Journal of
Medicinal
Chemistry

Volume 42, Number 1 January 14, 1999

Perspective

Emerging Therapies for the Prevention or Treatment of Postmenopausal Osteoporosis

Masahiko Sato,*,† Timothy A. Grese,† Jeffrey A. Dodge,† Henry U. Bryant,† and Charles H. Turner‡

Lilly Research Laboratories, Indianapolis, Indiana 46285, and Department of Orthopaedic Surgery, Indiana University Medical Center, Indianapolis, Indiana 46202

Received June 3, 1998

Introduction

Bone is a living, dynamic tissue that is continuously remodeled during the adult life of an individual. A helpful concept is the notion that bone is remodeled in quantum units called bone-remodeling units[1,2] in which osteoclasts and osteoblasts are the active participants. Osteoclasts are the bone-resorbing cells which tightly adhere to the bone surface and then secrete acid which 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) which becomes mineralized by extracellular processes. Osteoblasts usually replenish the bone excavated by osteoclasts. Osteoporosis is the consequence of an imbalance between osteoclastic and osteoblastic activity, coupled with an increased rate of bone turnover observed with menopause. 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. Because the rate of remodeling is about 10 times higher in cancellous 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 over 50 years of age such that elderly women have a lifetime risk of fractures of about 75%.[8,9] At any given age, the risk of osteoporotic fracture is about 2 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, greater susceptibility to rapid bone loss associated with menopause, and a greater propensity to fall than men. 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 common sites of fracture are the distal forearm, spine, and proximal femur. Without question,

† Lilly Research Laboratories.
‡ Indiana University Medical Center.

10.1021/jm980344o CCC: $18.00
Published on Web 12/18/1998

appendicular and spinal fractures are associated with significant morbidity;[13b] however, 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 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).[13b]

As a consequence, pharmacologists have pursued a number of therapeutic strategies in an effort to satisfy this largely unmet medical need. The FDA approval in 1995 of the bisphosphonate, alendronate (Fosamax), and the recent approval of the selective estrogen receptor modulator, raloxifene (Evista), suggest that very different pharmacological approaches can be utilized to prevent further bone loss in postmenopausal women. However, current therapies are not capable of actually replacing significant amounts of lost bone. The latter issue is likely to be of concern to the majority of osteoporotic patients who typically wait until considerable bone has been lost before seeking therapy. The most promising of these diverse prevention and treatment pharmacological strategies will be reviewed in the following sections.

Bone Safety and Efficacy Analyses in Animal Models and the Characterization of Bone Quality

The pharmacological effort to prevent or treat osteoporosis has fueled considerable improvement in the development of animal models for osteoporosis research, as well as in the analytical methods used to quantitate bone mass and bone quality.[18−20] The development of animal models has been fueled by the registration guidelines of regulatory agencies in the United States and Europe which require demonstration of long-term bone safety and efficacy data in rats and another remodeling species for the registration of a new osteoporosis therapy. Ovariectomized rats greater than 5 months of age have been shown to reproducibly lose primarily cancellous bone from axial and appendicular skeletal sites due to estrogen deficiency, not unlike postmenopausal women, Figure 1. Efficacy studies in ovariectomized rats have been predictive of therapeutic utility in postmenopausal women for estrogens,[21−23] calcitonin,[24] bisphosphonates,[25,26] tamoxifen,[27−29] and raloxifene.[30−34] Rodents have also been useful to char-

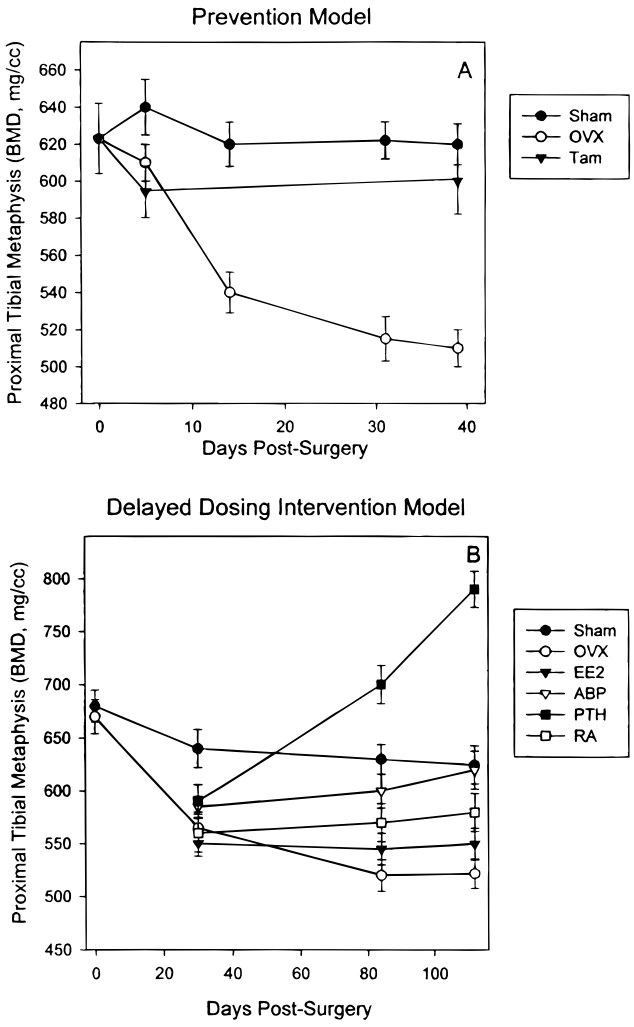


Figure 1. Ovariectomized rat models for the prevention or treatment of osteoporosis. A prevention model is shown in panel A in which 6-month-old rats were ovariectomized and scanned longitudinally by quantitative computed tomography (QCT). Plotted are the mean ± sem of the volumetric bone mineral density (BMD, mg/cm^3) of a site rich in cancellous bone, the proximal tibia metaphysis. This model mimics the situation of perimenopausal women in the process of losing bone. Analysis of the proximal tibia metaphysis showed a 17% reduction in volumetric BMD for ovariectomized rats (OVX) after 40 days postsurgery compared to sham ovariectomy controls (Sham). Tamoxifen (Tam, 1 mg/kg/day po) has been shown to prevent this loss of bone due to ovariectomy[27−29] like other agents including raloxifene,[31−33] bisphosphonates,[26] and calcitonin.[24] A delayed dosing intervention model with osteopenic, ovariectomized rats is shown in panel B. This model mimics the situation of osteoporotic women in need of treatment and illustrates mechanistic differences between agents that prevent bone loss and anabolic agents. In this latter model, 6-month-old rats were ovariectomized and allowed to lose bone for 1 month before dosing for 3 months with 17α-ethynylestradiol (EE2: 0.1 mg/kg/day po), alendronate (ABP: 30 mg/kg sc twice per week), human parathyroid hormone (1-34) (PTH: 30 mg/kg/day sc), or raloxifene (RA: 3 mg/kg/day po). ABP, RA, and EE2 prevent further loss of bone with initiation of dosing, although with differing kinetics. Because both Sham and OVX controls gradually lose bone due to aging, BMD levels of these groups can approach those of Sham in this model with time; however, efficacy of these agents are in marked contrast to an agent that stimulates bone formation like PTH which is capable of replacing lost bone and forming bone to well above baseline levels.

acterize the side effect complications in bone of other agents, such as fluoride.[35,36] However, given the differences in bone physiology between rodents and humans, animal data should be extrapolated with caution. Specifically, animals do not spontaneously fracture bones in response to declining estrogen levels or aging. Additionally, rats do not stop growing, although female rats do slow in growth by 5−6 months of age, and some perforation of the growth plate is seen at around 9 months of age.[18] Finally, the cortical bones of rats lack the Haversian-based remodeling observed for human cortical bone. Therefore, ovariectomized rats are useful to model cancellous bone responses to estrogen levels but appear to have limited utility to mimic the cortical bone of humans.

Larger animal species may be more likely to accurately model human cancellous and cortical bone physiology than rodents. Cortical bone safety concerns are the primary reason that a second, large animal study is required by the U.S. FDA for the registration of new pharmaceuticals. As regulatory agencies have tended to favor primates as a remodeling species, a growing body of data with monkeys have become available. Recent studies with domestically reared, aged Cynomolgus monkeys[37] showed a 10% bone loss for lumbar vertebra 2−4 with ovariectomy over 18 months, which resulted in reduced bone strength for vertebra and the femoral neck, Figure 2A. However, several studies of feral monkeys have shown that Cynomolgus monkeys and baboons do not lose bone after 2 years postovariectomy.[38−42] Rather, ovariectomized feral monkeys gain less bone than intact or sham-ovariectomized controls, resulting in a relative osteopenia with time, but show marginal or no bone loss when compared to baselines, Figure 2B. Apparently feral primates must be domesticated for several years, possibly to adapt to the superior nutrition in captivity, before mature females can be expected to lose bone following ovariectomy (C. Jerome, personal communication). Because domesticated primates are likely to be prohibitively expensive, feral monkeys were used for the registration efforts of alendronate/Fosamax and raloxifene/Evista; however, given the longitudinal kinetics of dual-energy X-ray absorptiometer measurements shown in Figure 2B, the best use of feral primates is probably not in the evaluation of agents that prevent loss of cancellous bone. Instead, nonhuman primates may be more useful to model the cortical bone properties of human bone; however, the osteonal density may not be identical and the turnover rate of the cortical bone of macaques may be slower than that of humans.[43] Clearly, additional large animal studies with proper controls and sufficient statistical power are critically needed to understand the possible relevance of monkeys and other species to model human bone physiology.

