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## Potential of Selective Estrogen Receptor Modulators as Treatments and Preventives of Breast Cancer

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### Abstract

Estrogen plays vital roles in human health and diseases. Estrogen mediates its actions almost entirely by binding to estrogen receptors (ER), alpha and beta which further function as transcription factors. Selective estrogen receptor modulators (SERMs) are synthetic molecules which bind to ER and can modulate its transcriptional capabilities in different ways in diverse estrogen target tissues. Tamoxifen, the prototypical SERM, is extensively used for targeted therapy of ER positive breast cancers and is also approved as the first chemo-preventive agent for lowering breast cancer incidence in high risk women. The therapeutic and preventive efficacy of tamoxifen was initially proven by series of experiments in the laboratory which laid the foundation of its clinical use. Unfortunately, use of tamoxifen is associated with de-novo and acquired resistance and some undesirable side effects. The molecular study of the resistance provides an opportunity to precisely understand the mechanism of SERM action which may further help in designing new and improved SERMs. Recent clinical studies reveal that another SERM, raloxifene, which is primarily used to treat post-menopausal osteoporosis, is as efficient as tamoxifen in preventing breast cancers with fewer side effects. Overall, these findings open a new horizon for SERMs as a class of drug which not only can be used for therapeutic and preventive purposes of breast cancers but also for various other diseases and disorders. Major efforts are therefore directed to make new SERMs with a better therapeutic profile and fewer side effects.

### Keywords

breast cancer; osteoporosis; estrogen receptor; tamoxifen; raloxifene; SERMs; endocrine therapy; drug resistance

## 1. INTRODUCTION

Breast cancer incidences and death rates have dropped significantly during recent years, which is associated strongly with improvement in early detection methods and decrease of menopausal hormone replacement therapy (HRT) [1, 2]. HRT, in the form of estrogen alone or estrogen plus progesterone, had been widely used since the 1960s until recent years, to treat conditions associated with aging as well as unpleasant menopausal symptoms. HRT was also known to protect post-menopausal women from osteoporosis and also thought to protect women from heart disease and Alzheimer's disease. However, the Women's Health Initiative (WHI) study indicated that taking estrogen with or without progesterone for 5 or more years placed the women at higher risk of breast cancer, Alzheimer's disease, heart

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disease, blood clot and stroke, although HRT is effective to reduce the risks of osteoporosis and colon cancer [3, 4]. The Million Women Study (MWS) conducted in the UK also showed that women taking HRT were more likely to develop breast cancer [5], endometrial cancer [6] and ovarian cancer [7]. In the US, the use of estrogen-plus-progestin HRT has dropped almost 50% when the WHI announced their findings in 2002, and this was followed by a sharp 7% decrease of new breast cancers in 2003 [2]. Although the decrease of HRT uses is not the sole reason leading to less breast cancer incidences, much effort has been focused on finding more effective and safer compounds to replace HRT which not only relieve menopausal symptoms but also prevent and treat hormone-responsive cancers. One most promising approach is to use selective estrogen receptor modulators (SERMs).

SERMs are synthetic compounds that bind to estrogen receptors alpha and/or beta (ER and/or ER $\beta$ ) and exert estrogenic or antiestrogenic activities in a tissue/cell-specific manner. The first SERM that has been used successfully in the clinic to prevent and treat breast cancer is tamoxifen, a failed postcoital contraceptive that evolved into the “gold standard” for breast cancer treatment [8, 9]. Tamoxifen is estimated to have saved the lives of over 400,000 women with breast cancer [8]. The second generation SERM, raloxifene (formally called keoxifene), failed as a treatment for breast cancer but is effective against osteoporosis and prevents breast cancer at the same time. Raloxifene is as effective as tamoxifen to reduce invasive breast cancer risks without an increase in the risk of endometrial cancer observed with tamoxifen [10]. Indeed a recent study suggests that raloxifene might even be effective in preventing endometrial cancer [11]. These findings have acted as a catalyst for the search of new SERMs which are estrogen-like in bones and circulating lipids but antiestrogenic in women’s reproductive organs and therefore are anti-cancer agents. However, there are problems associated with the current SERMs such as drug resistance and side effects. For example, both tamoxifen and raloxifene increase both hot flashes and blood clots [12].

Besides SERMs, other endocrine therapies target the ER indirectly to prevent and treat breast cancer. Aromatase inhibitors (AIs) that block the synthesis of estrogen from androgen in peripheral tissues have been extensively studied and show efficacy equivalent or superior to tamoxifen to treat postmenopausal breast cancer [13]. Since the mechanism for AIs to treat ER-positive breast cancer is to deplete estrogen in postmenopausal patients, they do not increase risks of endometrium cancer or blood clot and may be a better choice for postmenopausal breast cancer patients than tamoxifen. However, AIs are not effective in premenopausal women with actively functioning ovaries because AIs do not inhibit ovarian estrogen production. In addition, AIs lack the estrogenic protective function for cardiovascular diseases or osteoporosis. As a result, the side effects of AIs are mostly consistent with estrogen deprivation. AIs are associated with a greater incidence of bone loss and musculoskeletal symptoms, and probably higher risk of cardiovascular disease suggested by adjuvant trials comparing AIs and tamoxifen [14]. However, AIs are associated with a lower incidence of gynecological symptoms, thromboembolic diseases and hot flashes compared to tamoxifen in adjuvant setting [14]. Another strategy is to use selective estrogen receptor down-regulators (SERDs), such as fulvestrant, that cause degradation of ERs. Fulvestrant has been approved to treat advanced breast cancer after tamoxifen failure, and a recent phase III trial indicated that fulvestrant and AI exemestane were equally effective with a similar safety profile [15].

Resistance is a common problem associated with endocrine therapy therefore alternative treatment strategies without cross-resistance are necessary. Compared to the pure anti-estrogenic actions like AIs or fulvestrant, an ideal SERM with beneficial estrogenic effects has great potential for breast cancer prevention and treatment, especially in postmenopausal women as they often suffer from unpleasant symptoms resulting from lower estrogen. A

perfect SERM would reduce the risk of breast cancer, ovarian cancer and uterine cancer, as well as strengthen the bone, prevent coronary heart disease, strokes and Alzheimer's disease, and relieve menopausal discomfort like hot flashes and vaginal atrophy [12].

The complicated outcome of SERMs action cannot just be explained by turning on or off the ERs and their downstream genes. Although much new knowledge is being developed, we are still evolving in our understanding of the detailed mechanism of SERMs and their interaction with the ERs. In the past decade, another group of protein factors, nuclear receptor coregulators, have been identified that are essential for modulating the functions of SERMs and ERs. In this article, we will review the evolving understanding of the molecular mechanisms of SERMs action in the context of other signal transduction pathways and nuclear receptor coregulators, as well as the problems associated with the application of SERMs as a treatment or preventative for breast cancer. Finally, the new SERMs with potential as new agents to treat or prevent breast cancer will be described.

## 2. MECHANISM OF ESTROGEN ACTION

### 2.1. Structure and Function of ER

The existence of estrogen binding protein was first predicted by Elwood Jensen and colleagues in early 1960's [16]. The first ER cDNA, now known as ER $\alpha$ , was later cloned in the mid-1980's [17, 18]. In 1996, an additional ER was cloned from rat prostate [19] and designated as ER $\beta$ . The action of estrogen in cells is therefore almost entirely mediated by these two related but distinct subtype of estrogen receptors, ER $\alpha$  and ER $\beta$ . Both receptors function as ligand activated transcription factors which can bind the cognate DNA sequences known as estrogen responsive elements (ERE), and activate transcription. The ER proteins can be structurally subdivided into six domains on the basis of the functions controlled by the region, as shown in Fig. (1). The A/B domain contains one of the two transcriptional activation functions (AFs), designated as AF1 which is involved in estrogen-independent activation of transcription. Another activation function domain, AF2, is located in the E domain which also harbors the ligand binding domain (LBD), and is involved in estrogen/ ligand dependent activation [20, 21]. The ER $\alpha$  has 97% homology in the DBD and 61% homology in the LBD with ER $\beta$  suggesting differential ligand binding capability of ERs [21].

SERMs, the molecules which can bind to ER $\alpha$  and/or ER $\beta$  and can either stimulate estrogen-like actions (agonist) or oppose estrogen actions (antagonist) in various estrogen target tissues and cells. This pharmacologic knowledge advanced studies to decipher the details of the molecular mechanism of estrogen action in different cell and tissue types.

The structural studies of a SERM complexed with the LBD of ER $\alpha$  and ER $\beta$  reveal that re-orientation of the AF2 helix (helix 12) after the binding of the SERM to the hydrophobic pocket of the LBD [22, 23]. The interaction of amino acid Asp351 of ER $\alpha$  with the alkylaminoethoxyphenyl side chain of tamoxifen or raloxifene is crucial to prevent the recruitment of coactivators to the SERM-receptor complex surface [22, 23]. Using different mutants of ER $\alpha$  for the amino acid Asp351, it was shown that shielding and neutralization of Asp351 by the side chain of raloxifene is critical in defining the antiestrogenicity of this SERM. Furthermore, it has been shown that changing the Asp351 from aspartate to glycine (D351G) abolishes the estrogen-agonist activity of the tamoxifen-ER complex, while retaining its antagonistic property. The AF2 region of the agonist-bound receptor is particularly important for the interactions of steroid receptor coactivators (SRCs 1–3) via the interacting amino acid motif LxxLL, known as nuclear receptor interacting domain (NRID). It is important to note that the affinity of ERs for these NRIDs of SRCs is highly dependent upon the ER subtype, ER $\alpha$  and ER $\beta$ , and ligand bound to the ER [24–26]. Recruitment of these co-

activator(s) is also responsible for facilitating the activation of estrogen responsive genes by modifying the chromatin structure and activating the transcriptional machinery. Additionally, SERMs may also show differential AF1 activity mediated by co-repressor binding. Using ERE-reporter constructs, it has been shown that the AF1 domain of ER is actively involved in agonist-induced gene expression whereas the AF1 domain of ER is involved very weakly [27].

