OSTEOPOROSIS THERAPY: MECHANISTIC ANTIRESORPTIVES

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1. INTRODUCTION

Bone is a living, dynamic tissue that is continuously remodeled during the adult life of an individual. The remodeling process occurs in quantum units called bone-remodeling units [1,2] through the action of osteoclasts and osteoblasts. Osteoclasts are the bone-resorbing cells, which tightly adhere to the bone surface and then secrete acid that dissolves the hydroxyapatite mineral and proteolytic enzymes that degrade the organic matrix of bone. Osteoblasts are the bone-forming cells that synthesize a highly cross-linked, lamellar organic matrix (osteoid) that becomes mineralized by extracellular processes. Osteoblasts usually replenish the bone excavated by osteoclasts. Osteoporosis is a disease of the bone that leads to increased risk of fracture as a consequence of an imbalance between osteoclastic and osteoblastic activities, coupled with an increased rate of bone turnover observed. That is, a net loss of bone mass or inadequate architecture results due to either the excessive bone-resorbing activity of osteoclasts or the impaired bone-forming activity of osteoblasts, such that osteoblasts do not optimally replenish the lost bone. For women, this phenomenon is related to the decline of endogenous levels of the steroid hormone estrogen after menopause. Because the rate of remodeling is approximately 10 times higher in cancellous bone than cortical bone, bone loss following menopause is observed primarily in regions enriched for trabecular bone such as the vertebra and proximal femur. Gradually, perforations in or thinning of the trabecular bone spicules develop with the result that a weakened and inadequate architecture ensues.

Osteoporosis is currently defined by the World Health Organization as a condition observed for patients with spinal bone mineral density (BMD) of less than 2.5 standard deviations below the mean of young, normal adults of the same gender [3,4]. Osteoporosis is an ailment of increasing concern among elderly women and men in which bone has been lost to the extent that too little remains to support the mechanical usage requirements of the individual's activities. As a result, these individuals are at risk for spontaneous, atraumatic (or mild trauma) fractures. The inverse relationship between densitometric measures of bone mass and fracture risk was clearly shown for peri- and postmenopausal women in the process of losing bone due to declining levels of circulating estrogens [5–7].

Postmenopausal or type I osteoporosis is observed with escalating frequency in women elder than 50 years of age such that elderly women have a lifetime risk of fractures of approximately 75% [8,9]. At any given age, the risk of osteoporotic fracture is approximately two times greater in women than in men and in white people of Northern European ancestry than in Africans or Asians [10]. Women are at greater risk because of the lower peak bone density achieved in adulthood and greater susceptibility to rapid bone loss associated with menopause. Women also have a greater tendency than men to survive well into the age of vulnerability [11–13]. Therefore, for these reasons much of the past research activity in the field has been focused on postmenopausal osteoporosis.

The most serious consequences to the patient appear to result from hip fractures. Hip fractures account for the major proportion of the measured economic impact of osteoporosis because of the necessity of hospitalization [12,13]. Additionally, mortality within 4 months of hip fracture is currently 20%, with the majority of the survivors facing lifelong impairment. Risk assessment analyses have clearly shown that the risk of hip fractures increases exponentially with age and is currently 40% for white women aged 50 years or more in the United States [8]. As life expectancy continues to increase in most regions worldwide, the total of 323 million individuals aged 65 years or older in 1990 is expected to exceed 1.5 billion by the year 2050. Worldwide, the number of hip fractures may increase from 1.7 million in 1990 to 6.3 million

Burger's Medicinal Chemistry, Drug Discovery, and Development, Seventh Edition,
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by 2025 [14,15]. Assuming a 5% annual inflation rate, costs for hip fractures in the United States alone are projected to increase from an excess of $10 billion in 1990 to $240 billion by 2040 [16,17]. These may be conservative estimates because while most vertebral fractures do not lead to hospitalization, human costs were recently shown to be significant in terms of lost days due to back pain (2 days of bed rest, 10 days of limited activity).

As a consequence, a number of therapeutic strategies have been successfully pursued in an effort to satisfy this unmet medical need. Supportive clinical data with molecules with varying modes of actions such as the bisphosphonates, selective estrogen receptor modulators, and parathyroid hormone analogs suggest that very different pharmacological approaches can be utilized to prevent further bone loss in postmenopausal women. This review will focus on those therapies that act by inhibiting bone resorption. Subsequent chapters address therapies that result in bone formation.

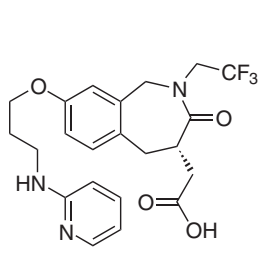
1.1. Calcitonin and Integrin Antagonists

Salmon calcitonin is among the most potent inhibitors of the bone-resorbing activity of osteoclasts *in vitro* [18–20] and is available as intramuscular injection and as nasal spray formulations to treat postmenopausal osteoporosis. While calcitonin has been shown to inhibit osteoclastic activity at low concentrations *in vitro*, calcitonin signaling is desensitized with continued exposure through the downregulation of calcitonin receptors [21–23]. This may help explain the somewhat limited clinical efficacy observed of 1–1.5% vertebral BMD increase over 3 years for treated patients. Nevertheless, despite this limited BMD efficacy observed for calcitonins and the poor bioavailability observed for nasal calcitonin [24], both formulations were shown to decrease significantly the incidence of vertebral fractures in osteoporotic women [25–28]. Calcitonin also has analgesic effects that appear to help alleviate bone pain in osteoporotic women, which may help explain calcitonin's popularity in some regions of Europe and Japan.

An alternative therapeutic strategy to inhibit osteoclastic bone resorption has been to target the integrin mediated attachment of osteoclasts to the bone surface [29]. The Arg-Gly-Asp (RGD)-containing snake venom protein, echistatin, was shown to be a potent inhibitor of the $\alpha_v\beta_3$ integrin mediated resorbing activity of osteoclasts *in vitro* [30,31] and *in vivo* [32,33]. While echistatin itself is not likely to be therapeutically useful [34], RGD peptides and integrin antagonists have been shown to prevent bone loss in ovariectomized animals [35,36]. More recently, $\alpha_v\beta_3$ antagonist with improved drug-like properties have been described that **1** and **2** in Fig. 1. Both demonstrated potent antagonist activity *in vitro*. Compound **2** has good oral bioavailability in rats, dogs, and monkeys and has demonstrated bone-related efficacy in rats and monkeys after oral administration [37].