Traditionally, the quantitative analysis of bone architecture and quality has been based on histomorphometry;[44] however, significant improvements have occurred recently in noninvasive diagnostic instrumentation.[45,46] Currently, the most useful instrumentation for the densitometric analysis of bone mass and structure requires ionizing radiation. Available devices at regional bone centers include single-photon absorptiometers, dual-photon absorptiometers, dual-energy

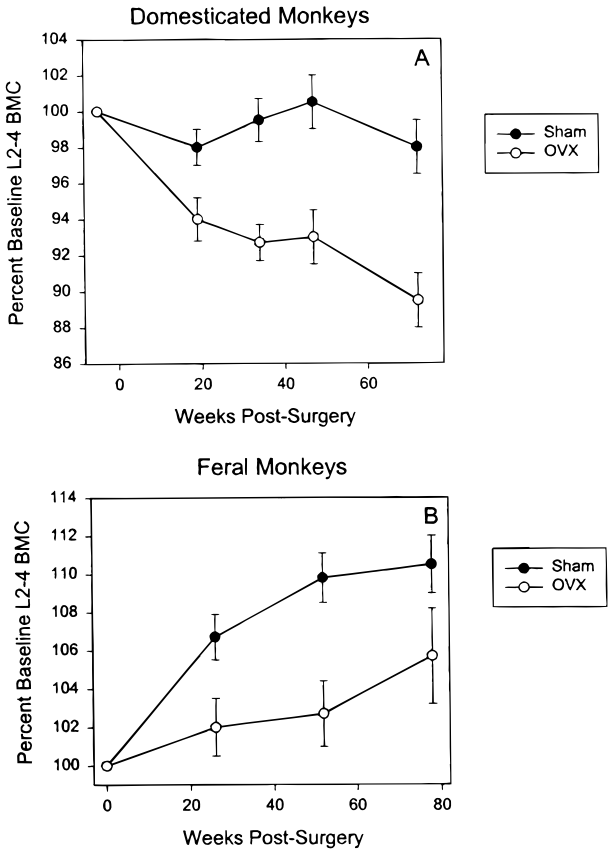


Figure 2. Model of ovariectomized Cynomolgus macaque (*Macaca fascicularus*). Panel A shows a longitudinal DXA analysis of L2-4 from Sham and OVX controls with domesticated Cynomolgus monkeys aged 9−15 years old, replotted with most data taken from ref 37. By 18 months postsurgery OVX controls have lost about 10% of the bone mineral content (BMC) measured at baseline, while the BMC for Sham has changed little. This model shows a clear bone loss due to ovariectomy, not unlike postmenopausal women. Panel B shows a longitudinal DXA analysis of L2-4 BMC for Sham and OVX controls conducted with feral Cynomolgus monkeys aged 9−11 years old. By 18 months postsurgery, Sham controls had increased BMC by 10%, while OVX controls increased BMC by 5%. This latter model shows that both Sham and OVX gain bone, with Sham gaining more bone than OVX. The basis for the difference in bone physiology between domesticated and feral Cynomolgus macaque remains to be elucidated but may be nutritionally based.

X-ray absorptiometers (DXA), and quantitative computed tomography (QCT) with precision and accuracy specifications, as reviewed previously.[45,46] The recently introduced ultrasound devices are low-cost, nonirradiating instruments which may have utility as diagnostic instruments but do not measure clinically important skeletal sites.[47−49] Additionally, the recent availability of DXA machines at some drug stores in the United States may make bone diagnostics more accessible to the general public.

Scientifically, perhaps the most significant advances have resulted from the combination of clinical bone densitometry and engineering techniques to characterize relationships between bone structure and mechanical integrity. Not surprisingly, the most informative analyses have been conducted with animal models, because of the obvious advantages to experimental design and manipulation of specific skeletal sites afforded by animals. The use of bone densitometry with

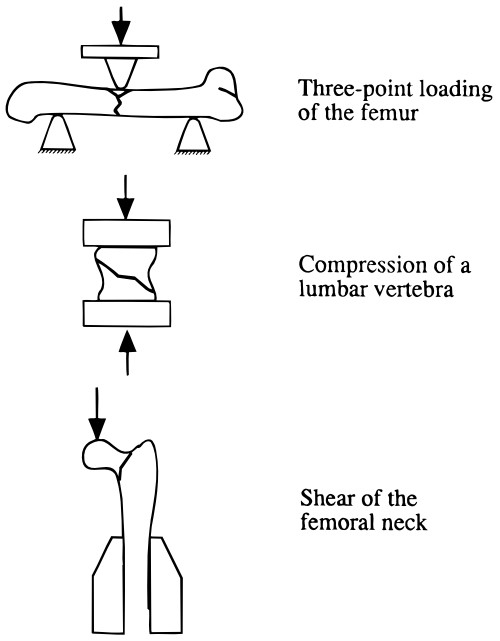


Figure 3. Biomechanical analyses. The biomechanical properties of excised bones can be quantitated using several different methods. Analyses include, but are not limited to, three-point bending to fracture of the midshaft of the diaphysis of a long bone such as femor, load to fracture in compression of lumbar vertebra, and load to fracture in a combination of bending and shear of the femoral neck.

animal models has led to rapid improvements in design, performance characteristics, and software features of DXAs[19] and QCTs.[50−54] Specifically, step sizes of 0.1 mm × 0.1 mm are now available for the bone, lean, and fat composition analyses by DXA, as well as spatial resolutions of up to 2 μm in three dimensions for X-ray tube based computed tomography (R. Turner, personal communication). As a result, high-resolution correlations in three dimensions can now be made between the spatial distribution of bone mass and the mechanical failure properties of specific bone sites.[54] More importantly, growing popularity has made these analytical devices and techniques now affordable by most research and clinical groups.

Another important advance has been the use of mechanical engineering techniques to rigorously characterize the tissue and material effects of pharmaceuticals on bone integrity.[20] However, because biomechanical testing is still largely destructive, these techniques are really only applicable to animal models, especially in experiments designed to test the safety and efficacy of compounds. Biomechanical parameters are now an accepted surrogate for, and an accurate predictor of, clinical fracture risk.[55] Measures of bone strength in animals include, but are not limited to, bending force of the midshaft of a long bone, bending/shear force to failure of the femoral neck, and compressive force to failure of lumbar vertebra (Figure 3). From these mechanical tests, several biomechanical properties can be quantitated, including ultimate force (load at failure, F_u), stiffness (S), work to failure (U), and ultimate displacement (d_u) (Figure 4). These measures are called extrinsic or structural properties of the bone because they reflect the biomechanical properties of the whole tissue (midshaft, femora neck, vertebra), which incorporates the bone distribution, size, and shape of the specimen.[20,55]

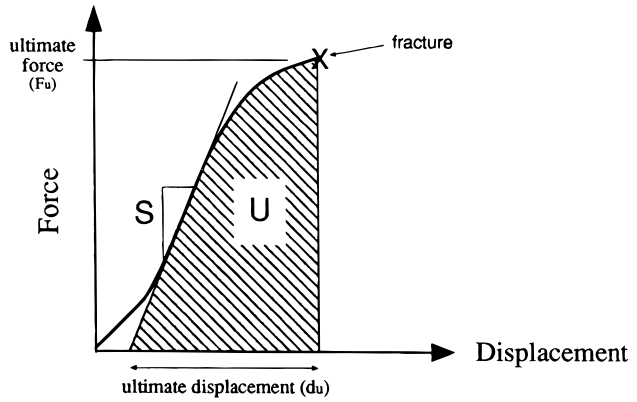


Figure 4. Force−displacement curve of a bone sample subjected to loading. Four fundamental biomechanical parameters can be determined from the force−displacement curve. The ultimate force (F_u) represents the height of the curve which is a measure of the strength of the sample. The ultimate displacement (d_u) represents the width of the curve and is inversely proportional to the brittleness of the specimen. The slope of the linear portion of the curve is the specimen stiffness (S), and the area under the curve is the work to failure (U). The latter parameter may be the best measure of resistance to fracture.

Should the applied load and displacement be normalized using engineering formulas to adjust for the size and shape of the bone (such as division of the load by the cross-sectional area), intrinsic material properties of the bone can be determined.[20] Considerable confusion exists as to the meaning and utility of these and other biomechanical parameters. For brevity, the current discussion will be limited to the structural biomechanical properties of bones described in Figure 4. Each of these measured parameters reflects a different property of the bone. For example, ultimate load reflects the general integrity of the bone tissue. Stiffness is closely related to the mineralization state of the tissue. Work to failure is the amount of energy necessary to break the bone, and ultimate displacement is inversely related to the brittleness of the bone.

The biomechanical condition of bone is poorly described by just one of these properties. For instance, hypermineralized bones from an osteopetrotic patient will tend to be very stiff, but also very brittle, resulting in reduced work to failure and an increased risk of fracture, Figure 5. On the other hand, a bone from a young child or a patient with osteomalacia will tend to be incompletely or poorly mineralized and weaker with a reduced ultimate force. However, these osteomalacic bones are very ductile with large ultimate displacement. This is why “greenstick” fractures are sometimes observed in elementary school children, where the bone bends and splits but does not fracture into two distinct segments. As the skeleton ages, it loses its ductility so the ultimate displacement and the work to failure of the bone tend to decrease with age.[56] Bones from osteoporotic patients have lower ultimate force and so are weaker than bones from normal individuals. Therefore, work to failure is reduced in each of these bone conditions, reflecting the increased risk of fracture for different mechanistic reasons for individuals with osteopetrosis, osteomalacia, and osteoporosis.

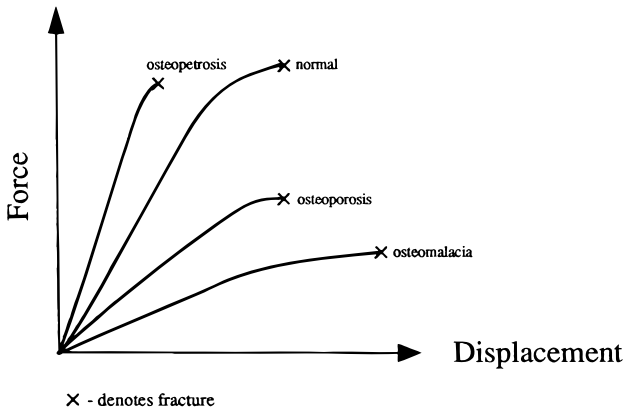


Figure 5. Force−displacement curve is descriptive of different metabolic bone diseases. Osteopetrotic bones have higher stiffness but are more brittle and, thus, fracture abruptly. Osteoporotic bones have a lower ultimate force than normal and, thus, are not as strong as normal bones. Poorly mineralized bones from osteomalacia patients are weak (lower ultimate force) but are more ductile (greater ultimate displacement). The work to failure is reduced by each of these bone disorders, reflecting increased risk of fracture.