Estrogen can also modulate the expression of genes by another mechanism in which the receptor complex can interact with other transcription factors such as activating protein 1 (AP1) or stimulating protein 1 (Sp1) through a process known as a tethering mechanism. Intriguing differences are observed in the mechanism of action between ER and ER through an AP1 site. In the presence of estrogen, ER induces AP1 driven reporter activity but ER has no effect [28]. The raloxifene bound ER complex can induce transcriptional activity through the AP1 site but the activity through ER bound to raloxifene is negligible.

ERs also act in a non-genomic manner initiated from the cell membrane. These actions are very fast (seconds to minutes) and occur without RNA or protein synthesis. They often mobilize second messenger molecules such as  $\text{Ca}^{2+}$  and cAMP, and are associated with protein kinase cascades such as PI3K/Akt and MAPK [29, 30]. Several explanations have been offered to explain these effects. There could be a subpopulation of nuclear ERs associate with the plasma membrane, either through posttranslational modification such as palmitoylation or mediated by scaffold proteins such as caveolin-1 and MNAR, since ERs do not have a transmembrane domain [29, 30]. Another membrane bound protein, G protein coupled receptor GPR30, was identified in recent years that mediates non-genomic actions of estrogen [31, 32]. The cellular localization of GPR30 is still controversial. Some evidence suggests it is at plasma membrane [33, 34] and other evidence suggests it is in the endoplasmic reticulum [32]. GPR30 binds to 17 $\beta$ -estradiol, tamoxifen and fulvestrant with high affinity [33] and is associated with breast cancer metastasis and transactivation of the epidermal growth factor receptor (EGFR) [35].

## 2.2. Co-Regulators

The co-regulators are protein molecules which can physically interact with the liganded or un-liganded ERs and modulate the transcription of the genes. The transcriptional activation or repression of the responsive genes is a combinatorial function of ligand-receptor interaction, recognition of cognate DNA sequence and recruitment of specific co-regulators onto the promoter of the gene. The assembly of the whole transcriptional complex is also dependent upon the affinity of the above mentioned individual components among themselves and their relative concentrations in the cell. Co-regulators play defining roles in the final tissue outcome in terms of transcriptional activation or repression mediated by estrogen or SERMs. The co-regulators can be broadly classified on the basis of their function, as co-activators which promote the activation of the transcriptional process, or co-repressors which are associated with repression of transcription of genes (Fig. (2)).

**2.2.1 Co-Activators**—Presently, around 200 co-activators are known, which are associated with 48 nuclear receptors [36]. The family of p160 proteins known as steroid receptor co-activators (SRCs) have been studied extensively. The relative abundance of SRC1 in uterine cells is responsible for the agonistic activity of tamoxifen, whereas in breast cancer cells, with low SRC1 levels, tamoxifen acts as an estrogen antagonist [37]. However, raloxifene, another related SERM, does not recruit SRC-1 even in the uterine cells [37], suggesting that the interaction with specific ligand which elicits a unique conformation of the receptor is critical for the interaction of co-regulators. These observations further provide an explanation for the earlier studies, where tamoxifen have been reported to induce growth

of endometrial cancer cells but not of breast cancer cells in athymic mice [38] and also that estrogen agonistic properties of raloxifene is less in endometrial cancer cells [39]. These finding also translate very well to clinical experience [40]. In addition, the SERMs can enhance the stability of the co-activators (SRC1 and SRC3) and thereby influence the transcriptional capability of other nuclear receptors [41]. Post-translational modifications of the co-activators, including but not limited to phosphorylation, methylation, ubiquitylation, sumoylation and acetylation, can also regulate the gene activation by influencing the ability of the co-activators to interact with ER and other components of the transcriptional complex [34–36]. The understanding of structure-function relationship of ligands at the ER has formed the basis of designing effective new SERMs with fewer side effects.

**2.2.2. Co-Repressors**—Co-repressors are functional counterparts of co-activators, which are associated with transcriptionally inactive promoters and help repress the expression of genes [42]. Fewer co-repressors have been reported compared to the co-activators. In the case of ER, the co-repressors are known to interact with the un-liganded and/or antagonist bound receptor. The two most extensively studied co-repressors in connection with ER are Nuclear receptor corepressor (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT). The ER bound to raloxifene or 4-hydroxytamoxifen (a potent antagonist metabolite of tamoxifen) is known to recruit NCoR and SMRT to the promoters of estrogen responsive genes and repress transcription [43–45]. It has been shown that inhibition of NCoR or SMRT with monoclonal antibodies can enhance the agonistic property of 4-hydroxytamoxifen [46]. Moreover, using fibroblasts from NCoR null mice, 4-hydroxytamoxifen was shown to be a relatively potent ER agonist [47]. The critical role of NCoR and SMRT in 4-hydroxytamoxifen-induced arrest of cell proliferation of ER positive breast cancer cells is confirmed because 4-hydroxytamoxifen-stimulated cell cycle progression now occurs in NCoR-and-SMRT-deficient breast cancer cells [48]. However not all estrogen responsive genes are activated by 4-hydroxytamoxifen in NCoR and SMRT deficient cells, clearly indicating that additional molecules are important in SERM-induced repression of estrogen responsive genes. Indeed, there are several other co-repressor proteins known for ER. Metastasis associated protein 1 (MTA1) is a corepressor found to mediate the ER transcriptional repression [49]. Another corepressor, known as repressor of estrogen action (REA) potentiates the inhibitory effects of anti-estrogens including 4-hydroxytamoxifen. Additionally, REA interacts with ER and competes with the co-activator SRC1 for binding to the estrogen bound ER [50, 51]. This again emphasizes the fact that the relative levels of co-regulators may be important in deciding the outcome of the SERM action. The proteasomal regulation of NCoR is another factor which may influence the SERM action. Degradation of NCoR occurs through the 26S proteasome, which is mediated by seven in absentia homologue 2 (Siah2) [52]. Interestingly, estrogen mediated upregulation of Siah2 in ER positive breast cancer cells has been implicated in the proteasomal degradation of NCoR, and subsequent de-repression of NCoR regulated genes [53].

In addition to acting as a “transcriptional adapter” between the receptors and the transcriptional machinery, the coregulator itself or its complex possess various enzymatic activities such as acetylation, phosphorylation, methylation or de-acetylation by which they are able to modify the local chromatin structure thereby making the local environment conducive for gene expression or repression. Intrinsic histone acetyl transferase activity was found to be associated with co-activator SRC1 which helps in the activation of transcriptional expression [54]. In contrast, the 4-hydroxytamoxifen bound ER complex which recruits the co-repressors NCoR and SMRT is associated with histone de-acetylases and other chromatin modifying enzymes [37, 55]. The deacetylase activity promotes transcriptional repression [37, 55]. Interestingly, another enzyme in the co-activator complex, CARM1 (coactivator associated arginine methyltransferase 1) has recently been

implicated in modifying the coactivator itself and inducing the degradation of the complex [56]. This suggests the ability of the enzymes in the complex to modify other proteins in its own complex apart from a role in the modification of chromatin.

With this background of the molecular biology of SERM action, it is now appropriate to describe our evolving understanding about drug resistance. This is important not only because tumor drug resistance is the consequence of long term SERM administration, but also because new knowledge will aid patients with the development of novel treatment strategies for SERM-resistant breast cancer.

### 3. DRUG RESISTANCE TO SERMs

There are three types of resistance to SERMs based on the mechanism: metabolic resistance, intrinsic resistance and acquired resistance [57].

#### 3.1. Metabolic Resistance

Metabolic resistance to tamoxifen is mostly related to CYP2D6, an enzyme product that metabolizes tamoxifen into its active forms 4-hydroxytamoxifen and endoxifen [58]. This has been extensively reviewed recently and will only be briefly mentioned here [13, 59]. CYP2D6 is genetically polymorphic and 5–8% of Caucasian subjects are CYP2D6 “poor metabolizers” thus are less likely to benefit from tamoxifen treatment, although it has been shown that these women tolerate tamoxifen better and tend to remain on the drug for longer [59]. The genotype of CYP2D6 has been shown in multiple clinical trials to be directly related to the outcome of tamoxifen use, however, the results are not always consistent. Eight studies indicated that CYP2D6 “poor metabolizer” genotypes have worse outcome of breast cancer patients who received tamoxifen but two studies contradicted this conclusion [60]. In addition to the genotype of CYP2D6, it is important to consider that other drugs may interact with the enzyme system and block the metabolic activation of tamoxifen. Unfortunately, selective serotonin reuptake inhibitors (SSRIs) that are used to relieve the menopausal side effects of tamoxifen are also metabolized by CYP2D6 and block the metabolic activation of tamoxifen. The proper choice of SSRI is therefore important so as not to impair tamoxifen metabolism. The SSRI of choice is venlafaxine that has only a low affinity for the CYP2D6 enzyme [61]. Although these emerging data about CYP2D6 genotypes and the drug interaction between tamoxifen and SSRIs are important, it is perhaps too early to use CYP2D6 status to routinely choose between tamoxifen and aromatase inhibitors to treat postmenopausal women with breast cancer. At present, an international consortium is evaluating the overall CYP2D6 status of completed clinical trials with tamoxifen to assemble a large scale retrospective analysis of the worth of genotyping. The aim is to answer the question of whether “poor metabolizers” should avoid tamoxifen use.

