in the treatment of breast cancer. During initial trials, it showed promise as being the "ideal SERM." However, during late Phase II testing and Phase III trials, it was proven to be less effective than tamoxifen, destroying its efforts to be used as a breast cancer therapeutic.¹³³ Arzoxifene uses a raloxifene base and then replaces the carbonyl group found on raloxifene with oxygen which results in a substantial increase in estrogen antagonistic potency compared to raloxifene. By doing this, bioavailability was also increased.^{134,135} Supporting this was the 10-fold improvement in IC₅₀ values from raloxifene to arzoxifene.¹³⁴ Arzoxifene is metabolized by the P450 system to produce its active metabolite, desmethylarzoxifene, which has 8× greater affinity for ERα than arzoxifene and approximately 24× greater affinity for ERα than the active metabolite of tamoxifen, 4-OHT ligand.^{135–137} Additionally, like raloxifene, desmethylarzoxifene can be further oxidized to diquinone methide.^{138–140}

The primary goal for the development of arzoxifene was for it to be used for metastatic breast cancer. In initial studies, it was shown that arzoxifene caused inhibition of growth in breast epithelial cells while also inhibiting the growth of basal cells in the absence of estrogen. This differed from tamoxifen, which inhibited the growth of epithelial cells and caused the stimulation of basal cell proliferation.¹³⁵ In addition to its effect in breast cancers, arzoxifene also inhibited the agonistic effects of estrogen in the uterus and on endometrial cell growth.^{134,135} The combinations of these effects led to the exploration of arzoxifene in Phase I trials. In a Phase I trial of 32 women with metastatic breast cancer (all of which had received prior hormone therapy or chemotherapy), arzoxifene was given. None of the women saw an objective response and 19% had steady disease for greater than 6 months (6–34 months; median 7.7 months).¹⁴¹ It also proved itself to be as effective as estrogen in anti-absorptive effects on bone in postmenopausal women. Like tamoxifen, arzoxifene was found to decrease FSH and LH levels and increase SHBG.¹³³ In addition, during other Phase I trials, it was found that arzoxifene caused decreases in proliferating cell nuclear antigen, IGF-1, and IGF binding protein 3.¹⁴² Though no objective response was seen, the hormonal data and results on endometrium prompted Phase II trials. In these trials, results showed that lower doses (20 mg versus 50 mg) of arzoxifene showed better objective response rates, with hormone panels for FSH, LH, and SHBG confirming Phase I results.¹⁴³ With regards to bone mineral density (BMD), the

study for Phase II was underpowered so there were not enough premenopausal women enrolled to make reliable inferences.¹⁴⁴ Initial reports of the drug showed possible efficacy in endometrial cancer treatment. During Phase II trials, the drug was given to 34 patients and overall response rate was reported to be 31% (one complete response and eight PR). Results were comparable or better than previous treatments with progesterone with or without tamoxifen. In spite of supportive results, the drug was not pursued further by the manufacturer.¹⁴¹

Though the drug was pulled after Phase II trials, some Phase III trials had begun and showed the drug had efficacy in the treatment of breast cancer. However, when compared head to head with tamoxifen, the results were disappointing. Tamoxifen proved superior in progression-free survival, time to treatment failure, and on study progression-free survival. However, it is important to note that there were no significant differences in overall response rate, clinical benefit rate, median response duration, or overall survival.¹⁴¹ The major side effects reported throughout the trials included hot flashes (non-dose dependent), nausea, cutaneous side effects, neuromotor toxicity, and weight gain.^{133,141} However, as stated earlier, the drug was not pursued further by the manufacturer in the treatment of breast cancer. There still remains potential for this drug in the treatment of uterine cancers and postmenopausal bone density loss.