1.2. Cathepsin K Inhibitors

Cathepsin K is a lysosomal cysteine protease that is highly expressed in osteoclasts [38–40]. Cathepsin K has been mapped to chromosome 1q21, and functional mutations to this gene



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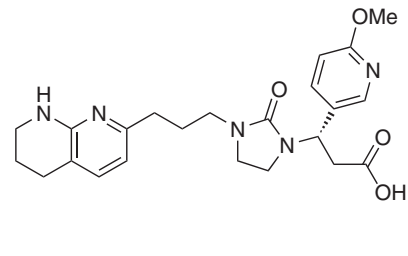

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Figure 1. Integrin antagonists.

occur naturally, resulting in pycnodysostosis, a rare skeletal dysplasia that is characterized by dwarfism, low rate of bone turnover, and osteosclerosis [41]. Chemical tools represented by peptide aldehyde inhibitors of this enzyme have been shown to inhibit resorbing activity of osteoclasts *in vitro* with IC_{50} of 20–100 nM and in rats [42]. Emerging evidence that cathepsin K is the primary enzyme involved in osteoclastic bone resorption has made it an important target for the treatment of osteoporosis [43]. Several studies have shown that cathepsin K deficiency leads to an increase in BMD [44]. Pharmacological studies of cathepsin K inhibitors in rats [45] and monkeys [46] have shown reductions in biochemical markers of bone resorption and increased BMD. Recently, clinical data have been disclosed for the cathepsin K inhibitor balicatib demonstrating a reduction of biochemical markers of bone resorption and increases in BMD over 1 year of treatment [47] In addition, a 3-week study of MK-0822 showed a 70–80% reduction in serum CTx and an 80% reduction in urinary NT.

Cathepsin inhibitors can be classified by structural class based on the electrophilic nature of subunit, or warhead, that interacts at the active site of the enzyme. Covalent inhibitors can be categorized into cyano or ketone-based molecules. There are also noncovalent inhibitors which are based on an aminoaniline structural subunit. Representative ketone inhibitors include those shown in Fig. 2 and include cyclohexanones **3** [48], azapanones **4** [49], dihydrofuranones **5** [50], and sulfonamidoketones **6** [51], to name a few. This class of inhibitors is generally characterized by electron withdrawing substituents such as alpha-heteroatom or carbonyl functionalities. Nitrile based inhibitors include dipeptide **7** [52] and aromatic nitriles [53]. Noncovalent competi-

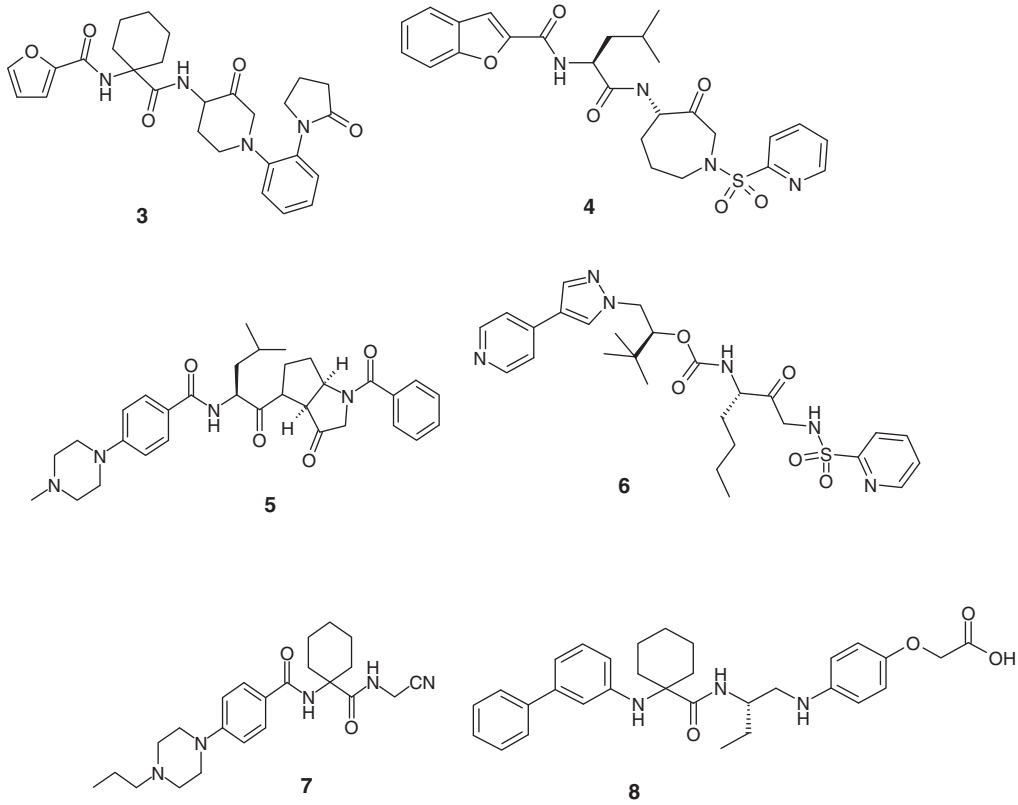

Figure 2. Cathepskin K inhibitors.

tive inhibitors include aminoethylaniline derivatives such as **8** [54] that achieve efficacy through lipophilic P1′ interactions.

1.3. OPG/RANKL/RANK Inhibitors

Osteoprotegerin (OPG) and receptor activator of nuclear factor-κB ligand (RANKL) are dominant regulators of bone resorption. Many hormones, cytokines, and growth factors mediate bone resorption by altering the ratio of RANKL to OPG. RANKL and OPG expression is also altered in numerous bone diseases, and these changes can reflect disease etiology or compensatory responses to disease. RANKL stimulates osteoclast formation, function and survival, and each of these effects is inhibited by OPG. OPG suppresses bone resorption and increases the density, area, and strength of both cancellous and cortical bones.

The discoveries of OPG and RANKL were significant breakthroughs that have expanded the understanding of bone remodeling. A paradox in bone biology was that most of the hormones, cytokines, and growth factors that regulated osteoclast activity had receptors on osteoblasts rather than osteoclasts. As a result, an unidentified osteoblast-derived protein factor was invoked to explain the response to proresorptive stimuli [55]. This factor was shown to be RANKL, a tumor necrosis factor (TNF) family member that is essential for osteoclast formation, function, and survival [56]. OPG is the counter regulatory partner to RANKL [57]. OPG is a soluble decoy receptor from the TNF receptor family with a mechanism of action that does not involve direct signaling activity. OPG binds to RANKL and prevents RANKL from binding and activating receptor activator of nuclear factor-κB (RANK). RANK is another member of the TNF receptor family that is present on osteoclasts and osteoclast precursors [58]. This triad of proteins—OPG/RANKL/RANK—has been shown in genetic and pharmacology studies to play a critical role in the regulation of osteoclasts and bone resorption. Thus, RANKL inhibitors provide therapeutic potential for the treatment of bone loss conditions such as postmenopausal osteoporosis. OPG and other RANKL inhibitors act systemically to inhibit RANKL at all skeletal sites, independent of local bone turnover rates or access to remodeling surfaces. OPG has been valuable in the understanding of the bone remodeling process as it rapidly reduces osteoclast numbers while having no direct effect on osteoblasts.