Estrogen Replacement Therapy for Osteoporosis

During natural or surgically induced menopause, declining levels of circulating 17β-estradiol and estrone associated with cessation of ovarian function[57] have been shown to induce an increase in the rate of bone remodeling in women and animals. Mechanistically, an increase in osteoclast number and activity is observed with some impairment of osteoblastic bone formation activity, which is also a possibility in elderly women (see review by R. Turner et al.[58]). Estrogens have clearly been shown to lower the rate of bone turnover and prevent this net bone loss; however, the molecular mechanisms responsible for the bone effects have not been clearly elucidated, as briefly discussed below.[58]

Estrogens have been shown to mediate effects through interactions with estrogen receptors which are a family of nuclear hormone receptors consisting of ERα, ERβ, and possibly other members.[59] The originally described estrogen receptor, ERα, has been shown to bind freely diffusable estrogen to form a high-affinity ligand−receptor complex in the nucleus.[58,60] This estrogen−receptor complex binds to specific DNA sequences called estrogen response elements (ERE) and functions as a transcription factor by modulating the expression of a variety of estrogen responsive genes such as the proto-oncogenes *c-jun* and *c-fos*.[58,61] Cells in conventional estrogen target tissues such as the breast and uterus have a large number of receptors, 10^4-10^5/cell, but bone cells have much lower receptor levels of 10^2-10^3/cell.[62−66] Recently, another receptor ERβ was identified in osteoblast-like cells that may be expressed at higher levels than ERα in the cancellous bone of rats.[67] Considerable activity is presently directed toward elucidating the ligand specificity of ERβ, the molecular pathways associated with ERβ regulation of bone cell function, and the specific aspects of estrogen efficacy in bone in vivo associated with ERα, ERβ, or other ER isoforms.

For many postmenopausal women, hormonal replacement has been the treatment of choice for osteoporosis. Despite the lack of direct prospective clinical trials on fracture incidence, numerous studies over the past 30 years strongly suggest that long-term hormone replacement inhibits bone loss and lowers the risk of fractures at both axial and appendicular sites in women deficient in estrogen.[68−77] Additionally, observational studies have strongly suggested that estrogens protect postmenopausal women from ischemic heart disease, improve HDL cholesterol levels and fibrinogen levels, and lower the risk of death due to coronary heart disease for women treated for up to 10 years, compared to those never administered estrogen.[68,78−83] Surprisingly, a recent, randomized, double-blind study (HERS[83a]) with conjugated equine estrogens (0.625 mg) plus medroxy progesterone acetate (2.5 mg of progestin) showed no benefit compared to placebo controls on the overall risk for myocardial infarctions or deaths due to coronary heart disease. This 4-year study of 2763 postmenopausal women with established coronary disease and mean age of 67 years also showed treatment-related increased risk of venous thromboembolism[80] and gall bladder disease.[83a] Possible explanations for the unexpected coronary data include suggestions that observational studies are flawed because women seeking treatment are healthier than those who do not, that estrogens may function better as preventative therapy in younger women, or that progestin attenuates the beneficial effects of estrogen on the cardiovascular system. The HERS study has raised questions about the utility of estrogen plus progestin for the secondary prevention of coronary disease; nevertheless, estrogen replacement will continue in all likelihood as an important therapy for the primary indication of postmenopausal osteoporosis.[83a]

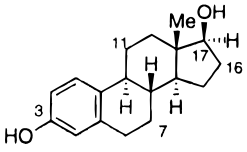
Estrogen is also associated with side effects including breakthrough bleeding, breast tenderness, and abdominal bloating, the result of which is that patient compliance can be problematic with most conventional hormone replacement therapy regimens. More importantly, estrogens have also been shown to significantly increase the incidence of endometrial cancer [84−86] and breast cancer with controversial effects on mortality.[87−90] Recent analysis of the risks associated with breast and endometrial cancer versus the benefits to coronary heart disease and osteoporosis suggested that the benefits associated with long-term estrogen useage decreased after 5−10 years.[83] Therefore, the risks of hormone replacement therapy may outweigh the benefits after 5−10 years of treatment, especially if the individuals are predisposed to breast cancer.[83,90,91] As a result, numerous efforts are currently under way to circumvent these undesirable side effects of estrogen while retaining the positive effects on bone and the cardiovascular system. One such study examined the use of low-dose esterified estrogens without coadministration of a progestin for 2 years.[92] In a placebo-controlled, double-blind study involving 406 postmenopausal women a low, 0.3 mg, daily dose of esterified estrogen produced a modest effect on spine bone mineral density (1.76% increase over baseline). This dose, however, failed to produce significant effects on either HDL or LDL cholesterol, although favorable trends were observed. Incidence of endometrial hyperplasia at the 0.3 mg esterified estrogen dose was not different from the placebo group. Higher doses of esterified estrogens (0.625 or 1.25 mg/

day) were associated with greater increases in lumbar spine bone mineral density and significantly increased HDL cholesterol (significant decrease in LDL cholesterol only occurred at 1.25 mg/day). However, the two higher dose groups were also associated with marked increases in incidence of endometrial hyperplasia. Current activity in the field suggests that several pharmaceutical groups have concluded that alternative therapies may become appropriate for many postmenopausal women after 5 years on estrogen or perhaps sooner.

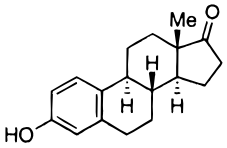
Conjugated Equine Estrogens. The leading formulation for osteoporotic women in the United States has been oral administration of conjugated equine estrogens (Premarin) at 0.625 mg;[93] however, recently approved transdermal estrogen formulations have also been shown to be efficacious.[94,95] Typically, progestins are coprescribed or administered for at least 10 days per menstrual cycle to minimize the estrogen-induced hypertrophy of the endometrium and reduce the risk for endometrial cancer.[91] Conjugated equine estrogens (CEEs) are a complex mixture of estrogen metabolites. The clinical use of CEEs has prompted interest in evaluating the individual structural components which include sulfate esters of two distinct estrogen structural classes: (1) ring B saturated steroids including traditional sex steroid hormones such as estrone, 17β-estradiol, and 17α-estradiol and (2) ring B unsaturated estrogens such as equilin (Eq), equilenin (Eqn), 17β-dihydroequilenin (17β-DHEqn), 17β-dihydroequilin (17β-DHEq), 17α-dihydroequilenin (17α-DHEqn), and 17α-dihydroequilin (17α-DHEq). Bhavnani et al.[96] examined these individual steroids, in their unconjugated form, to determine their relative binding affinities for the estrogen receptor and their in vivo effects on uterine hypertrophy in the immature rat. In this study, the majority of equine components mimicked 17β-estradiol in their ability to increase uterine weight relative to vehicle treated animals. The notable exception to this uterotrophic response was 17α-DHEqn which did not cause a significant effect at the dose examined (2 mg/kg). More recently, the sulfate ester conjugate of 17α-DHEqn has been shown to lower serum cholesterol in rats and improve arterial vasomotor function in macaques.[97,98]

Further studies on the relative effects of conjugated equine estrogens on bone versus uterus have shown that 17α-DHEqn is an estrogen agonist in rats.[99] In this animal study, uterotrophic effects were observed after 4 days of oral dosing with Eq, Eqn, 17β-DHEqn, and 17α-DHEqn. Increases in uterine wet weight from treated rodents relative to ovariectomized controls ranged from 263% for Eq to 100% for 17α-DHEqn. Serum cholesterol levels were lowered with similar potencies for all equine estrogens. Bone mineral density measurements indicated that 17α-DHEqn effectively prevented bone loss in ovariectomized rats in a dose-dependent fashion after 5 weeks of oral administration (BMD was 59.9% of Sham at 1 mg/kg and was 119% of Sham at 10 mg/kg). In addition, an average uterine weight gain of 100.4% relative to ovariectomized controls (OVX) was observed at the 1 mg/kg dose. These data demonstrate that 17α-DHEqn behaves as an estrogen agonist on bone but as a weaker agonist on the uterus in ovariectomized rats and further highlights the potential of individual components of CEE as tissue selective therapy for osteoporosis. While clinical data is limited, the three most prevalent constituents of CEE were shown to have potentially cardioprotective effects.[100] Therefore the therapeutic efficacy of CEE may be attributed to the multiple structural components of this mixture.

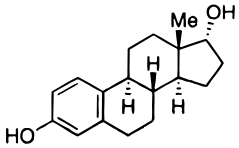
Steroidal Estrogens and New Synthetic Steroids. Early synthetic efforts to identify more selective estrogens focused on changes on the parent steroid to elicit tissue specific biological responses. For example, the estrogen metabolites estriol and 17α-estradiol were found to be time-dependent mixed agonist−antagonists of estrogen which stimulate uterine plasminogen activator activity but have little effect on true uterine hypertrophy and hyperplasia unless administered chronically at high doses.[101] Estriol caused significantly less uterine hyperplasia than 17β-estradiol and inhibited the development of breast cancer in rodent models.[102] The estrogen metabolite 2-methoxyestradiol has been implicated in the angiogenesis of vascular tissue, and a number of analogues have been reported which potently inhibit tubulin polymerization and cancer cell proliferation.[103,104]