Indole, tetrahydronaphthalene, and naphthol SERMs

Lasofloxifene

Lasofloxifene, similar to other SERMs, selectively binds human ER α and ER β with an affinity similar to estradiol.^{145–147} This SERM has been shown to function as a skeletal agonist and a breast and uterine antagonist.^{44,148–152} Lasofloxifene's structure is similar to that of endogenous estrogens, with a polyaromatic phenol scaffold that may be oxidized to catechols¹⁵³ which may cause toxicity. Lasofloxifene is a third generation nonsteroidal SERM with a similar structure to idoxifene. The osteoporosis prevention and lipid lowering and Postmenopausal Evaluation and Risk Reduction with Lasofloxifene (PEARL) studies were aimed at evaluating the efficacy of lasofloxifene use in the treatment of osteoporosis. The results of the osteoporosis prevention and lipid lowering, which assessed vaginal and bone effect of lasofloxifene in non-osteoporotic women, indicated that changes in BMD were significantly reduced in the lasofloxifene group compared to placebo. It was also determined that there was an improvement in vaginal pH after 2 years of therapy.¹⁵⁴ The

PEARL trials showed that in postmenopausal women with low bone density, lasofloxifene therapy was associated with reductions in all breast cancer (79%) and ER-positive breast cancer (81%), as well as the reduction in non-vertebral (24%) and vertebral fractures (42%), coronary heart disease (32%), and stroke (36%) compared to placebo.¹⁵⁵ Furthermore, the Comparison of Raloxifene and Lasofloxifene (CORAL) trial compared the effects of lasofloxifene, raloxifene, and placebo on BMD in postmenopausal women and found that after 2 years of treatment, lasofloxifene was associated with an improved lumbar spine BMD and reduced low-density lipoprotein cholesterol levels compared to placebo.¹⁵¹ In rat models, lasofloxifene, like most SERMs (including tamoxifen), also acted as an estrogen agonist in serum cholesterol¹⁵⁶ and had the effect of reducing total serum cholesterol and low-density lipoproteins. This suggests that there may be potential cardiovascular advantages associated with the treatment of osteoporosis with SERMs. Compared to placebo, lasofloxifene was associated with leg cramps, hot flashes, endometrial hypertrophy, uterine polyps, and vaginal candidiasis.¹⁵⁵ Furthermore, in rat models, lasofloxifene has no effect in the prostate.¹⁴⁵ The absence of effect in the prostate indicates that lasofloxifene may also be a useful therapeutic for men with some degree of hypogonadism.

Bazedoxifene

Bazedoxifene is a third generation SERM created as an indole-based ER ligand that was specifically designed to prevent and treat postmenopausal osteoporosis with reduced negative effects compared to previous SERMs, and can be given with conjugated equine estrogens for menopausal symptoms.¹⁵⁷ Bazedoxifene design differed from its predecessors in its core binding domain, which consists of a 2-phenyl-3-methyl indole, a side chain effector region connected to the core binding region via a methylene hinge, and a hexamethylenediamine ring at the side chain terminus.⁴⁴ Part of bazedoxifene's design was to prevent effects on the uterus such as those seen with levormeloxifene, idoxifene, and droloxifene. Bazedoxifene's binding affinity is slightly higher for ER α compared to ER β .^{158,159} The metabolism of bazedoxifene involves P450 glucuronidation at the indole 5 and 4' hydroxyl positions.¹⁶⁰

In general, bazedoxifene's estrogenic effects are agonist in bone and lipid metabolism and antagonistic in breast and endometrium.^{44,161,162} Bazedoxifene acetate is effective in preventing and treating osteoporosis, and improving lipid profile.⁴⁴ A 2-year, double blind study designed to assess the effects of bazedoxifene on BMD determined that BMD was

improved at all skeletal sites compared to placebo, which was similar to a 60 mg raloxifene (positive control) treatment. Furthermore, the incidence of new vertebral fractures was significantly lower in the bazedoxifene group compared to placebo. There was no difference in the incidence of vertebral fractures or breast or endometrial carcinoma between treatment groups.^{163–165} The SMART-1 trial investigated the use of bazedoxifene and conjugated estrogens compared to placebo on the effects of endometrial lining and BMD. The results indicated that the bazedoxifene plus conjugated estrogens group had reduced incidence of endometrial hyperplasia over placebo at 2 years and a significantly increased BMD in the lumbar spine and hip.^{166,167} In a separate 5-year international, double blind, randomized, trial (n=7,492) it was determined that bazedoxifene reduced the risk of new vertebral fractures at both a dose of 10 mg and 40 mg compared to raloxifene (60 mg) and placebo. Additionally, the risk of non-vertebral fracture risk was reduced by 44% with bazedoxifene (20 mg) compared to raloxifene. In the same sample, it was determined that bazedoxifene (20 mg) decreased the risk of new vertebral fracture by 50% in those who were at a higher fracture risk or had a previous vertebral fracture (n=1,771).¹⁶³ The most common adverse effects observed with bazedoxifene use are hot flashes and leg cramps, and the rates of endometrial hyperplasia, cancer, and polyps were low in these trials. The most serious negative effect was an increased risk of venous thromboembolism.^{163,168,169} Furthermore, transvaginal ultrasonography and mammography were administered to a subset (n=753) of patients taking bazedoxifene, raloxifene, and placebo in order to assess endometrial safety and breast density. The results showed that there was no between group differences in mean endometrial thickness or change from baseline in endometrial thickness associated with the use of either bazedoxifene or raloxifene.^{168,170} This finding was supported by a randomized, double blind, placebo and active controlled study that found that bazedoxifene had no activity on endometrial or breast tissue.¹⁷¹