#### 3.2. Intrinsic Resistance

Approximately 30% ER-positive breast cancer patients do not respond to tamoxifen [62]. This type of resistance is referred to as “de novo” resistance or intrinsic resistance. Clinical studies showed that only 40% patients with ER-positive, progesterone receptor (PR)-negative breast cancers are responsive to anti-estrogen treatment (tamoxifen or endocrine ablation) compared to 80% responsive rate in ER-and-PR-positive patients [58, 59]. Historically, the status of PR has been regarded as an indicator of a functional ER pathway, since expression of PR is regulated by estrogen. On the other hand, recent evidence suggested that the absence of PR is associated with excessive growth factor signaling such as overexpression of HER2 [63, 64], which has been known to impair estrogen induction of PR and reduce the effectiveness of tamoxifen treatment for breast cancer [65]. However, the negative association between PR and HER2 seems more evident in older women (> 45 yrs)

[66] and it remains controversial that PR-status could be used for clinical decision on choosing between tamoxifen or AIs [67].

Growth factor signaling, especially through epidermal growth factor (EGF) pathway, has been studied extensively in the past two decades and linked to SERM resistance. This has been recently reviewed [68] and will only be briefly summarized here. EGF binds to ErbB family of cell surface receptors that include four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/c-neu (ErbB-2), HER3 (ErbB-3) and HER4 (ErbB-4). Overexpression of HER2 has been clinically linked to less response to endocrine therapies and worse prognosis [69–71], so has the overexpression of EGFR [72]. Different ErbB family members can form heterodimers and activate multiple signalling pathways including PI3K/Akt and MAPK. The major molecular mechanisms leading to SERM resistance can be summarized as follows: 1. Activation of downstream kinase cascade results in the phosphorylation of ER at key residues (Ser106/107, 118, 167, 305 and Thr 311) which activates transcription in a ligand-independent manner. Phosphorylation may change the binding of ER with ligands, DNA and coregulators, which may ultimately alter the activity of SERMs [73]. For example, phosphorylation of ER at Ser167 by Akt and Ser118 by the MAPK pathway both cause ligand-independent activation [74–76]. A recent study showed that phosphorylation of ER at Ser305 altered the orientation between the C-terminus of ER and SRC-1 that led to the recruitment of ER transcription coactivators and RNA polymerase II even in the presence of tamoxifen [77]. 2. Phosphorylation of ER co-regulators is equally important as the phosphorylation of ER itself, since phosphorylated co-activators have increased activity in the presence of SERMs [78–80]. Phosphorylation of co-repressors such as SMRT is associated with the co-repressor's nuclear export and impaired transcriptional suppressing function [81]. 3. Other than enhancing the transcriptional activity of the ER by phosphorylation, overexpression of EGFR or HER2 increases the non-genomic actions of ER, and SERMs may now act as estrogen agonists via the membrane effects of ER [82, 83]. In addition to the EGF signal pathway, the insulin-like growth factor (IGF) signal pathway is also involved in tamoxifen resistance [84]. It can activate PI3K/Akt pathway [71] and turn on genes that are otherwise activated by estrogen [85, 86].

Dysregulation of ER co-regulators is another major contributor to intrinsic SERM resistance. Overexpression of both AIB1 (SRC-3, ACTR, p/CIP, RAC3, TRAM-1) and HER2 have been shown to convert tamoxifen into an estrogen agonist in breast cancer cells [79]. Elevated AIB1 was found to associate with tamoxifen resistance, DNA-nondiploidy, high S-phase fraction and HER2 amplification in samples from clinical study [87]. Although a study indicated that high expression of AIB1 was not associated with relapse during tamoxifen treatment [88], AIB1 was shown to associate with tamoxifen resistance in breast cancers that overexpressed ErbB family proteins [88, 89]. AIB1 might be a predictor marker for tamoxifen ineffectiveness in ER-positive, HER2-positive and PR-negative breast cancer. On the other hand, low expression of ER co-repressor NcoR is associated with shorter relapse-free survival in breast cancer patients who only received tamoxifen after surgery [90]. Based on the emerging importance of co-regulators and tamoxifen resistance, one novel approach to overcome tamoxifen resistance is by the use of disulfide benzamide (DIBA) to disrupt the zinc finger in the ER DNA binding domain. The approach facilitates ER dissociation from coactivator AIB1 and concomitant association of corepressor NcoR without changing the phosphorylation of HER2, MAPK, Akt or AIB1 [91].

Another group of regulators associated with tamoxifen resistance are microRNAs (miRNA). These are naturally occurring single-stranded RNAs with the length of 21–23 nucleotides that do not code for proteins. They regulate gene expression mainly by inducing target mRNA degradation or inhibiting translation (protein synthesis). Dysregulation of miRNAs is associated with many cancers including breast cancer [92, 93]. Two recent studies show that

miRNA-221/222 are upregulated in tamoxifen-resistant breast cancer cells and primary tumors, and they may contribute to tamoxifen resistance by down-regulating p27Kip1 or ER [94, 95].

### 3.3. Acquired Resistance

Breast cancer patients who initially respond to tamoxifen later develop “acquired resistance” that is characterized by tamoxifen stimulated growth. This can be replicated in the laboratory with MCF-7 xenograft tumors implanted in ovariectomized athymic mice. Tamoxifen initially inhibits estrogen stimulated tumor growth but eventually some tumors start to grow during tamoxifen therapy [96]. These tumors now grow in response to either estrogen or tamoxifen and stop growing with no treatment or during treatment with fulvestrant [96]. The laboratory model is consistent with the clinic observation that aromatase inhibitor or fulvestrant are equally effective after the failure of tamoxifen treatment [97, 98]. It therefore appears that ER remains fully functional in the laboratory model of acquired tamoxifen resistance. In clinical studies, only 17–28% patients with acquired tamoxifen resistance have a loss of ER function [99, 100], and it is more likely that acquired resistance is associated with the stimulation of other growth/survival pathways [101]. For example, activated mammalian target of rapamycin (mTOR, downstream of PI3K/Akt and MAPK pathway) and c-Src (downstream of EGFR/HER2) were observed in breast cancer cells and mTOR and c-Src inhibitors can restore tamoxifen sensitivity in these cells, respectively [102, 103]. Several genes involved in cell proliferation and survival have altered expression level in breast cancer cells with acquired tamoxifen resistance. Examples of genes which down regulation is associated with acquired tamoxifen resistance include cyclin-dependent kinase inhibitors p21Cip [104] and p27Kip [105]. Examples of genes which upregulation is associated with tamoxifen acquired resistance include cyclin-dependent kinase 10 (CDK10) [106] and anti-apoptotic protein survivin [107].

Laboratory observation showed that acquired tamoxifen-resistant breast cancer cells/tumors respond differently to estrogen, and three phases of tamoxifen-resistance have been described, which seems to depend on the length of tamoxifen exposure [12]. Tumors with phase I resistance are stimulated by estrogen and tamoxifen but inhibited by AIs and fulvestrant; tumors with phase II resistance are stimulated by tamoxifen but are inhibited by estrogen due to apoptosis; tumors with phase III resistance (autonomous growth) grow in a hormone-independent manner that is not responsive to either AIs or fulvestrant or SERMs, but estrogen still exerts apoptotic actions on those tumors [12]. The laboratory models suggest a new treatment strategy, in which limited duration, low-dose estrogen can be used to purge phase II- or phase III-resistant breast cancer cells so that the tumors will be responsive to antiestrogen therapy again. Phase II clinical study is ongoing to test this treatment plan [108].

Most studies on SERM-resistance are related to tamoxifen and little is known about raloxifene resistance. Based on a few studies on raloxifene resistance using cell culture and animal models, raloxifene-resistant tumors are likely to have similar properties as tamoxifen-resistant ones [109]. Raloxifene-resistant MCF7 cells generated by long-term exposure to raloxifene *in vitro* are also resistant to tamoxifen *in vitro* and *in vivo*. They exhibit phase II SERM-resistance as estradiol treatment causes tumor regression by inducing G2/M cell cycle arrest and apoptosis [110]. Another raloxifene-resistant breast tumor model generated by exposing MCF7 breast tumors to raloxifene *in vivo* exhibits phase I SERM-resistance whose growth is stimulated by tamoxifen, raloxifene and estrogen [109]. Interestingly, protein levels of EGFR and HER2 are also increased in this phase I raloxifene-resistant tumor model, which suggests raloxifene-resistant tumors share similar molecular mechanisms as tamoxifen-resistant ones [109].



Overall, the classifications of different forms of antihormonal drug resistance can be used as a basis to evaluate the pharmacology of new SERMs. The goal is to improve on tamoxifen, the pioneer that over the past 30 years found ubiquitous long term applications in the treatment and prevention of breast cancer.

## 4. NEW SERMs

The discovery of the first antibiotic penicillin initiated a search for further antibiotics to delay drug resistance and to target specific diseases. Similarly, the successful clinical application of tamoxifen in medicine has resulted in the investigation of numerous related molecules to develop the “ideal SERM”. However, it has been challenging to find a SERM that is superior to tamoxifen, which retains or extends its benefit to treat and prevent breast cancer but with fewer side effects. Tamoxifen maintains bone density in animals [111] and humans [112] so SERMs are being developed to treat osteoporosis, but the potential to prevent breast cancer and uterine cancer will also increase their clinical value and commercial success. The core structures of SERMs are diverse, including triphenylethylene, benzothiophene, chromene (benzopyran), naphthalene, indole and steroid, but each of the newer SERM is really a mimic of tamoxifen, raloxifene or estradiol. The development of dozens of SERMs have been discontinued due to ineffectiveness for human disease or severe side effects, but several new SERMs are under active investigations with great potential in breast cancer treatment and/or prevention, alone or in combination with other type of drugs. In addition, since the identification of ER in 1996 [19], ER subtype selective SERMs have been developed which could potentially be used as breast cancer preventives. Thus this area of medicinal chemistry remains an important topic of interest as new ER regulated targets emerge. We will review the current status of several agents that are either approved or in the process of drug development (summarized in Table).