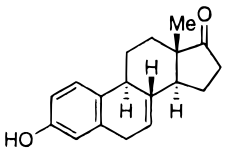
17β-estradiol



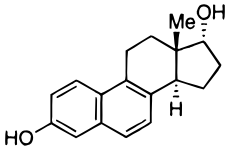
estrone



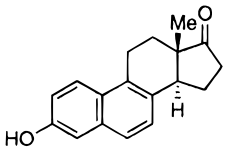
17α-estradiol



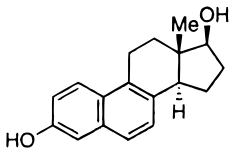
equilin



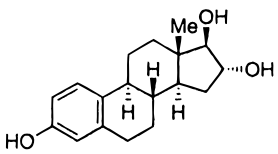
17α-dihydroequilinen



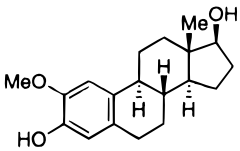
equilinen



17β-dihydroequilinen



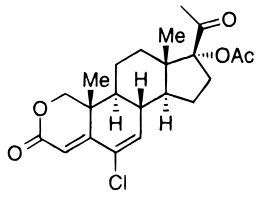
estriol



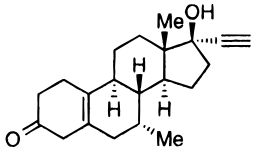
2-methoxyestradiol

Improvements in tissue selectivity have been observed with a family of D-ring halogenated estrones which have demonstrated potent lipid lowering yet diminished uterine hypertrophy relative to estrone.[105] Other attempts to attenuate the estrogenic activity of steroids on the uterus by opening of the steroid nucleus, such as 9,11-seco steroids, have met with only limited success.[106,107]

A synthetic steroid with weak estrogenic, progestational, and androgenic properties that has been examined clinically is Tibolone (OD-14).[108] Tibolone has been used to treat climacteric syndome without inducing endometrial stimulation.[109] At oral doses of 2.5 mg/day for 2 years in 140 postmenopausal women, DXA analysis showed that Tibolone was as effacious as oral or transdermal hormone replacement theory (HRT) in the lumbar spine and femoral neck, without producing concomitant endometrial stimulation and withdrawal bleeding.[108] Therefore, Tibolone may have advantages over HRT for postmenopausal women who are unwilling to accept the reoccurrence of vaginal bleeding. However, questions have been asked about Tibolone's long-term activity in the uterus, possible endometrial hypertrophy, and reduction of HDL cholesterol levels.



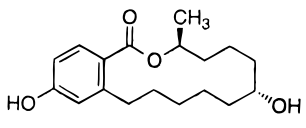
TZP-4238



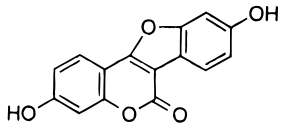
tibolone

A new synthetic steriod with progestational, antiandrogenic, and no to weak estrogenic properties that has been examined in ovariectomized rats is TZP-4238 (osaterone acetate).[110] This compound partially prevented cancellous bone loss in rats due to estrogen deficiency at 2.5 mg/kg po and stimulated periosteal bone formation beyond OVX controls. The femoral diaphysis had increased BMD and was significantly stronger than estrogen controls. TZP-4238 had no effect on uterine weight and lowered serum cholesterol levels, including a reduction in HDL levels. Further experiments are needed to elucidate cellular mechanisms behind the intriguing bone effects and ascertain if beneficial efficacy can be achieved in osteoporotic women.

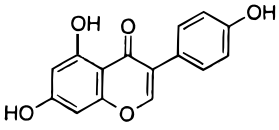
Environmental Estrogens and Phytoestrogens. Recently, the effects of environmental estrogens, including phytoestrogens, on bone and other nonreproductive tissue have received considerable attention. Although clinical studies are limited, preclinical data in nonhuman primates and rats indicate probable beneficial effects of some environmental estrogens and possibly other soy components on bone and cardiovascular tissues. For example, dietary soy proteins have been reported to improve cardiovascular risk factors in monkeys.[111] In addition, a case-controlled clinical study suggested that soy with fiber consumption reduced the risk of endometrial cancer in women.[112] A recent study of the effects of representative phytoestrogens and environmental estrogens on bone, lipids, and uterus in ovariectomized rats indicated that some of these agents were effective in lowering cholesterol and preventing bone loss.[113] Zeranol, an anabolic agent used for growth promotion in livestock, competed effectively for estrogen receptor binding and was a potent cholesterol-lowering agent ($ED_{50} = 0.2$ mg/kg). In addition, zeranol prevented trabecular bone loss in the OVX rats following 5 weeks of oral administration. Coumestrol has been shown to have beneficial bone effects in vitro[114] and in vivo.[113] One limitation observed for many agents in this class of compounds has been the lack of desirable tissue selectivity, in that estrogen-like bone and cholesterol-lowering effects have been typically accompanied by uterine hypertrophy.



zeranol



coumestrol

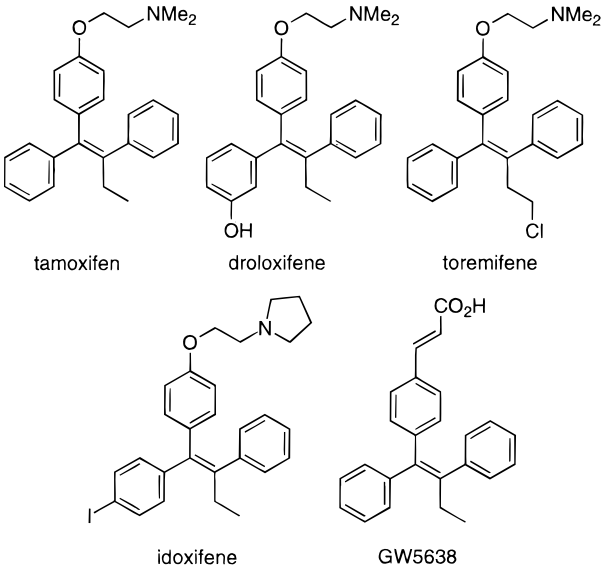


genistein

Prevention of Bone Loss with Selective Estrogen Receptor Modulators

Perhaps the most active pharmacological effort has been in the development of synthetic nonsteroidal compounds shown to have high-affinity interactions with the conventional estrogen receptor.[115−117] Because of the multiplicity of estrogen effects on bone, cardiovascular system, uterus, breast, and other tissues, researchers have searched for compounds with selective estrogen agonist activity in bone and in the cardiovascular system but with estrogen antagonist activity or no activity in reproductive tissues. Several synthetic compounds with this possible spectrum of activities have been described as selective estrogen receptor modulators (SERMs) and represent several widely varied structural families, including triphenylethylene derivatives such as tamoxifen/Nolvadex, dihydronaphthalene derivatives such as nafoxidine, benzopyrans derivatives such as levormeloxifene, and benzothiophene derivatives such as raloxifene/Evista.

Triphenylethylenes. Triphenylethylenes in various stages of development by different pharmaceutical groups include tamoxifen/Nolvadex, toremifene, droloxifene, idoxifene, and GW5638.[117,118] Tamoxifen has been shown to prevent bone loss[119,120] and has beneficial cardiovascular effects[121] in postmenopausal breast cancer patients. Tamoxifen strongly antagonizes the estrogen-stimulated proliferation of mammary tissue and is prescribed successfully to treat and prevent breast cancer.[115,122−124] However, tamoxifen functions as a partial estrogen agonist in uterine tissues with a 5-fold increased risk of endometrial cancer.[124−128] Additionally, tamoxifen has been shown to induce DNA adduct formation[129,130] and liver cancer in rats.[131] Toremifene, idoxifene, and droloxifene are more recent triphenylethylenes with reduced or nondetectable DNA adduct formation.[132−139] All three stimulate uterine hypertrophy to a lesser degree than tamoxifen in animal studies[140,141] and are being evaluated for the prevention or

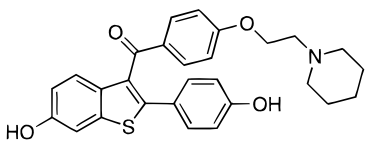


tamoxifen droloxifene toremifene

idoxifene GW5638

treatment of breast cancer. Toremifene lowered cholesterol levels and prevented bone loss in postmenopausal breast cancer patients but appeared to be as estrogenic in the uterus as tamoxifen.[142] Toremifene was recently approved for the treatment of advanced breast cancer in postmenopausal women, although advantages over tamoxifen for this indication were not clear in clinical studies.[143−145] Droloxifene and idoxifene have been shown to protect against bone loss due to ovariectomy and to lower serum cholestrol levels in rat models.[140,141,146−148] The newest member of this family, GW5638, appears to have markedly reduced uterotrophic effects while maintaining beneficial bone and lipid effects in rats, suggesting that GW5638 may be a promising member of this family to treat a spectrum of conditions observed in postmenopausal women.[118]

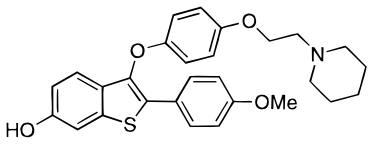
Benzothiophenes. Raloxifene is the best known member of the benzothiophene family that has previously been referred to as keoxifene, LY139481, or LY156758. Raloxifene had, at one point, been considered a candidate for the treatment of breast cancer[149] but was never developed for this indication. However, recently raloxifene has been shown to be efficacious in preventing bone loss in postmenopausal women.[150] Specifically, a double-blind study with 601 postmenopausal women showed a 2.4% increase in DXA BMD for the spine and hip compared to placebo controls after 2 years of treatment with 60 mg of raloxifene. Despite the modest effects on spinal BMD, raloxifene was recently shown to reduce fracture incidence by 50% after 2 years of treatment with the 60 mg dose.[150a]



raloxifene

Pharmacologically, raloxifene is distinguished from triphenylethylenes—tamoxifen, droloxifene, idoxifene, and toremifene—primarily on the basis of its uterine effects, where a qualitative difference has been observed.[30,33] In direct comparison with tamoxifen, droloxifene, and idoxifene, raloxifene functioned essentially as a complete antagonist of estrogen action in the immature female rat uterus, while the triphenylethylenes functioned as partial agonists, inhibiting the effects of estrogen on uterine weight gain only to the level of their own intrinsic agonist activity.[33,151] Similarly, in ovariectomized rats, the first generation triphenylethylenes were found to induce a larger maximal stimulation of uterine weight, larger uterine epithelial cell height, and uterine eosinophilia while raloxifene did not. Raloxifene does stimulate a modest increase in uterine wet weight of rats; however, this increase was not coincident with increases in other measures of uterine hypertrophy and may be attributed to water retention,[30] although this point has been disputed.[152] Transvaginal ultrasonography of postmenopausal women showed no stimulatory effect of raloxifene on endometrial thickness at any time during the 2 years of treatment.[150]