LY20266948

The goal in the development of LY2066948 was to develop the ideal SERM that did not have the toxic effects of tamoxifen or its other derivatives. The ligand binds both ER α and ER β with high affinity Ki 0.51 nm and 1.36 nm, respectively.¹⁷² LY2066948 shows potent uterine antagonistic effects.¹⁷³ This was demonstrated through both in vitro and in vivo studies. In in vivo studies following treatment with estrogen in immature female rats (3 weeks), a significant (3–4 \times) increase in uterine weight was seen. Treatment with

LY2066948 showed significant inhibitory effects on the estrogen stimulated uterine growth at a dose of 10 mg/kg.¹⁷³ The ED50 value for LY2066948 was determined to be 0.07+–0.02 mg/kg.¹⁷² With estrogen stimulus, potency compares well with tamoxifen and raloxifene.¹³⁴ Additionally, overall results show no significant ovarian stimulation.¹⁷² In vitro studies of LY2066948 in uterine tissues showed 87.5% inhibition of E2 stimulated response at 1 nM and IC50 of 10.7 nM. The IC50 of tamoxifen was 421 nm with only 53.4% inhibition.¹⁷² LY2066948 is similar in structure to raloxifene, and undergoes metabolism through the P-450 pathway in the liver. This metabolism leads to the eventual formation of the metabolite 3,4-o-quinone^{174,175} and, based on its structure, this metabolite is not as toxic as those produced by other SERMs.¹⁷⁶

Tamoxifen versus raloxifene

Raloxifene is used to reduce the risk of breast cancer in postmenopausal women with osteoporosis, and both tamoxifen and raloxifene are used to decrease the risk of breast cancer in high-risk women. In general, raloxifene has less estrogen-like effects compared to tamoxifen. The most notable distinction between these two drugs is that raloxifene does not share the pro-estrogenic effects of tamoxifen on the endometrium, which corresponds to an increased risk of endometrial cancer.¹²⁸ A potential mechanism for the difference in actions of these drugs is based on differential actions within the ligand binding domain (AF-2 domain) of ER α . ER α has an amino acid Asp-351 present within the ligand binding domain whose relationship to the anti-estrogenic side chain and the AF-2 site profoundly affects the nature of the estrogen-like outcome. In the case of raloxifene, removing the neutralizing change of the piperidine by substituting a cyclohexane resulted in increased estrogen-like actions, and moving the side chain of 4-OHT further away from Asp-351 also resulted in enhanced estrogen-like activity.^{177,178} Similarly, the effect of raloxifene is altered by changing the distance between the piperidine nitrogen and the negatively charged amino acid Asp-351. It is believed that the sidechain of raloxifene shields and neutralizes the Asp-351 to produce an anti-estrogenic ER α complex within the uterus.¹⁷⁸

Tamoxifen toxicity

Tamoxifen and TOR are both nonsteroidal triphenylethylene derivatives, differing only in the substitution of a chloride atom for hydrogen in an ethyl chain of TOR.⁸⁵ One major difference between the two drugs is that tamoxifen is associated

with an increased risk of endometrial cancer, and TOR has less genotoxic potential and may not have an association with endometrial cancer. Endogenous estrogen alone is known to be an endometrial carcinogenic,⁸⁴ and tamoxifen is a partial agonist of estrogen in endometrial tissue. However, estrogen-like activity is unlikely to be the only mechanism resulting in tamoxifen's effects on the endometrium since the tumors caused by endogenous estrogens are usually low stage and grade,¹⁷⁹ and tamoxifen associated tumors are more aggressive.^{180,181}