### 4.1. Tamoxifen-like SERMs

**4.1.1. Toremifene (Fareston)**—Toremifene (**2**) is a chlorinated tamoxifen analogue which has been approved in the US and several other countries for the treatment of metastatic breast cancer. Its structure is shown in Fig. (3). Toremifene is as effective as tamoxifen in the treatment of ER-positive breast cancer but with the potential of fewer genotoxic effects, since it does not produce DNA adducts in rat liver and human endometrium [113]. The mechanism for the reduced genotoxicity of toremifene can be explained as follows: Tamoxifen-DNA adducts are primarily formed via sulfonation of the -hydroxylated tamoxifen metabolites, but the -hydroxy metabolites of toremifene is poorly esterified or sulfonated, and even sulfonated -hydroxy toremifene, -sulfoxytoremifene, reacts poorly with DNA [114, 115]. However, there are some reports to show toremifene induces DNA damages and hepatocarcinogenesis in rats [116, 117].

The effects of toremifene and tamoxifen on bones are similar [118], as are the endometrial effects. However, a recent safety evaluation demonstrates that secondary endometrial cancer incidence is lower with toremifene than with tamoxifen and is similar to that with raloxifene [119]. Nevertheless, toremifene stimulates the growth of human endometrial cancer implanted in athymic mice in the same way as tamoxifen [120]. The positive effects of toremifene on lipid profiles are superior to tamoxifen's. Toremifene lowers the low-density lipoprotein (LDL) cholesterol to a level similar to that seen with tamoxifen, but unlike tamoxifen, toremifene slightly increases high-density lipoprotein (HDL) cholesterol and lowers triglycerides in the serum [121, 122].

Cross-resistance with tamoxifen is an important issue to consider when using toremifene for recurrent breast cancer because the majority of patients have received or failed adjuvant tamoxifen. Toremifene is completely cross-resistant with tamoxifen in human breast tumors

implanted in athymic mice [123], as well as in breast cancer patients [124, 125]. Therefore, toremifene would not be effective as a second-line endocrine therapy after tamoxifen failure and may offer no therapeutic advantages over tamoxifen as an adjuvant therapy.

In recent years, toremifene has been developed to treat other estrogen-related diseases. Toremifene is effective to treat mastalgia in some small phase II trials [126, 127], and is also effective at decreasing prostate cancer incidences in a high-risk population [128]. In addition, a recent multicenter randomized phase III trial showed that toremifene increased bone density and improved lipid profile in men receiving androgen deprivation therapy for prostate cancer [129, 130].

**4.1.2. Ospemifene (Deaminohydroxytoremifene, FC-1271a)**—Ospemifene (**3**), or deaminohydroxytoremifene, is a metabolite of toremifene (Fig. (3)). Like toremifene, ospemifene is generally well tolerated and has a favorable safety profile. It does not induce DNA adducts in mice [131], rats [132] and monkey [133]. Ospemifene exerts a very weak estrogenic effect on endometrial histology, like raloxifene and decreases cholesterol [134]. However, unlike tamoxifen or raloxifene, ospemifene has significant estrogenic effects on vaginal epithelium [134–136] and is being developed for postmenopausal vaginal atrophy, a chronic condition experienced by about 40% postmenopausal women. Ospemifene is being evaluated in a phase III trial that has already recruited 826 women. Early results suggested that a 12-week course of ospemifene treatment significantly relieves symptoms of dryness in the vagina.

Ospemifene has showed promise in the prevention and treatment of osteoporosis. Cell culture studies indicated that ospemifene inhibits osteoclast formation and bone resorption and protects osteoblast-derived cells from apoptosis [137, 138]. In a recent phase II trial to compare effects of ospemifene and raloxifene on biochemical markers of bone turnover in postmenopausal women, ospemifene showed similar effects as raloxifene in regulating most of the bone markers examined, and at the 90-mg dose, ospemifene increased procollagen type I N propeptide (PINP) more than raloxifene [139]. Ospemifene is currently in phase III development for the treatment of postmenopausal osteoporosis.

Studies based on animal models suggest ospemifene might be effective in breast cancer prevention. Ospemifene prevented dimethylbenzanthracene (DMBA)-induced mammary tumors in female Sencar mice as effectively as tamoxifen, while raloxifene was not effective [140]. In a transplantable mouse model of ductal carcinoma in situ (DCIS), ospemifene had inhibitory effects equivalent to tamoxifen in terms of tumor growth and progression [141]. Nevertheless, the chemoprevention effects of ospemifene in breast cancer need to be further studied and substantiated by clinical trials.

**4.1.3. GW5638 (DPC974) and GW7604**—GW5638 (**4**) is a derivative of tamoxifen with an acrylate side chain in place of the dimethylaminoethoxy side chain in tamoxifen. GW7604 (**5**) is the 4-hydroxy version of GW5638, analogous to the major metabolite of tamoxifen, 4-hydroxytamoxifen. Their structures are shown in Fig. (3). GW5638 functions as a full ER agonist in bone and the cardiovascular system but as an antagonist in breast and endometrial system in rodent models [142].

Although the structures of tamoxifen and GW5638/GW7604 are similar, GW5638/GW7604 acts with a mechanism different from tamoxifen/4-hydroxy tamoxifen as suggested by the following evidence: 1. GW7604 acts as an antagonist in MDA-MB-231 cells transfected with wild-type ER $\alpha$ , but 4-hydroxytamoxifen acts as an agonist [143]; 2. Phage display experiments indicate that GW7604 bound ER $\alpha$  or ER $\beta$  is associated with different peptides from 4-hydroxytamoxifen, raloxifene or fulvestrant bound ERs [144]; 3. GW5638 inhibits

the growth of tamoxifen-resistant breast tumor xenograft [144, 145]; 4. The crystal structure of the ER LBD bound by GW5638 shows that GW5638 induces a distinct conformation of H12 in the ER AF2 region, which increased exposure of hydrophobic residues and results in ER destabilization in MCF7 cells [146].

GW5638 and GW7604 are also classified as selective estrogen receptor down-regulators (SERDs) because they induce ER degradation, a property observed with the pure antiestrogen fulvestrant which was approved for the treatment of metastatic breast cancer [147]. However, a recent report [148] indicates that GW5638 induces ER degradation through a different mechanism from fulvestrant and another SERD RU58,668, as the protein/protein interaction surface on ER required for fulvestrant-induced degradation is not necessary for GW5638-induced degradation. The fact that GW5638 has a unique mechanism to antagonize estrogen function and induces ER degradation in breast cancer cells makes it a possible second line therapy after tamoxifen failure and as an alternative to fulvestrant. Currently, GW5638 is under clinical development under the name DPC974 [148].

## 4.2. Raloxifene-like SERMs

**4.2.1. Arzoxifene (LY353381)**—Arzoxifene (**7**) is a derivative of raloxifene with the ketone group replaced by an ether group and the hydroxy group is replaced by a methoxy group (Fig. (4)). These modifications have improved the pharmacokinetic properties [149]. Arzoxifene has antiestrogenic effects on breast and endometrium but pro-estrogenic effects on bone and lipids [150]. Arzoxifene is cross-resistant in some but not all tamoxifen-stimulated breast tumor xenografts [151]. Phase II clinical trials indicate that arzoxifene is effective to treat tamoxifen-sensitive or tamoxifen-refractory patients with advanced or metastatic breast cancer [152] and patients with recurrent or advanced endometrial cancer [153] with minimum toxicity. However, a phase III trial showed arzoxifene was inferior to tamoxifen to treat patients with locally advanced and metastatic breast cancer [154]. The main role of arzoxifene may reside in its chemoprevention potential since it is more potent than raloxifene in pre-clinical studies [149].

The breast cancer chemoprevention property of arzoxifene has been studied with animal models and small short-term clinical trials. Arzoxifene effectively prevented nitrosomethylurea (NMU)-induced mammary tumor in rats [140] and induced apoptosis of breast cancer cells in rodent models especially when used in combination with rexinoid LG100268, a selective ligand for the retinoid X receptors (RXR) [155]. In two phase I clinical trials of women with newly diagnosed ductal carcinoma in situ or T1/T2 invasive cancer, arzoxifene did not demonstrate a significant reduction of tumor cell proliferation compared to placebo in 2–6 weeks treatment [156]. However, there were some favorable findings, such as a decrease of serum insulin like growth factor I (IGF-1) vs IGF binding protein 3 (IGFBP3) ratio and an increase of sex hormone binding globulin [156]. Another interesting aspect of the pharmacology of arzoxifene is that it might have chemopreventive properties for ER-negative breast cancer when used in combination with LG100268. A recent study showed that both SERMs arzoxifene and acolbifene alone prevent ER-negative mammary tumor in a mouse model and the effect is synergized with LG100268 [155]. Although the SERMs by themselves are not functional in the treatment of established tumors, together with LG100268 they inhibit proliferation and induce apoptosis in the ER-negative mammary tumors [155]. The mechanism how SERMs prevent tumorigenesis of ER-negative breast tissue is unknown, but the results suggest that arzoxifene has the potential for further clinical development as a chemoprevention drug of both ER-positive and negative breast cancer, especially in combination with rexinoids.

**4.2.2 Lasofoxifene (CP-336156, Fablyn)**—Lasofoxifene (**8**) has a naphthalene core structure, which is different from all the other SERMs discussed in this article (Fig. (4)). However, the crystal structure shows that lasofoxifene fits into the ER LBD pocket in a similar manner as other ligands [157]. In addition, lasofoxifene-bound ER LBD has similar conformational features as other SERM-bound ER LBDs, such as tamoxifen or raloxifene, in which H12 in the “antagonist-bound” conformation and occludes the coactivator binding surface [157]. Lasofoxifene has a high affinity for ER with an IC<sub>50</sub> of 1.5 nM, which is comparable to 17 $\beta$ -estradiol and higher than tamoxifen and raloxifene [158]. It preserves bone density and lowers serum cholesterol, and also has chemopreventive and chemotherapeutic effects in rat mammary tumor models without any uterine hypertrophic effects [159]. Lasofoxifene is currently undergoing an extensive clinical evaluation for the prevention and treatment of osteoporosis [159]. One advantage of lasofoxifene over raloxifene is its increased oral bioavailability due to the nonpolar naphthalene structure that makes it a poor substrate for intestinal wall glucuronidation [160]. In addition to its effects on bone, lasofoxifene significantly improves symptoms of vaginal atrophy [161] and a recently completed phase III trial indicated that lasofoxifene decreased vaginal pH and improved the vaginal-cell maturation index in osteoporotic postmenopausal women. These effects may be due to the increased vaginal ER and androgen receptor protein levels [162]. Lasofoxifene acts as a chemopreventive and treatment in the NMU-induced rat mammary tumor model. The results are similar to the comparator drug tamoxifen [163]. Phase III trials are currently ongoing to evaluate its ability to prevent breast cancer and cardiovascular diseases in postmenopausal women [164].