Pharmacologic intervention with recombinant OPG or RANKL causes skeletal changes that are consistent with the phenotypes described in mice lacking or overexpressing OPG or RANKL. Recombinant RANKL is a valuable tool for evaluating bone remodeling events in animals. Soluble RANKL induces bone resorption within 60 min of injection in mice [59]. Overexpression of soluble RANKL in transgenic mice results in a skeletal phenotype with many similarities to postmenopausal osteoporosis, including reduced BMD, increased bone resorption, cortical porosity, and skeletal fragility [60]. Each of these skeletal changes is also exhibited in OPG knockout mice [61–63]. Biomechanical strength of the femoral diaphysis is reduced by the same degree (50%) in mice that overexpress soluble RANKL [60] and in mice that lack OPG [62].

Preclinical studies have highlighted the skeletal benefits of RANKL inhibitors in diverse disease models including bone metastasis [64], rheumatoid arthritis [65], ovariectomy [66], and inflammatory bowel disease [67]. OPG also increases bone strength, a phenomenon that has been illustrated most frequently in preclinical models of disuse osteopenia. The focus on RANKL inhibitors in these models might be related to the extremely rapid bone loss associated with skeletal unloading, particularly at cortical sites [68]. In contrast, OPG significantly increased the density and strength of cortical bone [69] in a rat unloading model that was nearly identical in design to one in which bisphosphonate treatment had no such effects [70]. OPG improved the density and strength of the femoral neck in immobilized [71] and nonimmobilized rats [72]. OPG also prevented bone loss and improved cortical bone strength in mouse models of skeletal unloading [73], even under the extreme conditions of microgravity [74].

A fully human monocolonal antibody (mAb) has been made against human RANKL. This mAb, known generically as denosumab, has been tested in postmenopausal women and in

men and women undergoing sex hormone ablation therapy for cancer. The antifracture efficacy of denosumab has been shown to reduce fracture in men and women using subcutaneous dosing every 6 months.

In summary, OPG and RANKL are important physiologic, pathologic, and pharmacologic regulators of bone resorption. Inhibition of RANKL consistently suppresses osteoclast numbers and activity, resulting in increases in bone mass, density, volume, and strength. The ability of OPG to increase bone strength in preclinical models suggests that RANKL inhibition via denosumab, a fully human mAb, might reduce fracture incidence and prevent bone loss in a variety of disease states.

1.4. Bisphosponates

Bisphosphonates are synthetic P–C–P compounds pioneered by H. Fleisch that have been shown to be highly potent inhibitors of osteoclastic resorption activity [75,76]. In particular, the aminobisphosphonates such as pamidronate, alendronate, incadronate, ibandronate, neridronate, the cyclic bisphosphonates tiludronate, and risedronate have been shown to be highly efficacious in preventing bone loss due to estrogen deficiency *in vivo* [75,77–79]. Clinical studies with the first-generation bisphosphonate, etidronate, showed beneficial effects on spinal BMD [80,81] and etidronate was shown previously to impair mineralization, resulting in osteomalacia at clinically relevant doses in pagetic and osteoporotic patients [82–85].

Animal and clinical data have been generated with the third-generation bisphosphonate, alendronate. Specifically, double-blind clinical studies in postmenopausal women showed that 10 mg of alendronate improves DXA BMD for vertebra by 9% and femoral neck by 6% compared to placebo controls, after 3 years of treatment [86,87]. More importantly, fracture incidence was reduced by 50% for the spine, hip, and distal radius, with even greater reductions of up to 90% observed for osteoporotic women with multiple spinal fractures [88]. Additionally, DXA BMD analyses of 1174 women younger than 60 years of age showed a 3.5% increase in the spine and 1.9% increase in the hip after 2 years of treatment with 5 mg of alendronate, indicating that alendronate prevents bone loss to nearly the same extent as HRT in younger postmenopausal women [89]. As a result of these impressive clinical data, alendronate is an attractive therapy for osteoporotic women.

Alendronate appears to be remarkably effective in retarding osteoclastic resorption of bone [90,91]. Pharmacokinetic and autoradiography studies have shown that alendronate is not metabolized and is rapidly cleared from the circulation through the kidneys with a half-life of 1–2 h and that approximately half of the compound localizes directly to bone, especially cancellous bone [90–96]. The probable antiresorptive mechanism is based on the observation that only osteoclasts show cytoplasmic labeling with alendronate; that is, only osteoclasts can secrete sufficient acid to dissociate the alendronate/bone complex [90]. However, as alendronate is concentrated beneath (or within) osteoclasts through multiple rounds of dissociation and reassociation of alendronate to bone, formation of the ruffled border is eventually inhibited, and therefore so is resorption activity [90,97]. Additionally, alendronate has also been shown to retard osteoclast differentiation by inhibition of tyrosine phosphatase activity [98,99], it may induce osteoclast apoptosis [100], and at high concentrations *in vitro*, alendronate may also have osteoblast-mediated inhibitory effects on osteoclasts [101,102]. It has also been shown that bisphosphonates can inhibit resorption through inhibition of farnesyl pyrophosphate synthesis [103].

Analyses of iliac crest biopsies from 231 osteoporotic women treated with alendronate showed a significant increase in wall thickness and reduced erosion depth with no effect on mineral apposition rate after 2–3 years of treatment, confirming that mineralization is normal with no osteomalacia [104]. In addition, newly formed bone was lamellar with no evidence of marrow fibrosis or cellular toxicity [104]. These findings partially explain the dramatic effects of alendronate on DXA BMD as a reduction in the remodeling space. That is, osteoblasts appear to continue through the slower formation/mineralization processes for months, even after osteoclasts have been inhibited to stop resorbing with alendronate

treatment. However, histomorphometry also showed an 81–95% reduction in osteoid volume (OV/BV), osteoblast surface (OS/BS), mineralized surface (MS/BS), bone formation rate (BFR/BS), and activation frequency (A.cf) for the 10 mg dose after 2–3 years [104]. These data indicate substantial reduction of bone turnover (both resorption and formation activities), with similar reduction of bone turnover observed in long-term animal studies [105–107].