Recent studies have suggested that distinct and specific structural features of raloxifene may be responsible for its improved tissue selectivity.[33,117,153] The strategy of incorporating the stilbene moiety of a triphenylethylene into a bicyclic ring system (i.e., naphthalene or benzothiophene) has, with varied success, been used to confer configurational stability upon the olefin, thus reducing metabolic conversion to potentially uterotrophic double-bond isomers.[154] Nevertheless, the well-established uterine stimulatory effects of nafoxidine, a nonisomerizable analogue of tamoxifen, demonstrates that this is an incomplete solution.[33] The carbonyl hinge, which is imposed between the benzothiophene moiety of raloxifene and its aminoethoxyphenyl side chain, has also been hypothesized to contribute to its profile of tissue selectivity. Comparison of the low-energy conformations of raloxifene and tamoxifen demonstrates that this subtle structural modification produces a major change in the orientation of the side chain, from coplanar with the stilbene nucleus in tamoxifen to roughly orthogonal in raloxifene. This orthogonal side chain orientation, which is consistent with the crystal structure of raloxifene bound to the estrogen receptor,[155] has been postulated to be a critical determinant of raloxifene's enhanced tissue specificity. Indeed, biological evaluation of a raloxifene analogue in which the carbonyl hinge has been excised demonstrated a profile of activity very similar to that of tamoxifen.[153] Likewise, several other estrogen receptor modulators which demonstrate tissue specific estrogen agonist activity without significant uterine stimulation have been shown to have similar side chain orientations.[156,157]

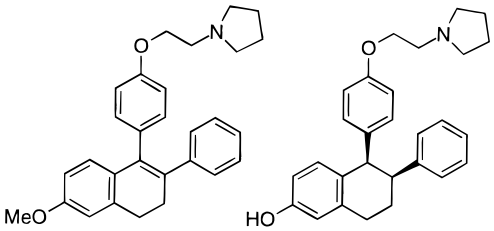


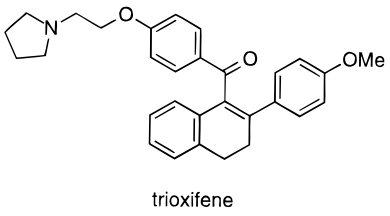
LY353381

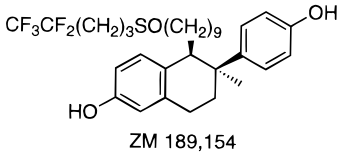
LY353381.HCl is a new benzothiophene analogue with efficacy similar to that of raloxifene but with improved potency in rat models.[158] This compound has beneficial effects on bone and serum cholesterol while functioning as an estrogen antagonist in the uterus.[158]

LY353381.HCl is currently in clinical trials with breast cancer patients. Additional studies are necessary to ascertain if LY353381.HCl has significant advantages over raloxifene or other more recent SERMs as therapy for postmenopausal women.

Naphthalenes. The best characterized naphthalene derivative is nafoxidine which had also been considered at one point for the treatment of breast cancer.[159] Nafoxidine was shown to significantly lower serum cholesterol levels but had complex bone effects in ovariectomized rats.[33] Specifically, 10 mg/kg nafoxidine prevented bone loss but 1 mg/kg did not. However, half of the animals also died at 10 mg/kg nafoxidine, suggesting toxicity issues. These data are consistent with in vivo data for ZM189154[160] in suggesting that not all compounds with high-affinity interactions with the conventional estrogen receptor have beneficial effects on bone. Another member of this family, trioxifene, has also been shown previously to prevent bone loss due to ovariectomy in rats.[151,161] However, both nafoxidine and trioxifene were shown to function as partial estrogen agonists on the uterus.

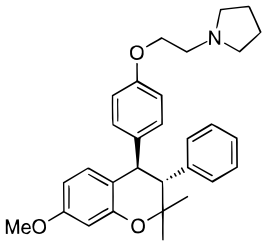


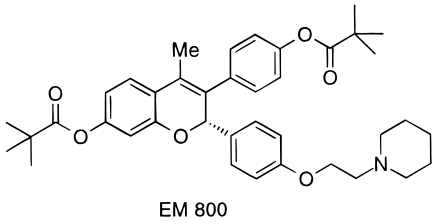




An interesting new member of this family is the reduced nafoxidine derivative CP-336,156 which has been shown to have potent SERM activity in rat models.[157,162] This compound was shown to have improved oral bioavailability with beneficial effects on bone and serum cholesterol levels at doses of 10 μg/kg/day.[162] Marginal effects on uterine weight and epithelial thickness were observed in ovariectomized rats, showing that CP-336,156 has beneficial effects on bone, cholesterol, and reduction of body fat, with little or no stimulation of the uterus. Clinical studies with CP336,156 will help to elucidate if these preclinical effects translate to the desired efficacy in osteoporotic postmenopausal women.

Benzopyrans. An interesting member of the benzopyran family of SERM is ormeloxifene, which was formerly known as centchroman. Ormeloxifene has been shown to prevent bone loss by directly inhibiting the bone-resorbing activity of osteoclasts.[163] However, this compound has been reported to stimulate uterine hypertropy in ovariectomized rats.[164] Recently, an enantiomer of ormeloxifene called levormeloxifene has been reported to prevent bone loss while lowering serum cholesterol levels in ovariectomized rats with reduced uterine stimulation relative to 17β-estradiol.[165−167] Levormeloxifene has been reported to be in clinical studies and was shown to reduce serum cholesterol and biochemical markers of bone turnover in postmenopausal women.[168] If uterine stimulation is not observed in women, levormeloxifene may serve as a potential therapy for postmenopausal women.





A newly synthesized member of this family is EM-800, which has potent antiestrogenic characteristics.[169,170] EM-800 antagonized the estrogen-stimulated proliferation of T-47D, ZR75-1, and MCF-7 human breast cancer cells and the alkaline phosphatase activity of the human endometrial adenocarcinoma Ishikawa cells.[170] EM-800 potently antagonized the estrogen-stimulated increase in uterine wet weight in female mice, was beneficial in bone, and lowered serum cholesterol and triglyceride levels in rats. Analysis of isomers showed that the (*S*)-(+)-enantiomer is much more potent than the (*R*)-(+)-enantiomer; however, this compound appears to be in the early preclinical stages of investigation.

Molecular Pharmacology. Part of the mechanistic basis to the tissue selective efficacy shown for these SERMs may be explained by the ligand-dependent conformations of the ligand−estrogen receptor (ER) complex. The distinct structural features of synthetic ER ligands are thought to effect conformational changes to the ligand−ER complex, which is the likely basis for the observed tissue selective pharmacology. Evidence for these different conformations was found in the unique in vitro protease digestion profiles for ERα complexed to nafoxidine, tamoxifen, or raloxifene.[171] Luciferase assays with various chimeric ER constructs were used to show mechanistic differences in the SERM-dependent transactivation of genes containing estrogen responsive elements.[171] On the basis of the collective in vitro data, McDonnell et al.[171] proposed four classes of ERα modulators referred to as type I, II, III, and IV. Additional studies showed that ERα complexed to

raloxifene or metabolites of 17β-estradiol can regulate the transcription of genes such as TGF-β3, which are devoid of the palindromic estrogen responsive element, through interactions with alternative DNA sequences in the upstream promoter region.[172,173] The collective SERM data suggest that a compound is not confined to a particular class, in that modifications to the core structure have powerful effects on the efficacy and tissue selectivity of the compound. Several groups are examining the molecular basis through structure/activity pharmacology studies to ascertain the chemical features responsible for the tissue selective pharmacology.[117] Presumably, different conformational states of the ligand−ERβ complex are also likely to exist; however, it remains to be seen what the effect of different conformations of the specific ligand−ERβ complex are on gene expression and whether ERα or ERβ specific pathways can help explain the selective efficacy of SERMs on the growing number of tissues shown to be responsive to estrogen.

Estrogen and SERM Effects in the Brain. Recently, considerable attention has been directed toward the finding that estrogen replacement therapy has beneficial effects on the central nervous system (CNS) of postmenopausal women. While conflicting reports have appeared in the literature, accumulating evidence indicate beneficial effects of estrogen on higher brain functions, including learning and memory. Early clinical reports with synthetic estrogens noted improved cognition in elderly women.[174] More recently in an epidemiologic study conducted in a well-defined Southern California retirement community, estrogen users had a 30% lower risk of Alzheimer's disease based on death certificate diagnoses.[175] Retrospective epidemiological studies with other cohorts of postmenopausal women have demonstrated approximately 50% reductions in relative risk of Alzheimer's disease.[176,177] Conversely, another epidemiological study employing a large health maintenance organization population failed to show any benefit of estrogen use on Alzheimer's disease.[178] Despite inconsistencies in the literature, most studies generally point toward a beneficial effect of estrogen on cognition in elderly women, although the magnitude of the effect is moderate. It is interesting to note that those cognitive functions (i.e., verbal memory) most commonly affected by progression of Alzheimer's disease are those most frequently noted as improving with chronic estrogen use.[179] In fact, preliminary data suggests that estrogen could prevent or delay the onset of Alzheimer's disease in women.[180] To date, there are no reports on the effects of SERMs on higher brain function. Because SERMs have mixed estrogen agonist/antagonist profiles, it is likely that both estrogen agonist and antagonist CNS effects will be observed for the different SERM molecules, possibly along structural families.

ERα and ERβ subtypes have been shown to be widely distributed in the brain.[181] Neurons in the ventromedial hypothalamus were found to contain predominately ERα, while ERβ was primarily detected in neurons of the cerebral cortex, supraoptic nucleus, and paraventricular nucleus.[181,182] Both ER subtypes were found in hippocampus, preoptic area, amygdala, various hypothalamic and brain stem nuclei, and certain neural pathways (i.e., stria terminalis). Of considerable interest is understanding the different functional activity associated with ligand binding to ER subtypes in the various brain regions. Estrogen effects in the brain are likely to be mediated by the traditional ER nuclear hormone pathway; however, potential nongenomic mechanisms have also been proposed for estrogen and SERMs in the brain. For example, estrogens have been reported to inhibit calcium currents in isolated neurons[183] which would appear to be mediated by a nongenomic pathway based on the rapidity of the response. Similar inhibitory effects of tamoxifen have been reported on calcium currents.[184] These "nongenomic" effects, however, are observed at pharmacological concentrations, suggesting lower affinity interaction or possibly reduced intrinsic activity relative to the classical ER mediated nuclear pathways. An understanding of pharmacokinetics and distribution patterns (ADME) is also important in the interpretation of the pharmacologic effects, but ADME data are not always readily available to the scientific community.