**4.2.3. Pipendoxifene (ERA-923)**—Pipendoxifene (**9**) has an indole core structure (Fig. (4)). It was designed by adding an alkylaminoethoxyphenyl side chain to zindoxifene (D-16726), a 2-phenylindol based SERM which failed as a treatment for breast cancer [165]. Pipendoxifene, also named ERA-923, mimics the structure of raloxifene and is devoid of uterotrophic activities in immature rats and ovariectomized mice compared to raloxifene [166]. It inhibits the growth of tamoxifen-sensitive and -resistant tumors in rats and mice [167] and is under phase II clinical development for the treatment of tamoxifen-resistant metastatic breast cancer. In a recent study, a combination of pipendoxifene and temsirolimus, which is a mammalian target of rapamycin (mTOR) inhibitor, synergistically inhibited growth of MCF-7 cells and xenograft models even at suboptimal doses, primarily by causing G1 cell cycle arrest [168]. This suggested that combination of a SERM and an mTOR inhibitor might be of clinic value as breast cancer treatments.

**4.2.4. Bazedoxifene (TSE-424, WAY-140424)**—Bazedoxifene (**10**) is another indole SERM, designed and synthesized at the same time as pipendoxifene with a slight structural difference, as shown in Fig. (4) [166]. This SERM is being actively developed to treat osteoporosis with the potential to prevent breast cancer. Bazedoxifene binds to ER and ER with an affinity lower than raloxifene but is less selective for ER [169]. It inhibits estrogen-mediated proliferation of breast cancer MCF7 cells and increases bone density with little uterine or vasomotor effects in rat models [169]. A Phase III trial with 497 healthy postmenopausal women showed that 6-month bazedoxifene treatment decreases endometrium thickness and uterine bleeding, suggesting antagonistic effects of bazedoxifene in endometrium [170]. Bazedoxifene is currently under review by the Food and Drug Administration (FDA) for the prevention and treatment of postmenopausal osteoporosis. The completed 3-year phase III trial which enrolled 7,492 postmenopausal women with moderate to severe osteoporosis showed bazedoxifene significantly reduced the incidences of vertebral and non-vertebral fracture compared to placebo, while raloxifene was not effective against non-vertebral fracture [171]. No safety concerns related to breast or endometrium were observed, however, a statistical insignificant increase of venous

thromboembolic events was observed with groups treated with either bazedoxifene or raloxifene in the same study [172]. Based on studies using rodent models, combination of bazedoxifene and conjugated estrogens exerted positive vasomotor, lipid, and skeletal responses with minimal uterine stimulation [173]. This suggested that pairing SERMs and estrogen might be effective in the treatment of menopausal symptoms and prevention of osteoporosis. However, further studies are needed to examine the effectiveness of bazedoxifene in breast cancer prevention.

**4.2.5. Acolbifene (EM-652, SCH57068) and EM-800 (SCH57050)**—Acolbifene (EM-652) (**11**) and its orally active prodrug EM-800 (**12**) have a chromene core structure (Fig. (4)). They were initially misclassified as pure antiestrogens and their side chain was depicted by analogy with the pure antiestrogen fulvestrant [174]. However, the structure of acolbifene is actually similar to that of raloxifene, and unlike fulvestrant, the antiestrogenic side chain of acolbifene does not mask the mutant ER amino acid D351Y to produce an estrogenic action [175]. In addition, acolbifene and EM-800 act as antiestrogens in mammary and uterine tissues, but have estrogenic effects to prevent bone loss and have a favored function in the regulation of lipid metabolism by lowering plasma cholesterol and triglyceride in rodent models [176, 177]. Therefore, acolbifene and EM-800 should be classified as SERMs.

Acolbifene has the highest ER-binding affinity among all known compounds [178]. Preclinical studies indicated that acolbifene and EM-800 were more potent than tamoxifen, idoxifene, raloxifene, GW-5638, toremifene and droloxifene to inhibit the growth of breast cancer cell lines MCF-7, ZR-75-1, MCF-7 and T47D as well as ZR-75-1 xenograft in mice models [179, 180]. Interestingly, acolbifene caused disappearance of 65% ZR-75-1 xenograft in ovariectomized nude mice, while other SERMs tested (tamoxifen, toremifene, raloxifene, droloxifene, idoxifene and GW 5638) only decreased the tumor growth rate stimulated by estrone [180]. Acolbifene was evaluated as a second line therapy for tamoxifen-refractory breast cancers, since it was regarded as a pure anti-estrogen. In a small clinical trial involved 43 postmenopausal or ovariectomized women with breast cancer who had received tamoxifen for over a year but relapsed, the objective response to EM-800 was 12% with 1 complete response and 4 partial responses [181]. In a phase III trial to compare acolbifene with the aromatase inhibitor anastrozole in breast cancer patients who did not respond to tamoxifen, acolbifene did not show superior antitumor activity to anastrozole and the study was halted [182]. However, acolbifene and EM-800 may be more suitable as first line therapy and a phase III trial for untreated metastatic breast cancer patients is planned [182].

Recent studies indicate that acolbifene might be used in combination with other drugs. Acolbifene synergizes with rexinoid LG100268 in the prevention and treatment of mice with ER-negative mammary tumor [155]. It also synergizes with dehydroepiandrosterone (DHEA), which is a naturally produced prohormone for androgen and estrogen, in the prevention of dimethylbenzanthracene (DMBA)-induced mammary tumors in the rats [183]. A phase III trials of acolbifene plus DHEA for vaginal atrophy and uterine safety has been planned.

**4.2.6. CHF4227**—CHF4227 (**13**) is a SERM with a chromene (benzopyran) core structure, as shown in Fig. (4). Compared with raloxifene, CHF4227 binds to ER and ER with higher affinity and inhibits the uterotrophic action of 17 $\alpha$ -ethynyl estradiol with more potency [184]. CHF4227 significantly prevents the development of DMBA-induced mammary tumors in rats [184]. It preserves bone mass without affecting uterine weight and decreases serum cholesterol and fat mass in ovariectomized rats [185]. A recent phase I study showed CHF4227 is well-tolerated, as 28 days of treatment has a positive effect on the

serum lipid profile and bone markers without any negative effects on the endometrium or the fibrinolytic system. Additionally, CHF4227 does not cause vaginal bleeding or hot flashes [186]. These results suggest that CHF4227 is safe and worthy of further clinical development for osteoporosis and the chemoprevention of breast cancer.

**4.2.7. SP500263**—SP500263 (**14**) was discovered in a screen to identify ER agonist in bone cells [187]. It has a chromene core structure and binds to both ER and ER with high affinity similar to raloxifene's (Fig. (4)) [187]. SP500263 inhibits the growth of breast cancer MCF7 cells and xenografts in nude mice, and does not stimulate uterine weight gain in immature rats or ovariectomized adult rats [188, 189]. SP500263 also blocks osteoclastogenesis in human bone cell model [190]. These preclinical results suggest that SP500263 has potential for the treatment of both breast cancer and osteoporosis. However, clinical value of this drug has yet to be determined.

### 4.3. Steroidal SERMs

**4.3.1. HMR3339**—All of the SERMs described to this point are non-steroidal. Recently, steroidal SERMs have been described (Fig. (5)). In rats, HMP3339 (**15**) not only increases bone mineral density but also restores the mechanical strength at multiple sites even after ovariectomy, and it affects both cortical and cancellous bones, while raloxifene was effective only at cancellous sites [191]. HMR3339 has entered clinical investigation for the prevention of osteoporosis and cardiovascular diseases. In a series of small phase II trials with healthy postmenopausal women, HMR3339 was found to reduce total cholesterol, LDL cholesterol, C-reactive protein (CRP, a pro-inflammatory cytokine and a cardiovascular disease risk factor), asymmetric dimethylarginine (AMDA, a nitric oxide synthase inhibitor) and homocysteine [192–194]. Elevation of AMDA or homocysteine is linked to a high incidence of cardiovascular disease but raloxifene treatment does not reduce the level of either AMDA or homocysteine [192]. HMR3339 reduces concentrations of procarboxypeptidase U (pro-CpU, an inhibitor of fibrinolysis), antithrombin and fibrinogen to a degree similar to raloxifene and shows beneficial effects on some markers of fibrinolysis [195, 196]. Therefore, HMR3339 has potential to prevent cardiovascular diseases and possibly also osteoporosis. However, whether or not there is potential as a cancer preventive has not been determined.

**4.3.2 PSK3471**—PSK3471 (**16**) is a newly developed SERM with a structure similar to HMR3339 (Fig. (5)). It was reported to prevent gonadectomy-induced bone loss in male and female mice, and antagonize estradiol-stimulated MCF-7 cell proliferation [197].