Part of the explanation for alendronate effects on bone remodeling may be attributed to the extraordinarily long half-life of 10 years or more *in vivo* for alendronate in bone [77–79,92,95]. This means that the remodeling of bone labeled with alendronate will be inhibited for a long time, possibly leading to increased fragility and accumulation of microdamage [108]. Other side effects observed for daily alendronate (**9**, Fig. 3) include erosive esophagitis that is associated with the oral formulation. Previously, oral bioavailability on the order of 1% or less and irritation of the upper gastrointestinal tract has been described for several bisphosphonates [92,93,109–111]. To address the latter issue, bisphosphonates such as alendondrante (**9**) and risedronate (**10**) have been shown to be effective following once-weekly dosing thereby establishing these less frequent dosing as the standard for this class of drugs. Newer bisphosphonates shown in Fig. 3 such as ibandronate (**11**), minodronate (**12**), and zolendronate (**13**) are currently under clinical investigation [112,113].

1.5. Selective Estrogen Recpetor Modulators (SERMs)

With the first preclinical and clinical descriptions of the unique profile of raloxifene in estrogen deficient animals and postmenopausal women [114,115] the concept of selective modulation of the estrogen receptor (ER) was born which shifted thought around use of ER-based ligands in postmenopausal women and opened the door for use in chronic diseases such as osteoporosis. Accordingly, the initial goals of a SERM-based therapy for osteoporosis required the molecule to have estrogen-like efficacy on bone and concomitant fracture reduction without estrogen-like stimulatory effects on uterus or mammary tissue. As of the writing of this chapter, only four molecules with SERM-like profiles have achieved clinical use (Table 1) and only one, raloxifene, has attained approval for use in the treatment and prevention of osteoporosis. However, other molecules have been evaluated clinically, or are currently under clinical evaluation, for postmenopausal osteoporosis and will be reviewed here as well. The various classes of

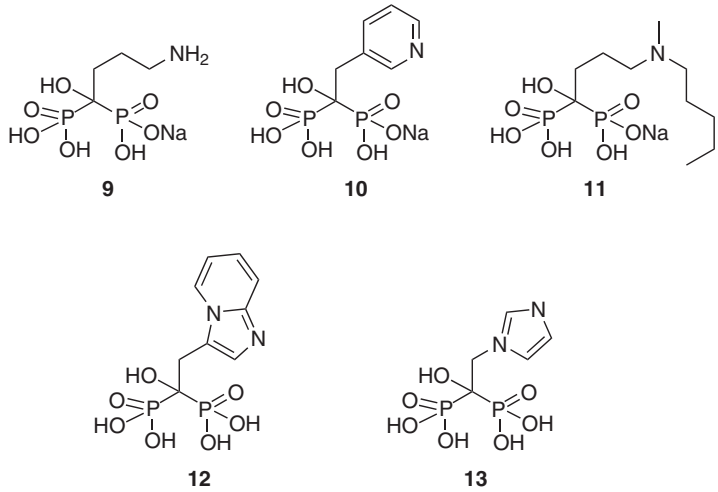

Figure 3. Bisphosphonates.

Table 1. SERMs Currently Approved for Human Use

SERM	Trade Name	Approved Indications	Daily Dose (mg)
Clomiphene	Clomid®	Induction of ovulation.	50–100
Raloxifene	Evista®	Treatment and prevention of osteoporosis in postmenopausal women with osteoporosis. Reduction in risk of invasive breast cancer in postmenopausal women with osteoporosis. Reduction of invasive breast cancer in postmenopausal women at high risk for invasive breast cancer.	60
Tamoxifen	Nolvadex®	Metastatic breast cancer treatment. Adjuvant breast cancer treatment. Ductal carcinoma *in situ*. Breast cancer risk reduction in high-risk women.	20–40
Toremifene[a]	Fareston®	Metastatic breast cancer treatment.	60

[a]Toremifene (Fareston®) is currently not approved in the United States, but is approved for metastatic breast cancer treatment.

SERMs are shown in Fig. 4 along with the corresponding structure.

The effects of SERMs on biologic systems are predominately mediated by specific, high-affinity, interactions with ER's that are primarily located in target cell nuclei [116]. Certainly non-ER mediated effects, such as antioxidant properties [119] and nonnuclear ER-mediated effects, such as nitrous oxide production by cardiovascular endothelial cells [120], have been described and may be important contributory factors to the overall pharmacology of SERMs. However, most attention has focused on the "nuclear hormone receptor" aspects of SERM mechanism. This nuclear hormonal action involves the complex interplay of a number of protein and genomic elements that allow SERMs to regulate gene transcription and subsequent protein production by the cell. Recent advances in understanding of the molecular biology of SERM action illuminate three key elements that distinguish estrogen and SERM effects. These three elements include: (1) high-affinity interaction with the ER, (2) ER-ligand dimerization and the association with a tissue-specific set of coregulatory proteins, and (3) binding of the ER/adaptor protein complex to specific DNA response elements located in the promoter regions of nuclear target genes and ensuing regulation of gene transcription. Depending upon the cellular and promoter context, the DNA-bound receptor can induce or inhibit the transcription of specific genes within the tissue.

The ability to specifically bind to the ER is perhaps the single most important feature of all molecules with a SERM profile. In the absence of ligand, the ER exists in a large protein complex, comprised of the receptor bound to heat shock proteins [116]. Binding of a ligand to the ER induces a conformational change that results in dissociation of the heat shock chaperone proteins from the ER One of the most important determinants of the ultimate pharmacological response is the shape of this ligand–ER complex, which is unique with each individual ligand [117,118]. The ligand-binding domain (LBD) of the ER consists of a hydrophobic core made up of parts of five distinct helices (helix-3, -6, -8, -11, and -12). When the LBD of ERα is bound to estrogen, helix 12 adopts an orientation that lies over the binding pocket of the receptor and allows for interaction of cellular proteins with the coactivator recognition groove. In contrast, when the 4-hydroxy metabolite of tamoxifen (likely the active metabolite of tamoxifen at ERα [121] is bound to the ERα, helix 12 adopts a distinct alignment from that of the estrogen bound receptor that occludes interactions with the coactivator recognition groove [122]. Raloxifene, when bound to the LBD of ERα protrudes from the ligand-binding cavity and physically prevents the alignment of helix-12 over the binding cavity, thus shifting helix-12 away from the pocket it normally occupies when 17β-estradiol is bound [119]. Thus, the conformation or shape of the ligand-ER complex provides an important structural