A hallmark CNS-related symptom associated with estrogen deficiency is vasomotor events, commonly referred to as hot flashes or hot flushes.[185] Clearly, one of the leading reasons that postmenopausal women seek hormone replacement therapy is to relieve these rapid, unpredictable, alterations in peripheral circulation and temperature. Unlike HRT, SERMs do not appear to offer beneficial effects with respect to postmenopausal vasomotor symptoms. Vasodilation (hot flashes, hot flushes, feeling of warmth) was a reported side effect for 600 mg/day raloxifene,[186] but reported hot flashes at 60 mg/day were not different from placebo controls after 2 years in postmenopausal women.[150] Increased incidence of hot flashes have also been reported in premenopausal women who receive tamoxifen for breast cancer treatment.[187]

Estrogen elicits other effects in the brain. In rats, estrogen produces a distinct hypophagic pattern which is paralleled by raloxifene.[188] Estrogen also has been associated with beneficial effects on mood, particularly with respect to an antidepressant action.[189] As with cognitive effects, clinical studies on these beneficial effects of estrogen on affect are inconsistent. One key variable is likely to be the inclusion of a progestin as part of a hormonal replacement regimen. Progestins are typically included to cause shedding of the endometrium to reduce the risk of endometrial cancer due to estrogen-induced hyperplasia of this tissue. However, the combination of a progestin and estrogen may negate some of the beneficial actions of estrogen in the CNS because negative mood effects have been associated with progestins.[190] The lack of need for combined use of a progestin with SERMs, like raloxifene, may represent an advantage for these agents; however, additional clinical studies are required to clarify these issues.

Resorption Inhibitors: Calcitonin, Integrin Antagonists, Capthepsin K Inhibitors, and Bisphosphonates

Calcitonin and bisphosphonates are two successful examples of several pharmacological strategies used to target inhibition of the osteoclastic resorption of bone. Salmon calcitonin is among the most potent inhibitors of the bone-resorbing activity of osteoclasts in vitro[191−193]

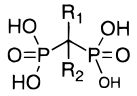
and is available as intramuscular injection and as nasal spray formulations to treat postmenopausal osteoporosis. While calcitonin has been shown to inhibit osteoclastic activity to 10^{-12} M concentration, in vitro studies have shown that calcitonin signaling is desensitized with continued exposure through the down-regulation of calcitonin receptors.[194] This may help explain the marginal clinical data observed of 1−1.5% vertebral BMD increase over 3 years for treated patients. Nevertheless, despite the sometimes nonsignificant BMD efficacy observed for calcitonins and the poor bioavailability observed for nasal calcitonin,[195] both formulations were shown to decrease significantly the incidence of vertebral fractures in osteoporotic women.[196−199] The 16−30% reduction of fracture incidence was a surprise, suggesting that DXA-measured BMD underestimates the benefit to mechanical integrity of small increases in bone mass in women.[199,200] A possible explanation is that DXA measures projected area parameters and not three-dimensional volumetric parameters such as QCT.[19,45] Therefore, DXA BMD can underestimate the architectural contribution of small increases of bone in osteoporotic women. For example, small strategic additions of bone to the cortical shell could have major effects on vertebral strength and toughness that would not register as change in DXA BMD because of positioning error. These clinical data suggest that DXA BMD may not be a reliable surrogate for fracture incidence in the evaluation of pharmaceuticals. Calcitonin also has analgesic effects which 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.[226] 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[227,228] and in animal models.[229,230] While echistatin itself is not likely to be therapeutically useful,[231] RGD peptides and integrin antagonists have been examined in animal models and shown to prevent bone loss due to ovariectomy.[232] While scientifically exciting, it remains to be seen if sufficient target tissue specificity or bioavailability to osteoclasts can be achieved for this pharmacological approach to become a viable therapy useful to patients with bone disorders. This is because multiple cell types have been shown to express RGD-binding integrins and peptides are susceptible to protease degradation, are immunogenic, and may be difficult to deliver specifically to osteoclasts active in resorption.

Another strategy has been to target a member of the papain superfamily of cysteine proteases, cathepsin K, which is highly expressed in human osteoclasts but expressed in low levels in spleen, liver, kidney, muscle, heart, lung, skin, colon, and rheumatoid synovium.[233−235] Cathepsin K has been mapped to chromosome 1q21, and functional mutations to this gene occur naturally, resulting in pycnodysostosis, a rare skeletal dysplasia that is characterized by dwarfism, low rate of bone turnover, and osteosclerosis.[236] 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 vivo in the adjuvant-arthritic and thyroparathyroidectomized rat models.[237] These results are highly intriguing but require further studies in order to ascertain possible specificity and usefulness to osteoporotic or arthritic patients.

Other strategies to prevent bone loss have been described in the literature, including inhibitors of vacuolar H^+-ATPases,[238] minocyclin/tetracyclines,[239] and others. However, these studies appear to be in the early stages of preclinical investigation and will not be discussed further here.

Table 1. Structures for Several Important Bisphosphonates



bisphosphonate	R_1	R_2
alendronate	OH	$CH_2CH_2CH_2NH_2$
neridronate	OH	$CH_2CH_2CH_2CH_2CH_2NH_2$
tiludronate	H	SC_6H_4-*p*-Cl
risendronate	OH	CH_2-3-pyridiyl
etidronate	OH	CH_3
clodronate	Cl	Cl
pamidronate	OH	$CH_2CH_2NH_2$
ibandronate	OH	$CH_2CH_2N(CH_3)CH_2CH_2CH_2CH_2CH_3$
incadronate	OH	$CH_2CH_2NH_2$

Bisphosphonates

Another area of considerable pharmacological activity includes bisphosphonates, which are synthetic P−C−P compounds pioneered by H. Fleisch that have been shown to be highly potent inhibitors of osteoclastic resorption activity (Table 1).[200] In particular the aminobisphosphonates—pamidronate, alendronate/Fosamax, incadronate, ibandronate, neridronate—and cyclic bisphosphonates—tiludronate, risedronate—have been shown to be highly efficacious in preventing bone loss due to estrogen deficiency in vivo (Table 1).[200,201] Clinical studies with the first generation bisphosphonate, etidronate, showed beneficial effects on spinal BMD,[202] and etidronate has been approved for the treatment of osteoporosis in some European countries. However, definitive fracture data have not been published yet, and etidronate was shown previously to impair mineralization, resulting in osteomalacia at clinically relevant doses in Pagetic and osteoporotic patients.[203]

More recently, impressive animal and clinical data have been generated with the third-generation bisphosphonate, alendronate/Fosamax, as reviewed previously.[200,201] 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.[204,205] 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.[206] Additionally, DXA BMD analyses of 1174 women under 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.[207]

As a result of these impressive clinical data, alendronate/Fosamax was approved for both the treatment and prevention of osteoporosis and appears to be attractive therapy for osteoporotic women who cannot or will not take estrogen.

At efficacious doses in vivo and in coculture experiments in vitro with human osteoclast precursors,[210,211] alendronate appears to be remarkably effective in retarding osteoclastic resorption of bone. 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 about half of the compound localizes directly to bone, especially cancellous bone.[210−215] 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.[210] 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.[210,216] Additionally, alendronate has also been shown to retard osteoclast differentiation by inhibition of tyrosine phosphatase activity,[217,218] it may induce osteoclast apoptosis,[219] and at high concentrations in vitro, alendronate may also have osteoblast-mediated inhibitory effects on osteoclasts.[220,221] Therefore, alendronate inhibits resorption, possibly through several cell pathways, with further studies needed to clarify the specific molecular interactions involved.

Side effects observed with alendronate appear to be different than previously described for etidronate, possibly due to the amino side chain and improved potency (Table 1). Analyses of iliac crest biopsies from 231 osteoporotic women 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.[208] In addition, newly formed bone was lamellar with no evidence of marrow fibrosis or cellular toxicity.[208] 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 a 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.[208] These data indicate substantial reduction of bone turnover (both resorption and formation activity), with similar reduction of bone turnover observed in long-term animal studies.[34,39,209] Hypothetically, increased mineralization could result from this reduction in bone turnover, possibly leading to increased brittleness, as suggested in vertebra from a 10-month rat study.[34] Clinical data have not shown alendronate effects on brittleness; however, long-term clinical studies of comparable duration have not yet been conducted (10 months in a rat corresponds to about 7.5 years in women).