### 4.4. ER Subtype Selective SERMs

ER and ER have a different tissue distribution and have overlapping but distinct biological functions [198]. Unlike ER, ER expression is not routinely examined in the clinic and its function in breast cancer remains unclear. ER expression is found in both normal and breast cancer specimens but does not correlate with ER expression [199]. It seems that ER functions differently if it is expressed alone or co-expressed with ER in breast cancers. In ER-positive breast tumors, ER often antagonizes the pro-proliferation actions of ER [200, 201] and its expression is associated with better response to endocrine therapy and a favorable clinical outcome in most cases [202]. Thus ER seems to function as a tumor suppressor. However in ER-negative breast tumors, several studies indicated that the expression of ER correlates with proliferation markers such as Ki67 and cyclin A [202, 203], which suggested that ER might stimulate cancer growth. In the latter situation, ER could serve as an endocrine therapy target in those patients who would otherwise be regarded as ER-negative and have limited choice but chemotherapy. The presence of ER in ER-negative breast cancers may partly explain why some “ER-negative” patients respond

to SERMs. The reason that ER $\alpha$  functions differently in the absence or presence of ER $\beta$  might be due to the different activities between the ER $\alpha$ /ER $\beta$  heterodimer and ER $\alpha$  or ER $\beta$  homodimers.

A new direction to consider is the estrogen related receptor (ERR $\alpha$ ) [204, 205]. There is emerging evidence that ERR $\alpha$  is critical for the growth of ER-negative breast-cancer MDA-MD-231 xenografts in mice [206], as ERR $\alpha$  appears to be involved in angiogenesis by inducing the expression of vascular endothelial growth factor (VEGF) [207, 208]. Novel therapeutic agents targeted to ERR $\alpha$  would be valuable to treat breast cancer.

Several ER-subtype selective SERMs have been reported, although it is difficult to design subtype selective ligands given the fact that only two amino acids are different in the ligand binding pocket between ER $\alpha$  and ER $\beta$  (despite that they have 61% amino acid identity in LBD). All the SERMs discussed previously were designed against ER $\alpha$  and have low subtype selection in terms of binding affinity. In contrast to the focus on ER $\alpha$  and breast cancer, most of ER subtype selective SERMs are developed for diseases other than breast cancer. In animal models, ER $\beta$ -selective agonists ERB041 and diaryl-propionitrile (DPN) have been shown to have anti-inflammatory properties and antidepressant-like effects, respectively [209, 210]. An ER $\alpha$  agonist, 8-vinylestra-1,3,5 (10)-triene-3,17 $\beta$ -diol, stimulates ovarian follicular development in hypophysectomized rats and gonadotropin-releasing hormone a ta gonist-treated mice [211], thus this drug could be used to enhance fertility [198]. A few ER $\alpha$  agonists are being developed for clinical applications in Alzheimer's disease and rheumatoid arthritis [212]. For breast cancer prevention and treatment, it is conceivable that ER $\alpha$  agonist might have potential for ER $\alpha$ -and-ER $\beta$ -positive tumors, especially in combination of an ER $\alpha$  selective antagonist, since the preclinical studies indicate a protective role of ER $\alpha$ . However, this strategy poses a difficult pharmacologic issue of tissue pharmacodynamics. Nevertheless, a couple of ER $\alpha$  modulators have been shown with positive effects to treat advanced postmenopausal breast cancer, which will be discussed below.

**4.4.1. Trilostane (Modrenal)**—Trilostane (**17**) (Fig. (6)) is an inhibitor of 3 $\beta$ -hydroxysteroid dehydrogenase, a critical enzyme in the conversion of DHEA to estradiol in breast tumors [213]. Trilostane increases the maximum binding of estradiol to ER $\alpha$  but not ER $\beta$  in MCF-7 breast cancer cells [214], and it increased the expression of ER $\alpha$  in MCF-7 cells and rat uterine [215]. Trilostane is approved in UK to treat advanced postmenopausal breast cancer after relapse to initial hormone therapy and is currently under investigation to be used in prostate cancer and premenopausal breast cancer [213].

**4.4.2. TAS-108 (SR16234)**—Another type of subtype selective SERM that might be relevant to breast cancer is a combined ER $\alpha$  antagonist but ER $\beta$  agonist. TAS-108 (**18**) (Fig. (5)) is a steroidal antiestrogen for ER $\alpha$  and a partial agonist on ER $\beta$  [216]. TAS-108 has pure antiestrogenic effects for ER $\alpha$  in the presence or absence of estrogen but exhibits partial agonist activity on ER $\beta$  using *in vitro* reporter assay. TAS-108 inhibits the growth of tamoxifen-resistant breast cancer cells, DMBA-induced mammary tumor in rats and estrogen-stimulated growth of MCF7 xenografts with little uterotrophic effect [216, 217]. Phase I trial indicate that TAS-108 has anti-tumor activity, is well tolerated, and does not have effects on an endometrial thickness based on an evaluation using trans-vaginal ultrasound [218, 219]. Similar results were obtained in Phase II trials that recruited postmenopausal women with advanced breast cancer, according to presentations at San Antonio Breast Cancer Symposium (SABCS) in December, 2008. A phase III trial is planned [217]. TAS-108 did not increase bone loss like fulvestrant, which could be due to its agonistic property on ER $\beta$ . Another advantage over fulvestrant is that TAS-108 is orally

administered [220]. TAS-108 is therefore a promising breast cancer drug, even for patients who have relapsed after tamoxifen.

## 5. CONCLUDING REMARKS

Endocrine therapy targeting to ER has been very successful in the treatment and prevention of breast cancer [221, 222]. It is very effective and less toxic compared to combinational cytotoxic chemotherapy that was the only option 30 years ago. In the ensuing period, multiple strategies have been developed to antagonize estrogen action. Most experience has accumulated with the competitive inhibitor of estrogen action tamoxifen, but targeting aromatase to deplete estrogen with AIs in postmenopausal patients or to induce ER degradation with SERDs have been valuable innovations in therapies. The goal for treatment is to create a “no-estrogen environment”. However, SERMs that maintain the beneficial effects of estrogen but antagonize the harmful effects of estrogen have great potential in the prevention of multiple diseases in common. It is clear that many new SERMs are being developed that could provide better choices for patients in the future.

To overcome the unwanted side effects and problems with drug resistance, combination therapy might be another important direction in addition to the development of new SERMs. For example, combination of SERM acolbifene and DHEA could be protective against breast cancer and osteoporosis with beneficial effects to stimulate vaginal maturation and decrease skin dryness [182]. As traditional HRT is less acceptable to regulatory authorities because of the increased risk of breast cancer, a combination of HRT and a SERM may be a reasonable idea to relieve unpleasant menopausal effects while decrease breast cancer risks. With regards to avoiding drug resistance, combining a SERM and an inhibitor targeting significant survival signal transduction pathway is under active evaluation. By way of example, a combination of tamoxifen and inhibitors of the HER2 signal transduction pathway may prevent acquired tamoxifen resistance [223]. Similarly, SERM pipendoxifene and mTOR inhibitor temsirolimus synergistically inhibits the proliferation of MCF7 breast cancer cells and xenograft at suboptimal concentrations [168]. Additionally, combinations of a SERM (arzoxifene or acolbifene) and a rexinoid LG100268 are effective to prevent and treat ER-negative mammary tumors in animal models [155]. The potential combination seems endless but the marriage of molecular biology and medicine holds great promise for advances in targeted therapeutics based on the SERM model.

In summary, it is clear that the original idea of targeting specific hormone receptor with selective medicine has proven its worth by advancing medicine with the SERMs tamoxifen and raloxifene. Now there are a whole range of new SERMs poised for clinical applications. But this is not the end of the story. Novel selective modulators of all members of the nuclear receptor superfamily are under investigation addressing the treatment or prevention of diseases never before considered possible [57, 222].