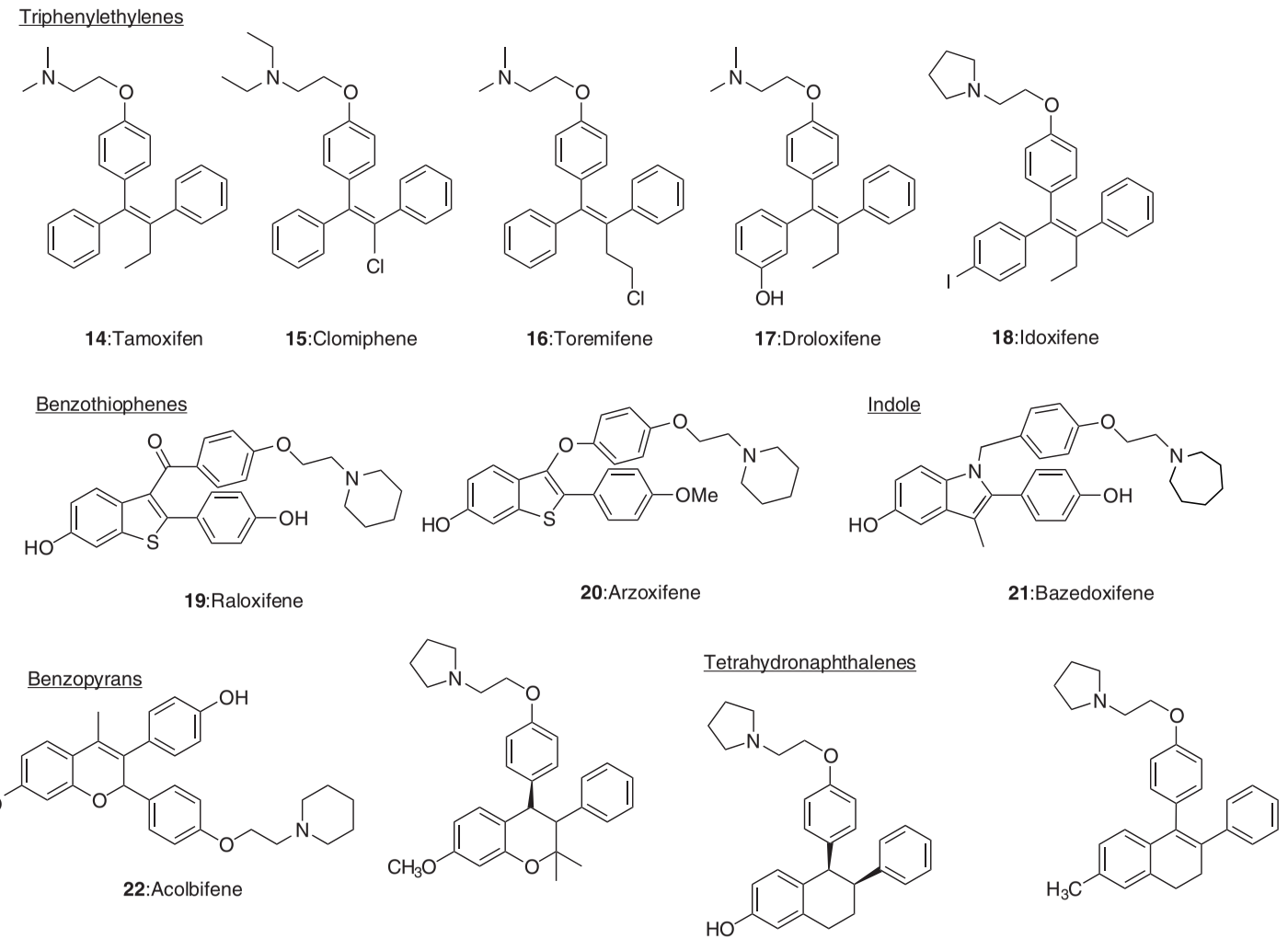

Figure 4. SERMs.

basis of SERM activity via determination of which particular subsequent protein-protein interactions are permitted. This is also a primary basis for the wide array of different pharmacological profiles produced by different SERMs, as the confirmation of the ER-SERM complex is distinct for each molecule [120]. It is important to recognize that a second form of the ER is known to exist, ERβ [123], which may also form heterodimers with ERα [124]. ERα and ERβ display unique patterns of tissue distribution typically with expression levels of one subtype dominating [125], although it should be noted that most tissues contain at least small amounts of both subtypes, and with the role of putative α:β heterodimers unknown, it is possible that low expression subtype, may be a key rate-limiting step in ultimate nuclear activity. ERα and ERβ are also each known to have multiple isoforms that are splice variants [126,127], with the potential of further differences in ligand bound three-dimensional structures adding an additional layer of complexity to ER-mediated activation or inhibition of estrogen response genes. However, to date, all of the SERMs that have reached advanced clinical evaluation show high affinity for both ERα and ERβ with sufficient circulating and tissue exposure to insure binding of both subtypes indicating that, for these molecules at least, differential ERα or ERβ activation does not explain the tissue selective pharmacological effects.

In addition to the ER's themselves, a number of other coregulatory proteins, such as coactivators (which enhance transcription) and corepressors (which reduce transcription) play an essential role in determining the ultimate response of an individual cell to liganded ER. The C-termini of both ERα and ERβ harbor the ligand-dependent AF-2 domain. Specific interactions between amino acid residues within the ER and a distinct ER recognition groove of coactivator proteins (identified by a signature LxxLL coactivator motif) are necessary for maximal ligand-dependent activation of estrogen target gene promoters [128]. Specific ER-associated coactivator proteins include various 160-kDa proteins, such as: SRC-1, TIF-2, AIB1, and ACTR [129–131], a 300-kDa protein (CBP) and an RNA coactivator (SRA-1 [132]). SRC-1 was the first steroid receptor coactivator to be cloned, and exhibits preferential interaction with ligand-bound ER, a hallmark feature of this family of coactivators. These coactivator/ligand/ER complexes serve three functions. First, they can act as bridging molecules for interactions with other members of the transcription machinery [133]. Second, they can help unravel target regulatory regions and increase accessibility to these areas of the chromatin covered with histones, such as via inherent histone acetyl transferase activity [134]. Finally, coactivator/ligand/ER complexes can mediate crosstalk between AF-1 and AF-2 within the receptor molecule, which enables the ER to achieve its complete activation potential [135]. Corepressors are the counterpart of coactivators, and possess a transrepressor function. Corepressors also contain a signature motif related to the LxxLL sequence found in coactivators. This motif, known as the corner box (L/IxxI/V-I), mediates the interaction between the ER and specific corepressor proteins such as N-CoR, SMRT, REA, and SHP [136,137].