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.[201,212,215] 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, if overprescribed or administered too long.[222] Other side effects observed for alendronate include erosive esophagitis which 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.[212,213,223] Despite specific instructions on the label insert to take alendronate on an empty stomach while upright at least 30 min before breakfast, gastrointestinal complications continue to be reported.[224,225]

Several other bisphosphonates have been in clinical trials, with tiludronate, risedronate, and incadronate in apparently the latter stages of development (Table 1). While highly efficacious, additional clinical data will be required to ascertain if the newer bisphosphonates have significant advantages over alendronate, as poor oral bioavailability and long half-life in bone may be general characteristics for this class of compound.[200,201,212]

Bone Formation Strategies: Fluoride, Parathyroid Hormone, and Others

Fluoride. Other pharmacological strategies have attempted to replace lost bone in osteoporotic women by inducing bone formation activity with controlled dosing regimens of fluoride or parathyroid hormone. Fluoride therapy remains a potential, but controversial, treatment for osteoporosis, with moderate to high doses of fluoride showing increased bone formation and bone mass.[240] Farley et al.[241] suggested that fluoride's anabolic effects were due to a direct effect on bone cells, which were supported by subsequent tissue culture studies[242,243] but were contradicted by others.[244,245] Worrisome are the findings in animal studies that showed no effect[246] to significant deterimental effects of fluoride on bone strength.[247−252] The reduced bone strength may be caused by mineralization defects that can result from high serum fluoride levels[252−255] or possibly indirectly through secondary hyperparathroidism.[256] Fluoride also adversely affected the bone strength of well-mineralized bone,[251] possibly by altering mineral crystal size and packing.[257−259] That is, fluoride tends to increase mineral crystal dimensions[258,259] and may alter the electrostatic bonding between mineral crystals and the collagen matrix.[260] Either mechanism may diminish the mechanical properties of bone tissue.[261]

A clinical trial of fluoride therapy using 75 mg/kg/day NaF resulted in increased incidence of appendicular fractures after 2 years,[262] probably due to impaired mineralization of bone.[255] In another clinical study, a lower dose, slow-release formulation of NaF in combination with calcium citrate showed increased vertebral BMD and significant reduction in the incidence of new vertebral fractures in postmenopausal women.[263] As a result, the Metabolic Disease Advisory Panel of the FDA unanimously recommended approval of this formulation for the treatment of postmenopausal osteoporosis in 1996, but the FDA has not yet approved fluoride therapy

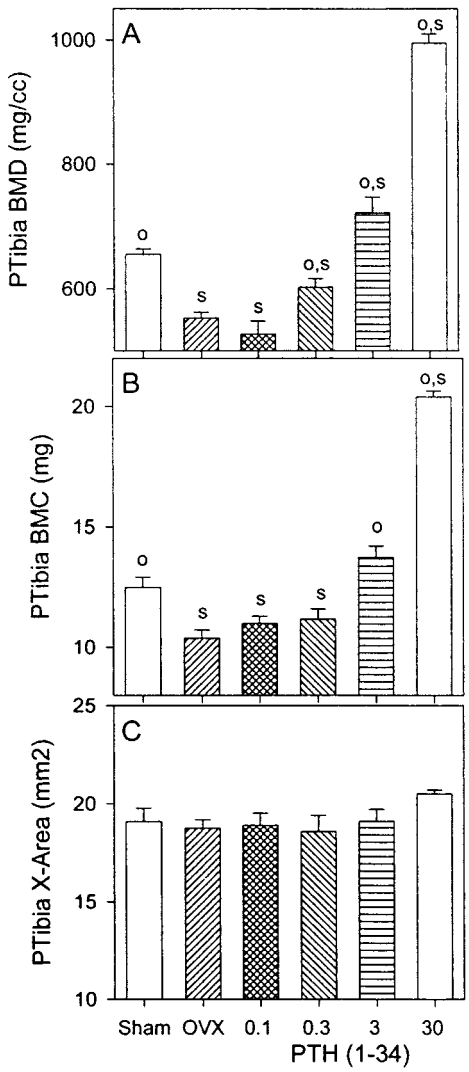


Figure 6. Dose−response curve for PTH (1-34) in osteopenic ovariectomized rats. Dose-dependent effects of the amino-terminal fragment of human parathyroid hormone (PTH (1-34)) are shown for a delayed dosing intervention model with osteopenic ovariectomized rats, as shown in Figure 1B. Specifically, 6-month-old rats were ovariectomized and allowed to lose bone for 1 month before dosing with the indicated amounts of recombinant human PTH (1-34) (0.1−30 mg/kg/day sc) for the following 3 months. A 1 mm cross-section of the proximal tibial metaphysis was analyzed at termination by QCT to quantitate the (A) volumetric bone mineral density (BMD, mg/cm^3), (B) bone mineral content (BMC, mg), and (C) cross-sectional area (X-Area, mm^2). Comparisons were made to Sham and OVX controls, with significant differences ($p < 0.05$) from OVX designated as "o" and significant differences from Sham designated as "s" (Fisher's PLSD). These data show the possible utility of PTH to prevent further loss of bone, to replace lost bone to Sham levels, or to add more bone, depending on the dose.

for osteoporosis in the United States. More recently, a clinical study using a slightly different slow-release formulation of fluoride revealed no effect of therapy on the vertebral fracture rate.[264] As a result, many clinicians in the field appear to be unconvinced as to the safety and efficacy of fluoride.[265]

Parathyroid Hormone. Intermittent subcutaneous administration of parathyroid hormone (PTH) and several amino-terminal fragments, such as PTH (1-34), have been shown to stimulate bone formation in numerous preclinical studies and several clinical studies; see Figures 1B and 6. In animals, intermittent administration of human PTH (1-34) was shown to significantly increase bone mass in ovariectomized rat models.[266] X-ray densitometry, histomorphometry, and other techniques were used to show that PTH (1-34) stimulates trabecular bone formation in the proximal tibia,[50,267−270] femoral neck,[271,272] distal femur,[268,273−275] and vertebrae.[275−279] Even in aged rats with established osteopenia, intermittently dosed PTH was shown to be capable of restoring lost trabecular bone to control levels[268,272,279−281] provided that remnants of trabecular bone spicules remained.[282] Examination of bone quality by mechanical testing showed that PTH treatment dose-dependently increased vertebral strength, decreased brittleness, and induced a substantial increase in work to failure of the vertebrae to levels well above both ovariectomized and baseline controls.[283]

Substantial gains in the cortical bone of rat models have also been reported with PTH (1-34).[284−292] The increase in cortical bone mass was attributed to an increase in cortical wall thickness and moment of inertia through stimulation of bone formation on both endocortical and periosteal surfaces.[283,286] Consequently, these PTH (1-34) effects have translated to substantial gains in the mechanical properties of the rat diaphysis.[283,292]

As shown in Figures 1B and 6, PTH (1-34) differs mechanistically from preventative agents in the ability to replace bone lost to estrogen deficiency to well above baseline levels. Dose−response analysis shows that PTH (1-34) is more efficacious in the skeleton than preventative agents (Figures 1B and 6). These rat studies have been invaluable in elucidating the cell processes by which PTH builds bone. Specifically, PTH (1-34) stimulates appositional bone formation activity on existing bone surfaces without necessarily first stimulating bone resorption activity.[293] If bone surfaces no longer exist as in the case of severely osteopenic animals, trabecular bone spicules are not restored in these sites such as vertebra.[282] PTH appears to stimulate bone modeling (appositional bone formation without resorption first) by inducing a dramatic increase in osteoblast number and osteoblastic activity.[270,294−296] That is, bone-lining cells are induced to become osteoblasts without stimulating proliferation of precursors.[296,297] Interestingly, this effect of PTH is dependent on intermittent administration, as continuous infusion or infusion of PTH for more than 2 h/day results in hypercalcemia and bone loss, as osteoclasts are stimulated to resorb bone.[266,298] Continuous infusion of PTH is reminiscent of hyperparathyroidism which is characterized by continuously elevated PTH levels and pathologic bone, as reviewed by Heath.[299]

The results of these animal studies have been found to parallel clinical data generated in postmenopausal women.[300−305] Daily subcutaneous injections of low doses of PTH (1-34) were shown to enhance bone formation in the spine and femoral neck of osteoporotic women and men.[306] Recently, PTH (1-34) was shown to induce impressive gains in vertebra (13% increase in BMD) and in the hip (2.7% increase in BMD), with significant reduction in incidence of vertebral fractures in postmenopausal women.[305] Osteoporotic women already taking HRT for at least 1 year were administered 25 mg/day of PTH (1-34) in addition to estrogens for the

following 3 years.[305] Benefits to bone mass and fracture incidence might have been greater had the comparator been a true placebo group without estrogen treatment. Earlier clinical studies suggested that the significant enhancement of trabecular bone mass occurs at the expense of cortical bone;[301,306] however, no evidence for this was observed in rigorously analyzed animal studies or more recent clinical studies which showed no loss of bone mass at any skeletal site;[305] see also the review by Reeve.[307]

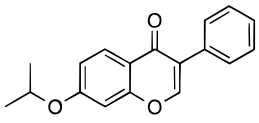
Considerable pharmacological activity is directed at PTH, fragments of PTH, analogues of PTH, and related proteins. For example, PTH (1-38)[308,309] and PTH (1-31)[310,311] have been shown to be anabolic in the rat skeleton, and in particular, PTH (1-38) has been shown to be clinically efficacious.[312] A new analogue of PTH (1-34), SDZ PTS 893, has been shown to be 4 times more potent than human PTH (1-38) in rat models[313] and efficacious at 0.2 mg/kg dosed 3 days/week after 40 weeks of treatment in ovariectomized monkeys.[314] Alternatively, amino-terminal fragments of parathroid hormone-related protein (PTHrP (1-34), (1-36), (1-74)), which is a second high-affinity ligand that activates the PTH/PTHrP receptor,[315] have also been shown to be anabolic in the skeleton.[316,317] A C-terminally substituted analogue of PTHrP (1-34), RS-23581, appears to be anabolic in the rat skeleton and may be more potent than bovine PTH (1-34),[318] although hypercalcemia remains a concern for PTHrP and analogues.[319,320]

Long-term safety and cortical bone effects remain concerns for PTH alone or in combination with other agents that will require additional clinical studies in osteoporotic women. It also remains to be seen what the effect of mode of administration (injection by syringe) will have on patient compliance, as these proteins will probably have to be administered parenterally. Nevertheless, the proper formulation of PTH has the potential to become a valuable addition in the treatment of osteoporotic patients.

Other proteins and peptides such as growth hormone, IGF-1, TGF-β, BMPs, Noggins, osteoprotegerin, and others are also under investigation. These agents appear to be under development primarily for slightly different indications, are presently in the early preclinical stages of experimentation, or were not found to be efficacious as potential therapy in bone clinical studies, and so they will not be discussed further here.