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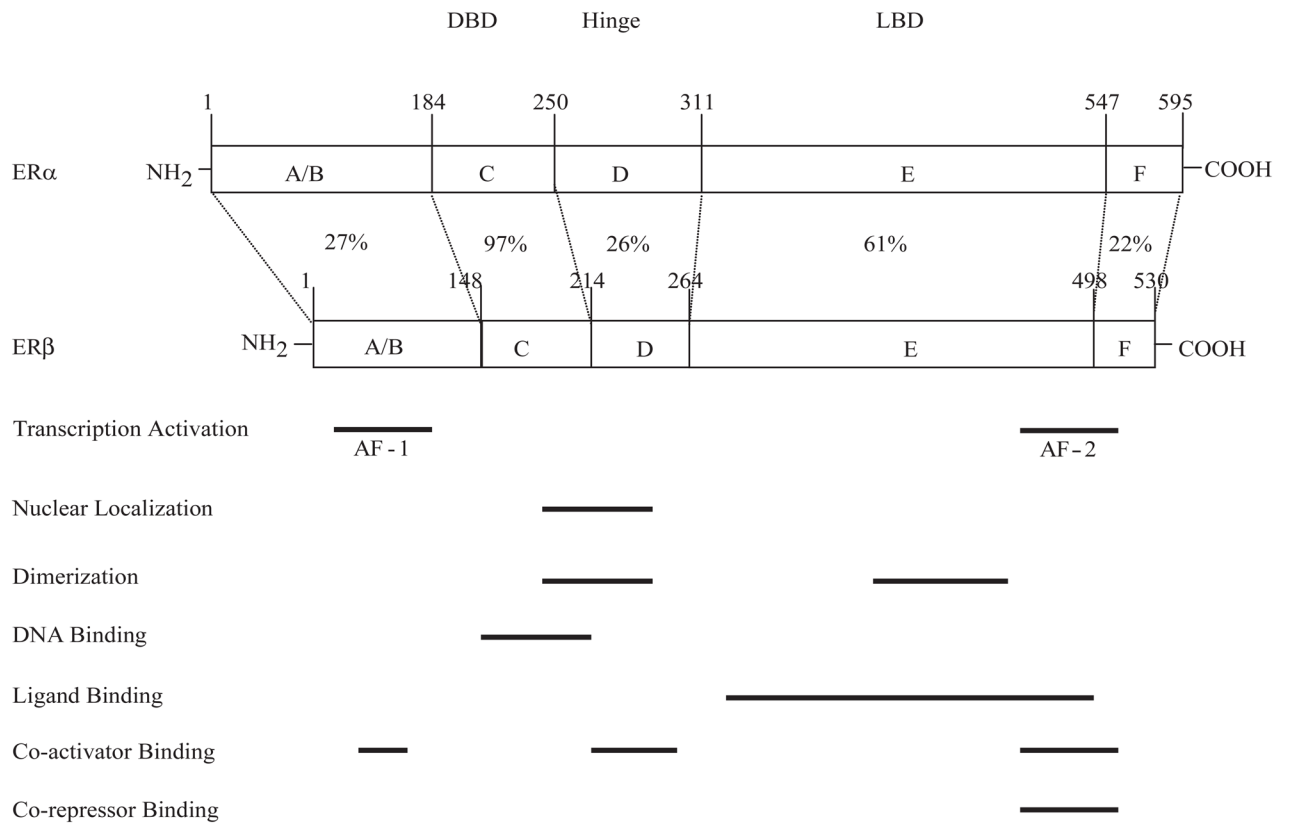
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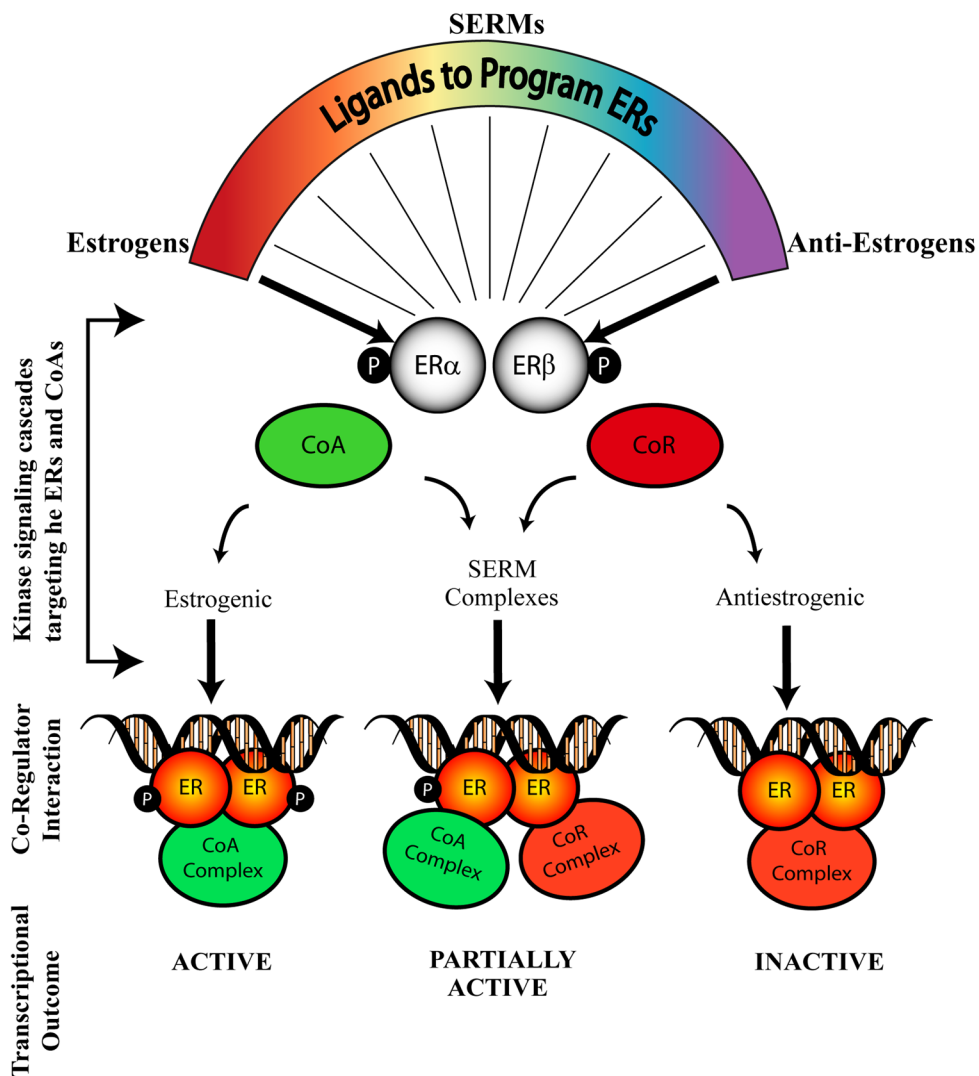
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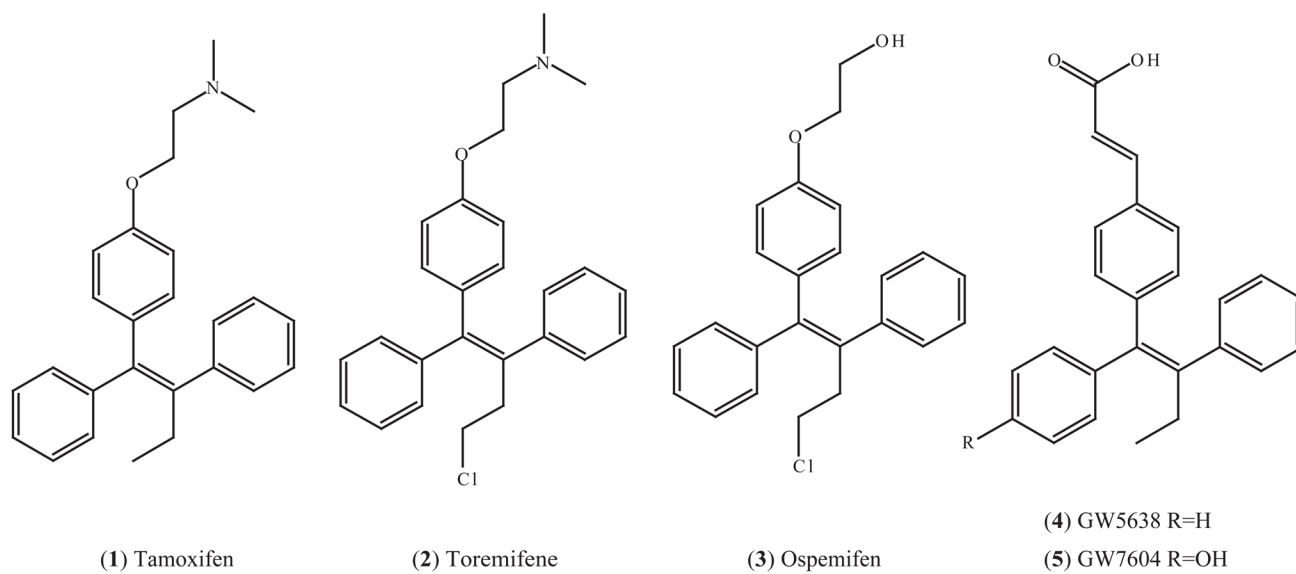


**Fig. (1).** Schematic comparison of human ER- and ER- structure. The structural domains are shown, and the percentage of amino acid identity shared by the two ERs is indicated for each domain. The horizontal bars highlight areas of different functions.