The relative expression of the different cofactors and the ability of the ER-ligand complex to interact with those cofactors play a major role in the tissue selective agonist/antagonist profile of the various SERM molecules, as despite the presence of numerous cellular proteins with transcriptional coregulatory activities, there are numerous examples of tissue selective activities [138,139]. An additional point of significance is that coactivators such as ACTR and AIB1 are amplified in various breast and uterine tumors [139,140]. The important nature of the tissue-relevant cofactor context was best demonstrated by Shang [141], who compared the effects of two SERMs, tamoxifen and raloxifene, to estrogen in two tissue contexts: a breast cancer cell line and a uterine endometrial carcinoma cell line. In the mammary cells, which are induced to proliferate in the presence of estrogen, 17β-estradiol recruited coactivators leading to increased gene expression. In these same cells, where tamoxifen and raloxifene both display estrogen antagonist pharmacology, the ligand-SERM complex with both molecules recruited corepressors and not the coactivators observed with

17β-estradiol on ER-mediated transcription. However, in a uterine cell line where tamoxifen exhibits estrogen agonist pharmacology and raloxifene behaves as a complete antagonist, tamoxifen was associated with the recruitment of a coactivator protein complex that included SRC-1, AIB1, and CBP that resulted in histone acetylation. SRC-1 in particular may be an important coactivator in the uterine cell stimulatory response to tamoxifen, as this coactivator is expressed at higher levels in uterine cells. Of note, the coactivator requirements for estrogen stimulated gene expression in uterine cells were distinct from those for tamoxifen, indicating multiple signaling mechanisms even for the agonist response. Conversely, raloxifene failed to recruit a coactivator construct, rather inducing a corepressor construct associated with histone deacetylase activity in the uterine cell line [141]. Thus, the relative abundance of ER-associated coactivators and corepressors are an important factor in the tissue specific pharmacology of SERMs.

Crystal structures of various ligands bound to the ER indicate that small molecules can induce a spectrum of receptor conformations. As described above, the specific SERM-ER conformation has tremendous impact on cofactor recruitment and ultimate genomic activation or inhibition by the SERM. Chemical scaffolds that have produced SERMs in current clinical use, or at least that have reached phase 3 clinical evaluation in humans are depicted in Fig. 4 and include: triphenylethylenes (i.e., tamoxifen, droloxifene, idoxifene, clomiphene, toremifene), benzothiophenes (raloxifene, arzoxifene), tetrahydronaphthylenes (lasofoxifene, nafoxidine), indoles (bazedoxifene), and benzopyrans (acolbifene, levormeloxifene). Key structural features of these molecules are typical for the entire class with the most important features being: (1) the hydroxyl moieties and (2) the basic side chain.

The hydroxyl moieties on the "A" and "D" rings are required for the high affinity interaction with the ER [142] and align in the binding pocket of the ER in a manner that parallels the binding of the hydroxyl groups of 17β-estradiol, with the 3-hydroxyl on the "A" ring of 17β-estradiol being the most important [119]. As shown in Fig. 4, the location of the hydroxyl groups for 17β-estradiol and an energy-optimized orientation of raloxifene align very closely, allowing raloxifene to interact with the same peptide residues in the ER-binding pocket as those which bind estradiol. Note that those molecules lacking hydroxyl groups are likely hydroxylated *in vivo* as result of cytochrome P-450 metabolism, such as tamoxifen to 4-hydroxytamoxifen, which is the likely active metabolite of this SERM.

The basic side chain, on the other hand, appears to be very important for determining the SERM-ER conformation that ultimately determines the tissue selective pharmacology of the various SERMs. Specifically, the basic side chain of raloxifene [119,142] protrudes from the ER-binding pocket physically occupying the space helix 12 occupies when 17β-estradiol is bound to ER, thus forcing ER helix 12 to assume an orientation perpendicular to that which occurs with 17β-estradiol bound to the receptor. Thus, it is not only the chemical constituency of the basic side chain an important feature but also the orientation of the basic side chain in space. For example, analogs of raloxifene with an orthogonally constrained basic side chain show normal binding to the ER, the expected bone protective activity and lack of significant uterine stimulation, much as is observed with raloxifene in ovariectomized (OVX) rats [142]. This is in contrast to the orientation of the basic side chain in SERMs such as tamoxifen, which are more planar in nature to the stilbene core of the molecule. Of note, an analog of raloxifene with a forced planar orientation of the basic side chain that spatially overlaps with the location of tamoxifen's basic side chain, produced a profile in OVX rats very similar to that of tamoxifen: bone sparing, but uterine stimulatory [142].

Given the wide distribution of ER and the pleiotropic nature of estrogen and its multiple metabolites, SERMs may theoretically affect multiple organ systems. As the focus of this chapter is on skeletal pharmacology, emphasis here will be placed on the pharmacologic effects of SERMs on bone and on other tissues of relevance to safety in the clinical setting. Accordingly, emphasis will be place on those SERMs where osteoporosis and bone has been the primary focus of research. As raloxifene

represents the most extensively studied SERM in humans to date with clinical indications for prevention and treatment of postmenopausal osteoporosis as well as risk reduction of breast cancer in osteoporotic women and women at high breast cancer risk, and tamoxifen has been available as a breast cancer treatment adjunct and breast cancer preventative, the bulk of existing preclinical and clinical research with relevance to bone is available for these two SERMs and the bulk of this review of the SERM activity profile here will focus on these two molecules.