The increased incidence of endometrial cancer observed during tamoxifen administration are believed to be due to the formation of major DNA adducts primarily formed via sulfonation of α -hydroxylated tamoxifen metabolites such as α -OHTAM and hydroxyl-N-desmethyldtamoxifen.^{81,91,182–184} As previously noted, tamoxifen's activity is dependent on its metabolism by P-450 enzymes within the liver. Endogenous estrogens, which are also known as carcinogens, are also metabolized to form 2, and 4-hydroxy catechols.¹⁸⁵ It is believed that estrogens 4-hydroxy catechol metabolites display the most genotoxicity due to o-quinones', a 4-hydroxy catechol metabolite, ability to react with DNA to form genotoxic DNA adducts.¹⁸⁵ Metabolism of tamoxifen produces reactive carbocation, o-quinone, and quinone methide intermediates.^{186–189} It appears that these metabolites have the potential to form genotoxic DNA adducts.

These DNA adducts generate primarily guanine-to-thymine transversions in mammalian cells that have a large mutagenic potential and have been detected in the endometrium of women treated with tamoxifen.^{190–193} If not repaired, these adducts may cause mutations that can lead to endometrial cancer.¹⁹⁴ Conversely, TOR lacks the DNA adduct-forming ability and the genotoxicity of tamoxifen and has not been associated with the increased risk of endometrial cancer.^{91–95} Similarly, tamoxifen has been confirmed to function as a potent hepatocarcinogen, which may be a result of its DNA adduct-forming ability, and the lack of hepatocarcinogen ability of TOR may again result from its inability to form DNA adducts.⁸⁵ Supporting genotoxicity and hepatocarcinogenesis associated with tamoxifen, it was determined that tamoxifen increases point mutations and deletion mutation in the liver of gpt delta rats, and this effect was not found with TOR administration.¹⁹⁵ Both tamoxifen and 4-OHT can be metabolized by P450 to 3,4-dihydroxytamoxifen that can proceed to o-quinones via oxidation, and this metabolite was determined to have an extended half-life and the potential to cause genotoxicity, and may cause alkylation of amino acid residues on proteins.¹⁹⁶ Another potentially more

carcinogenic metabolite of tamoxifen are the carbocations, which are produced from sulfonation, and the subsequent loss of a sulfate group following hydroxylation of tamoxifen by P450 at the α -position.¹⁸⁸ This creates a highly reactive electrophilic carbocation that has a high affinity for binding the exocyclic amino group of quinine in DNA, leading to DNA adduct formation and potentially, mutagenesis.¹⁹⁷ Lastly, quinone methides, which differ from quinones due to a methylene group replacing the carbonyl oxygen, are formed following a two-electron oxidation of 4-OHT.^{174,187} It appears that quinone methides have the ability to form benzylic adducts of macromolecules and therefore may contribute to DNA adduct formation,^{198,199} although their extent of involvement has not been determined.

Metabolites of the benzothiophene SERMs

Raloxifene is extensively metabolized through glucuronidation pathways, and may proceed through oxidation by the liver to electrophilic diquinone methide and o-quinones.¹²⁵ Diquinone methide, which is also a metabolite of arzoxifene, is an electrophilic, active intermediate that may be a P450 3A4 inhibitor in human liver microsomes and may result in adduct formation with apoprotein.^{138–140} However, the half-life of diquinone methide is less than 1 second, suggesting that it does not significantly contribute to the toxicity profile of raloxifene. The other metabolite of raloxifene, a relatively stable o-quinone, is a known toxin; however, it is the minor product of raloxifene metabolism.¹²⁵

Conclusion

ER ligands have historically been classified by their actions, either as an agonist or an antagonist, but in reality, it appears that each falls somewhere within a continuum of agonist and antagonist and is dependent on multiple aspects of each tissue and drug. The ideal SERM would regulate menopausal symptoms, protect the skeleton, and prevent breast cancer without the negative effects associated with hormone therapy.

Disclosure

The authors report no conflicts of interest and have nothing to disclose.

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