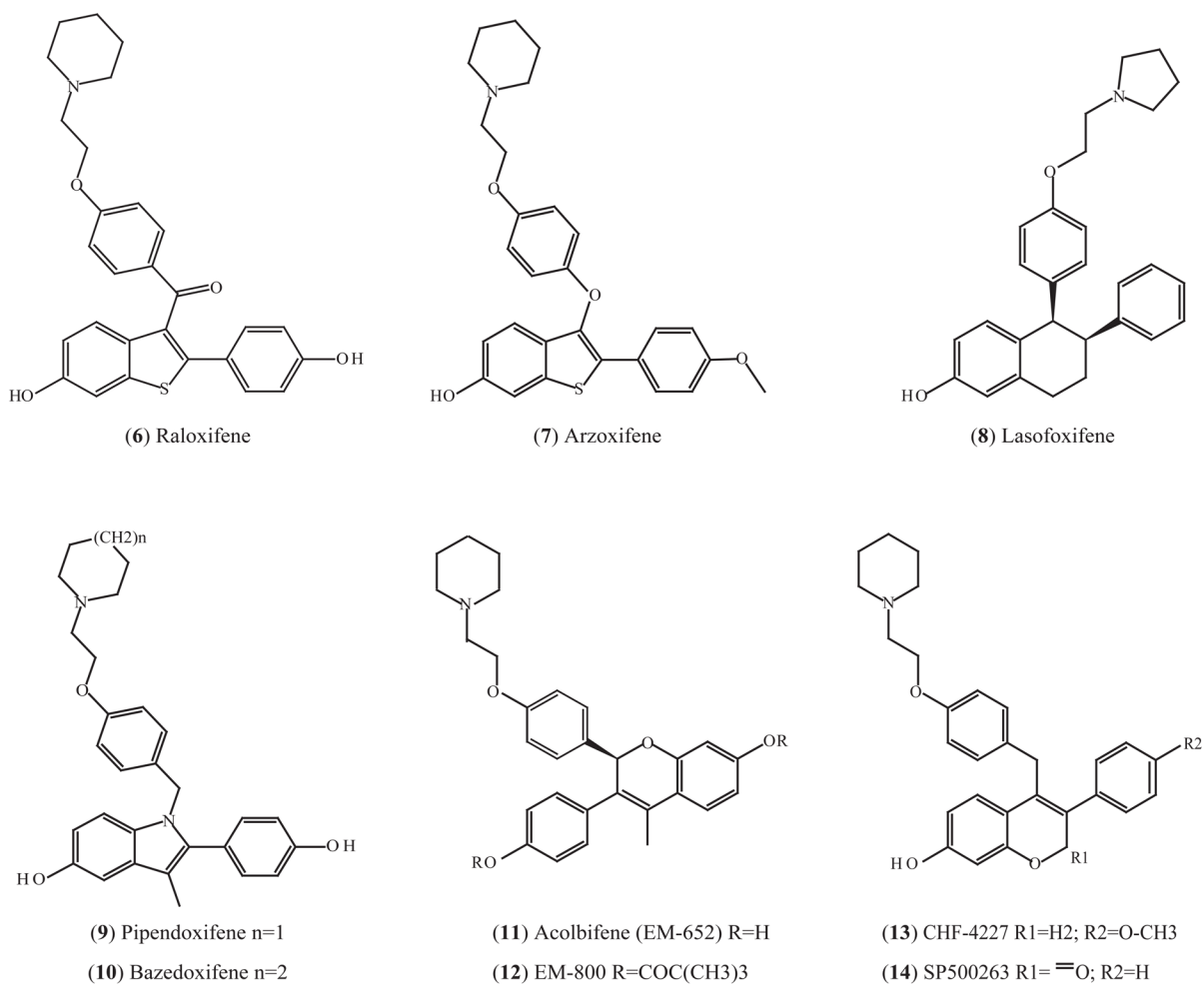


**Fig. (2).** Schematic representation of different liganded-ER complexes interacting with co-regulators and consequent transcriptional activities. ERs that bind to estrogenic ligands interact with co-activators (CoA) and activate transcription. Anti-estrogen liganded-ER complexes interact with co-repressors (CoR) and inactivate transcription of responsive genes. Selective estrogen receptor modulators (SERMs) bind to ERs and interact with either co-activator or co-repressor complexes eliciting partial transcriptional activity depending upon the cellular context.

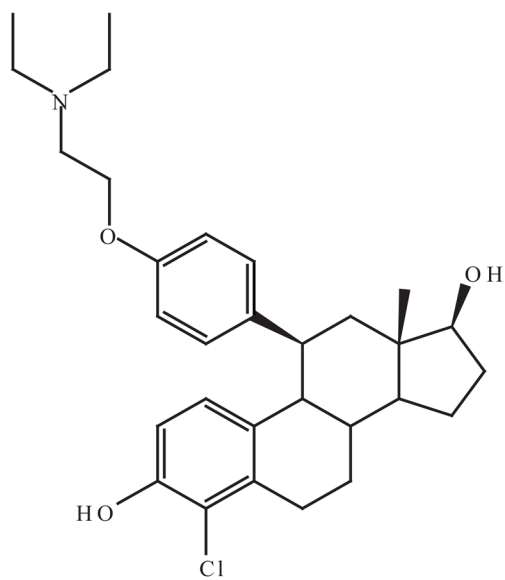




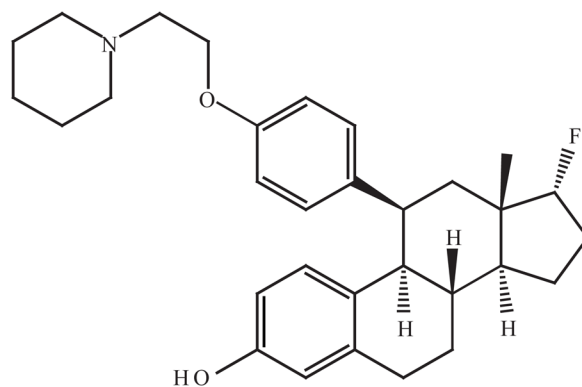
**Fig. (3).** SERMs with a structure mimicking tamoxifen containing a triphenylethylene core.



**Fig. (4).**  
SERMs with a structure mimicking raloxifene.

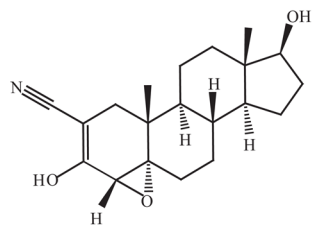


(15) HMR3339

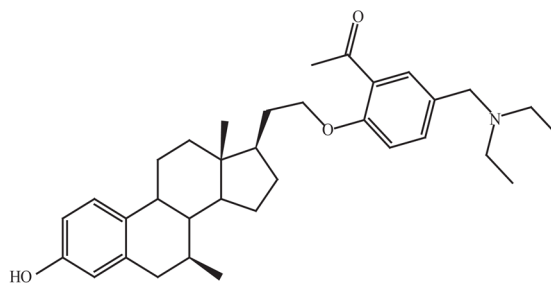


(16) PSK3471

**Fig. (5).**  
Steroidal SERMs



(17) Trilostane



(18) TAS-108 (SR16234)

**Fig. (6).**  
Structure of ER  $\alpha$ -selective agonists.

Table

## Current status of new SERMs

Drug Name	Category (Structure)	Effects	Preclinical Results	Clinical Status
Toremifene	Tamoxifen-like	Breast cancer treatment  Heart protection  Mastalgia treatment  Prostate cancer prevention  Relieve side effects of androgen deprivation therapy	Fewer genotoxic effects than tamoxifen [113], bone effects similar to tamoxifen [119]	FDA approved for metastatic breast cancer  Phase II trial (65 women) better than tamoxifen regulating lipid metabolism [121, 122]  Phase II trials (62 and 195 women) effective [126, 127]  Phase II trial (514 men) decreases prostate cancer incidence [128]  Phase III trial (1,389 men) improves lipid profiles [130]  Phase III trial (1,392 men) increases bone mineral density [129]
Ospemifene	Tamoxifen-like	Vaginal atrophy treatment  Osteoporosis treatment  Breast cancer prevention	Estrogenic effects on vaginal epithelium that is not observed with tamoxifen or raloxifene [134–136]  Inhibits tumor growth in animal models as effective as tamoxifen [140, 141]	Phase III trial (826 women) relieves vaginal dryness  Phase II trial (118 women): Comparable to or slightly better than raloxifene [139]  Phase III trial planned (detail not available)  Not available
GW5638 (DPC974) & GW7604	Tamoxifen-like	Breast cancer treatment (2 <sup>nd</sup> line therapy)	Works as a SERM and as a SERD [148], effective in tamoxifen-resistant tumors [144, 145]; functions as an ER agonist in bone and cardiovascular system but an antagonist in breast	Phase I trial (9 patients who failed first-line hormone therapy) low toxicity [ASCO]

Drug Name	Category (Structure)	Effects	Preclinical Results	Clinical Status
			and endometrium [142]	meeting 2002, abstract 452]
Arzoxifene (LY353381)	Raloxifene-like	Breast cancer treatment  Breast cancer prevention	Antiestrogenic in breast and endometrium, estrogenic in bone and lipids [150]  Effective to prevent ER-positive and ER-negative mammary tumors especially in combination with LG100268 [140, 155]	Phase III trial (200 patients) inferior to tamoxifen [154]  Phase I trials (50 and 76 women) low toxicity and favorable biomarker profile [156]
Lasofloxifene (CP-336156, Fablyn)	Raloxifene-like	Osteoporosis treatment and prevention  Vaginal atrophy treatment  Breast cancer treatment and prevention Heart disease prevention	Higher potency than tamoxifen and raloxifene [158]; higher oral bioavailability than raloxifene [160]    Effects similar to tamoxifen to prevent and treat NMU-induced mammary tumor in rats [163]	Phase III trial (1,907 women) significantly increases bone mineral density compared to placebo, no endometrial effects, no association with thromboembolic disorder [159]  Phase III trial to compare with raloxifene (CORAL trial, details not available)  Phase III trial (445 patients) improves vaginal atrophy compared to placebo  Phase III trial (PEARL trial with 8,556 women), reduces ER-positive breast cancer incidence compared to placebo; slightly decreases major coronary disease risk; reduces vertebral and non-vertebral fractures; increases risks of venous thromboembolic events but not stroke; no endometrial effects [SABCS 2008, abstract 11]

Drug Name	Category (Structure)	Effects	Preclinical Results	Clinical Status
Pipendoxifene (ERA-923)	Raloxifene-like	Breast cancer treatment	Inhibits tamoxifen-sensitive and -resistant tumors in mice and rats no uterotrophic activities compared to raloxifene [167]	Phase II trial to treat tamoxifen-refractory breast cancer in postmenopausal women (details not available)
Bazedoxifene (TSE-424 WAY-140424)	Raloxifene-like	Osteoporosis treatment and prevention  Breast cancer prevention	Increases bone density with little uterine or vasomotor effects  Inhibits estrogen-stimulated breast cancer cells growth [169]	Phase III trial (7,492 women) reduces vertebral and non-vertebral fracture incidences, while raloxifene is not effective against non-vertebral fracture [171]  Phase III trial (497 women) reduces endometrial thickness, unique property among known SERMs [170]  Not available
Acolbifene (EM-652, SCH57068) & EM-800 (SCH57050)	Raloxifene-like	Breast cancer treatment (2 <sup>nd</sup> line therapy)  Breast cancer treatment (1st line therapy)  Breast cancer prevention	Highest affinity for ER, inhibit growth of multiple breast cancer cells <i>in vitro</i> and <i>in vivo</i> [180]	Phase III trial, less effective than anastrozole to treat tamoxifen-resistance breast cancer, study halted [182]  Phase III trial planned [182]  Phase II trial (started in February, 2009) for premenopausal women
CHF4227	Raloxifene-like	Breast cancer and osteoporosis prevention	Prevents DMBA-induced mammary tumors and preserves bone mass in rats [184, 185];	Phase I trials (24 and 56 women) beneficial on bone markers and lipid metabolism; no effects on endometrium; not causing hot flashes
SP500263	Raloxifene-like	Breast cancer and osteoporosis treatment	Inhibits breast cancer cell growth <i>in vitro</i> and <i>in vivo</i> without stimulating uterine weight gain	Not available





Drug Name	Category (Structure)	Effects	Preclinical Results	Clinical Status
				tolerated [SABCS, 2008, abstract 2131 and 2132]