1.5.1. Preclinical Studies Much as in postmenopausal women, estrogen deficiency in OVX animals leads to a rapid increase in bone turnover, where excessive osteoclast resorptive activity results in a marked decline in trabecular bone mass and strength, with concomitant increase in fractures. In rats, ovariectomy produces a rapid osteopenic response, which can be discerned within 5 weeks. Most of the various SERMs discussed in this review have been evaluated in the OVX rat, and demonstrate estrogen-like protection from bone loss induced by estrogen deficiency. In the OVX rat model, SERMs such as raloxifene [114], arzoxifene [143], tamoxifen [144], droloxifene [145], idoxifene [146], clomiphene [147], bazedoxifene [148], lasofoxifene [149], levormeloxifene [150] toremifene [151] and acolbifene [152] all prevent the loss of bone in vertebrae, distal femur and proximal tibia, all trabecular-rich bone sites. In addition to maintaining bone mass, SERMs also preserve bone strength through improvements in bone microarchitecture [153]. For example, in OVX, mice administration of raloxifene not only improved vertebral bone mineral density but also increased trabecular thickness and maintained plate-like trabecular structures (versus rod-like), both of which correlate with improved biomechanical strength of bone [154]. In each case (bone mass and bone strength), the absolute magnitude of the effects of most SERMs on bone in OVX rats are indistinguishable from those of estrogen and can approach values attained for sham-surgery controls, when the SERM (or estrogen) is administered in a prevention mode. However, differences in potency for these bone protective effects can occur, with third-generation SERMs such as arzoxifene, bazedoxifene, and lasofoxifene producing equivalent efficacy to raloxifene in OVX rat trabecular BMD responses at approximately 10% of the dose [143,149]. Similarly with bazedoxifene, improved biomechanical properties in trabecular bone were observed relative to estrogen after 1-year of treatment in OVX rats [155]. Several SERMs have been extensively evaluated in other estrogen deficient animal models such as the monkey [156–158] yielding results largely similar to those observed in the OVX rat model.

As with estrogen, the primary activity of SERMs responsible for the beneficial effect on bone is antiresorptive. *In vivo* studies demonstrated that biochemical markers of bone turnover (i.e., serum osteocalcin, urinary collagen cross-links) were suppressed in a manner similar to that observed with estrogen [159]. Histomorphometric analysis of bone from raloxifene-treated, OVX, rats confirmed the antiresorptive mechanism of action for raloxifene [160]. Similar studies with the other SERMs discussed here indicate the same antiresorptive mechanism for bone protection. Of likely importance with respect to long-term safety in the skeleton is the finding that SERMs produce their inhibitory action on bone resorption with minimal suppressive effects on bone formation leaving bone formation rates at levels comparable to sham-operated control animals [160]. The molecular fingerprint of SERMs in estrogen-deficient rat trabecular bone, as assessed by DNA microarray, is unique for each SERM, although it is clear that some SERMs are less suppressive of bone formation. For example, in OVX rats raloxifene returned a cluster of genes associated with bone formation to ovary-intact control levels, as opposed alendronate, estrogen, or even another SERM (acolbifene), which exhibited a greater suppressive effect on bone formation-associated genes [161]. The overall SERM profile on bone then represents a sharp distinction from the marked suppression of bone formation that occurs with other bone antiresorptives, such as the bis-phosphonates [144]. The end result likely is greater opportunity for skeletal repair and remodeling with chronic SERM use, which permits the

skeleton to retain its critical self-healing properties.

1.5.2. Clinical Studies The abundance of preclinical information on the effects of SERMs on bone has easily been matched by a plethora of long-term clinical trials that have been conducted on a number of different SERM molecules, either as the primary element of registration trials for postmenopausal osteoporosis or as part of the safety assessment for use in breast cancer. Certainly, the most extensively studied SERM on the human skeleton has been raloxifene hydrochloride, which has been investigated in nearly 40,000 clinical trial subjects enrolled in prospective, randomized trials (placebo or active comparator) that have ranged duration of 1–8 years. In postmenopausal women, raloxifene hydrochloride (60 mg/day) exhibits an antiresorptive action as evidenced by reductions in the accelerated bone turnover as measured by biochemical markers of bone resorption [162] while only modestly suppressing bone formation. In calcium tracer kinetic studies in postmenopausal women, Heaney and Draper [163] provided evidence for suppression of bone resorption with raloxifene hydrochloride while bone formation was not affected in studies of up to 31 weeks duration. The observation of resorption inhibition with minimal formation suppression by raloxifene hydrochloride was confirmed by histomorphometric analysis of iliac crest bone biopsies [164,165]. This antiresorptive activity is associated with approximately a 2.5% increased vertebral BMD, relative to placebo-treated controls. This increase in spine BMD that occurs following raloxifene hydrochloride treatment in postmenopausal women is less marked than observed with alendronate [166]. However, this magnitude of BMD improvement in the spine underestimates the mechanical improvement produced by raloxifene hydrochloride, as evidenced by the 30% reduction in new vertebral fractures (versus placebo) in postmenopausal women without prevalent fractures and 55% reduction in new vertebral fractures in women with prevalent fractures [162], a rate comparable to that produced by other currently available antiresorptive agents for osteoporosis. This particular observation has led to an increased attentiveness to potential effects of raloxifene hydrochloride (and putatively other SERMs as well in the future) on bone quality and may be related to microarchitecural improvements as were observed in OVX mice [154]. The eventual resistance of bone to fracture is the result both of the content, or mass of the material (i.e., BMD), and the quality of that material. However, while BMD is a noninvasive, easily quantifiable, parameter in clinical trials, bone quality remains a more qualitative feature to date—only revealed by the eventual incidence of fracture. To that regard, a number of efforts have targeted better understanding, and quantifying, bone quality where raloxifene hydrochloride has shown some benefits over other antiresorptive therapies such as histomorphometric analyses of trabecular bone architecture and microcrack frequency in bone [167,168]. One area where some aspect of bone quality is beginning to be elucidated is the proximal femur, where imaging technologies have been applied to postmenopausal clinical trial subjects to show an increase in resistance to axial and bending stresses in raloxifene treated women [169], indicating improved structural components of bone strength and stability with the SERM. Raloxifene hydrochloride does produce positive effects on hip BMD, which increased 2.1% versus placebo after 3 years in postmenopausal women [162], although without a significant effect on nonvertebral fracture rates [162]. Finally, in addition to reduction of vertebral fracture in osteoporotic women, raloxifene hydrochloride also provides fracture risk protection to osteopenic women. Raloxifene hydrochloride did not lead to a significant overall reduction in nonvertebral fractures in the large, randomized, placebo-controlled registration studies that demonstrated the benefit on vertebral fractures. However, an interesting trend was noted in a subset of women who entered the trials with severe vertebral fractures. In this subset of more severely osteoporotic women, raloxifene hydrochloride produced a 50% reduction in nonvertebral fractures [170].

A number of other SERMs have unsuccessfully attempted to register for an osteoporosis prevention/treatment indication that are either currently in phase 3 clinical trials or

awaiting regulatory approval. Those molecules that have failed to achieve regulatory approval for osteoporosis primarily failed on the basis of safety and risk/benefit analysis, as each demonstrated some level of improvement on skeletal parameters. Prior to discontinuation of levormeloxifene phase 3 clinical trials due to gynecological-associated adverse events, phase 2 clinical trials demonstrated positive effects of this SERM on BMD and bone turnover [171]. A beneficial effect of levormeloxifene on biochemical markers of cartilage degradation was indicated in follow up analyses of these trials [172]. Idoxifene, a triphenylethylene also discontinued in phase 3 for uterine adverse events, produced clinically relevant increases in BMD in osteopenic postmenopausal women [171]. The most recent SERMs to report advanced clinical testing results for osteoporosis are the third-generation molecules, lasofoxifene and bazedoxifene, both very potent SERMs with relatively high bioavailability [174,175]. In a 2-year trial in 410 postmenopausal women lasofoxifene at 0.25 or 1 mg/day suppressed bone turnover comparably to raloxifene, but lasofoxifene increased lumbar spine BMD by 3.6% and 3.9%, respectively, which outpaced the increase observed with raloxifene. A 2-year BMD trial and 3-year fracture prevention trial demonstrated the skeletal protective effects of bazedoxifene relative to raloxifene. In the 3-year trial, nearly 7500 women were treated with 20 or 40 mg/day bazedoxifene, placebo or raloxifene at 60 mg/day. In this trial, the bazedoxifene produced a significant reduction in the relative risk reduction for new vertebral fractures of 37% for the higher dose and 42% for the lower dose, with raloxifene producing a comparable 42% in relative risk of new vertebral fractures [176]. Mean lumbar spine BMD was significantly improved, relative to placebo, by bazedoxifene with a magnitude of response comparable to raloxifene, and biochemical markers of bone turnover were also significantly lowered with bazedoxifene [177].

A number of clinical trials have focused on the bone sparing effects of two triphenylethylene SERMs: tamoxifen and toremifene. Both of these agents are indicated for use in women with breast cancer but not for osteoporosis, however, a number of studies have evaluated effects on BMD in breast cancer patients as part of the safety evaluation of these agents. While most studies demonstrate a skeletal benefit for these two agents, trials have typically been small and not placebo controlled in design. There is a consistent benefit observed with tamoxifen and toremifene primarily at trabecular bone sites, which is consistent with observations made with raloxifene in postmenopausal women. After 3-year use, tamoxifen or toremifene in stage II–III breast cancer patients was associated with less than expected decline in vertebral BMD [178]. In shorter trials (1 year), similar effects were observed with the effect of tamoxifen somewhat stronger than that of toremifene (2% higher BMD with tamoxifen versus toremifene that basically prevented age-related decline over the 1-year trial [179]. While many studies have reported similar benefits, particularly with tamoxifen, on BMD in postmenopausal breast cancer patients [180], there is at least one indication that use of tamoxifen in normal premenopausal women is associated with a reduction in bone mineral density [181]. Finally, there is recent interest in the potential application of bone sparing effects of SERMs for use in men as an adjunct to androgen deprivation therapy for prostate cancer. In these trials, toremifene was associated with improved BMD by 2.3% in lumbar vertebrae in men undergoing androgen deprivation therapy [182] and raloxifene increased bone mineral density in gonadotropin-releasing hormone (GnRH) agonist treated men [183].

1.5.3. SERMs and Breast Cancer A number of environmental and genetic factors are associated with increased risk of developing breast cancer in women, including advanced age, family history of breast cancer, and a greater lifetime estrogen exposure (assessed via surrogate indicators such as estradiol levels, use of estrogen therapy, age at menopause and body mass index). The best current tool for overall assessment of breast cancer risk is the Gail model, where a risk factor of ≥ 1.67 defines a woman at high risk [184]. Tamoxifen was the first SERM to show reduced risk of breast cancer through a number of large, placebo-controlled, trials. In the Breast Cancer Prevention Trial, tamoxifen was evaluated in

a cohort of 13,388 women at increased risk of breast cancer and produced a 49% reduction in the relative risk of invasive breast cancer, and a 69% reduced risk of ER-positive mammary tumors [185]. However, despite this substantial reduction in risk, and inclusion of breast cancer risk reduction as an approved use for tamoxifen, the clinical use of tamoxifen for this indication has been rather lackluster—primarily due to a side effect profile that tilts the risk/benefit ratio in a negative direction in the mind of most physicians and women. The increase in endometrial cancer in postmenopausal women likely stems from the uterine stimulatory properties of tamoxifen and represents one area for improvement in other SERMs. To this regard, raloxifene hydrochloride has recently received approval for reducing the risk of invasive breast cancer in postmenopausal women with osteoporosis and in postmenopausal women at high risk for invasive breast cancer. After 8 years of following 4011 postmenopausal women with osteoporosis, a 66% reduction in the incidence of invasive breast cancer was observed with raloxifene use [186]. In the Study of Tamoxifen and Raloxifene (STAR) Trial, a head-to-head comparison of the two SERMs was conducted in 19,000 postmenopausal women at high risk of breast cancer, where tamoxifen and raloxifene hydrochloride were found to produce similar reductions in the incidence of invasive breast cancer [187], with the primary benefit being due to a reduced risk of ER-positive invasive breast cancers [188]. The most significant differences between raloxifene hydrochloride and tamoxifen in the STAR trial were significantly fewer uterine-associated adverse events with raloxifene hydrochloride (most notably the lack of endometrial cancer) while tamoxifen appeared to have a greater effect on noninvasive breast cancer incidence than raloxifene [187]. These differences between tamoxifen and raloxifene hydrochloride, although subtle indicate a difference from preclinical and even early clinical indicators, and as such, demonstrate the need for thorough clinical evaluation before accurate therapeutic risk/benefit assessment and approval of indications can be made for human use. To this regard, several SERMs in development, such as acolbifene and bazedoxifene [189,190], have preclinical and early clinical profiles that are promising for potential use in reduction of risk for breast cancer, however, until sufficient clinical evaluation has been completed, it is too early to predict the ultimate utility of these molecules to this regard.

2. SUMMARY

SERMs are a diverse class of molecules that affect a broad spectrum of biological systems with potential therapeutic benefit for a variety of diseases. Current concern over long-term use of estrogen-containing regimens has created an opportunity for application of SERMs to chronic indications such as osteoporosis treatment or prevention. The unique SERM profile also allows their use in other chronic indications of interest to postmenopausal women, most notably, breast cancer risk reduction and treatment. However, safety considerations are a very important consideration for SERM use in these chronic indications. The pleiotropic nature the ER and its role in numerous physiologic systems raises the importance of considering potential SERM benefits and/or adverse events in the cardiovascular system and other tissues.

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