Alternative Pharmacological Approaches

Ipriflavone. Ipriflavone is a synthetic derivative of plant isoflavones that has been approved for the treatment of involutional osteoporosis in some European countries and in Japan. A recent multicenter clinical study in postmenopausal women showed that ipriflavone maintains or induces a slight 1% increase in DXA BMD of the spine after 2 years of treatment, compared to a −1% loss for the placebo control group.[321] Controversy exists on its mechanism of action, but in vitro and animal studies suggest that ipriflavone is a weak resorption inhibitor[322,323] that may stimulate bone formation in some cell systems.[324] However, analysis of serum and urine markers showed only a reduction in bone turnover, including significant reduction of skeletal alkaline phosphatase levels.[321,325] Ipriflavone may be utilized best in combination therapy with low-dose estrogen.[321]



ipriflavone

Nonpharmacological Approaches: Exercise and Bone Mass

An alternative nonpharmacological approach has included attempts to build bone through mechanical stimulation based on the notion that the architecture of bone tissue adapts in response to the functional demands placed on the skeleton. This tenet has been interpreted by many to suggest that exercise or mechanical stimulation will build bigger, stronger bones.[326,327] Unfortunately, the results from exercise intervention studies in the elderly have been disappointing, demonstrating modest or insignificant increases in bone mass even with substantial increase in muscle strength.[328,329] Evidence has accumulated suggesting that the bone-building effects of exercise depend greatly upon the type and intensity of exercise, the age of the individual, and the severity of bone loss at the start of the regimen.

Cross-sectional studies comparing active, athletic, and sedentary individuals have shown as much as 10−15% differences in BMD of the hip and spine.[330−332] These results have led to the belief that increased physical activity causes substantial changes in bone mass. However, the results might also reflect genetic selection, i.e., individuals who have larger muscles and stronger bones are more likely to choose an athletic lifestyle. More impressive were the results from Jones et al.[333] showing bone hypertrophy of about 30% in the dominant arm of professional tennis players, suggesting that indeed exercise can have dramatic effects on bone mass. However, prospective exercise trials in adults have demonstrated only modest improvement in bone mass.[329,334−340] It is unclear why the dramatic exercise-related differences in tennis players' bones cannot be duplicated by exercise regimens, yet there are at least two plausible explanations for this discrepancy. First, it has been suggested that the bone changes seen in the playing arm of tennis players are due to repair of overuse injuries;[341] thus one may have to exercise to the point of tissue injury to achieve dramatic changes in bone mass. Alternatively, it has been suggested that exercise affects bone mass most effectively in adolescents and young adults, but further accumulation of bone after cessation of growth does not necessarily occur.[342] All of the tennis players studied by Jones et al.[333] began playing the sport when they were children, and the bone hypertrophy observed in their dominant arm may have occurred well before they reached skeletal maturity. Therefore, this thesis is supported by animal studies[343,344] and recent observations of junior tennis and squash players.[345,346]

Different types of exercise have variable effects on the adult skeleton. Walking has little or no effect on bone mass in the spine or peripheral skeletal sites.[347,348,349] Aerobic training and weight lifting have been shown to

increase bone mass in the spine and hip by 1−2% per year in some studies.[329,335,338] However, positive changes in bone mass occurred only when the exercise regimen caused substantial change in muscle strength (30−60%, Lohman et al.;[329] 10−30%, Friedlander et al.[335]) or aerobic performance (16%, Snow-Harter et al.;[338] about 10%, Friedlander et al.[335]). In another study, weight lifting sufficient to increase muscle strength by 14% did not increase bone mass significantly.[337] Animal studies have shown that loading regimens which generate high strain rates maximize the osteogenic effect;[350,351] thus the most effective exercises for enhancing bone mass should be those that involve impact loading, such as high-impact aerobics, gymnastics, or volleyball. A cross-sectional study showed that young women who participated in gymnastics or volleyball had significantly higher bone mass in the hip and spine compared to competitive swimmers.[352] In a separate study of young women, gymnastics promoted a more rapid accretion of bone mineral than did running or swimming.[353] In older (>50 years, Welsh and Rutherford;[340] 39 years, Heinonen et al.[336]) subjects, high-impact aerobic exercise significantly increased hip BMD, although these increases were only modest (1−2%/yr). Another study in postmenopausal women showed no significant effect of high-impact exercise on bone mass at the hip after 1 year.[334]

Is exercise a therapy for osteoporosis? The answer is complex. Clearly exercise has a dramatic effect on bone mass in the growing skeleton and can improve peak bone mass, which will reduce the risk of osteoporosis.[342] However, once the skeleton stops growing, the effect of exercise on bone mass is modest, and considerably less than that reported after 3 years of treatment with alendronate.[201,207] Furthermore, the type of exercise that most effectively builds bone mass—impact loading—may also increase the risk of osteoarthritis.[354] In fact, analysis of the Framingham study cohort showed an association between physical activity and knee osteoarthritis in elderly subjects.[355] It appears that the type of exercise that is best for building bone mass may be bad for the joints. Therefore, prescription of weight-bearing exercise to improve bone architecture is probably misguided, especially for severely osteoporotic women. This does not mean that exercise should not be prescribed to reduce the risk of fractures for mildly osteoporotic women. Osteoporotic fractures, especially at the hip, are most often caused by mild trauma due to a fall or impact.[356] While exercise might not improve bone mass, it may slow the rate of bone loss and can dramatically improve balance and stability, thus reducing the risk of falling. Low-impact exercises such as walking and weight lifting, which do not damage the joints, can substantially increase muscle strength and balance and possibly reduce the risk of falling.[357] Future studies are needed to address the role of exercise in the prevention of falls and osteoporotic fractures.

Conclusions

Research into the medical issues faced by postmenopausal women represents one of the fastest growing areas of investigation in the biomedical field. Women are just beginning to enjoy the fruits of these activities with the approval of Fosamax in 1995, nasal calcitonin (Miacalcin) in 1996, and Evista in 1997. A rapid increase in the U.S. FDA registration of new osteoporosis agents is anticipated, as several dozen clinical trials of considerably diverse agents are in progress. Research into SERMs represents a departure from previous activities which have tended to focus exclusively on bone loss in osteoporosis. Current investigations appear to span the gamut from agents designed to prevent bone loss or treat only bone to those designed to also prevent or treat cardiovascular disease, breast and uterine cancer, improve CNS activity, improve muscle mass, and coordination. Calcitonin and alendronate reduce the risk of vertebral fractures in postmenopausal women by 30−50%. Newer agents under investigation have the possibility of reducing the fracture risk by even more.

We have attempted to review the major classes of pharmacological and nonpharmacological research activities that have been published. The authors apologize for any omissions which were not included because of our bias or ignorance. Specifically, there are exciting new developments with vitamin D analogues in Japan and Europe, which have not been discussed here but were reviewed recently by others.[358−361] Part of the rationale for the inclusion of the nonpharmacological section was to reiterate the importance of lifestyle changes in addition to pharmacological intervention. That is, in addition to the obvious activities that are deterimental to bone (heavy drinking, heavy lifting, smoking, etc.), proper diet (calcium intake of 1.5 g/day) and exercise will continue to be important in improving the quality of life for the individual. As the number of available therapeutic options continue to increase, the postmenopausal woman should be encouraged to actively participate with her physician in the decision making regarding the most appropriate course of treatment for her condition.

Biographies

Masahiko Sato, Ph.D., is a senior research scientist in the skeletal research group of the Endocrine Division of Lilly Research Laboratories and also an adjunct associate professor in the Department of Anatomy, Indiana University School of Medicine. Previous to joining Lilly in 1991, he was a research fellow in the bone division of Merck Research Laboratories.

Timothy A. Grese, Ph.D., is a research scientist in the Chemistry Division of Lilly Research Laboratories.

Jeffrey A. Dodge, Ph.D., is a research scientist in the Chemistry Division of Lilly Research Laboratories.

Henry U. Bryant, Ph.D., is the director of the skeletal research group in the Endocrine Division of Lilly Research Laboratories.

Charles H. Turner, Ph.D., is a professor and the director of Orthopaedic Research in the Department of Orthopaedic Surgery, Indiana University School of Medicine.

References

(1) Frost, H. M. Remodeling dynamics. In *Bone Remodeling Biodynamics*; Frost, H. M., Ed.; Little Brown: Boston, 1963; pp 65−78.
(2) Parfitt, A. M. Quantum concept of bone remodeling and turnover: implications for the pathogenesis of osteoporosis. *Calcif. Tissue Int.* **1979**, *28*, 1−5.
(3) Kanis, J. A.; Melton, L. J., III; Christiansen, C.; Jonston, C. C.; Khalaev, N. The diagnosis of osteoporosis. *J. Bone Miner. Res.* **1994**, *9*, 1137−41.
(4) Kanis, J. A.; Devogelaer, J. P.; Gennari, C. Practical guide for the use of bone mineral measurements in the assessment of treatment of osteoporosis: a position paper of the European Foundation for Osteoporosis and Bone Disease. *Osteoporosis Int.* **1996**, 6, 256−261.

(5) Hui, S. L.; Slemenda, C. W.; Johnston, C. C., Jr. Age and bone mass as predictors of fracture in a prospective study. *J. Clin. Invest.* **1988,** *81*, 1804−9.
(6) Slemenda, C. W.; Hui, S. L.; Longcope, C.; Wellman, H.; Johnston, C. C., Jr. Predictors of bone mass in perimenopausal women, a prospective study of clinical data using photon absorptiometry. *Ann. Intern. Med.* **1990**, *112*, 96−101.
(7) Marshall, D.; Johnell, O.; Wedel, H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *Br. Med. J.* **1996**, *312*, 1254−1259.
(8) Melton, L. J., III; Chrischilles, E. A.; Cooper, C.; Lane, A. W.; Riggs, B. L. How many women have osteoporosis? *J. Bone Miner. Res.* **1992**, *7*, 1005−1010.
(9) Eddy, D.; Cummings, S. R.; Dawson-Hughes B. Guidelines for the prevention, diagnosis and treatment of osteoporosis: Cost-effectiveness analysis and review of the evidence. *Osteoporosis Int.*, in press.
(10) Johnell, O.; Gullberg, B.; Allander, E.; Kanis, J. A. The apparent incidence of hip fractures in Europe: A study of national register sources. *Osteoporosis Int.* **1992**, *2*, 298−302.
(11) Cummings, S. R.; Nevitt, M. C.; Browner, W. S.; Stone, K.; Fox, K. M.; Ensrud, K. E.; Cauley, J.; Black, D.; Vogt, T. M. Risk fractors for hip fracture in white women. *N. Engl. J. Med***. 1995**, *332*, 767−773.
(12) Barrett-Connor, E. The economic and human costs of osteoporotic fracture. *Am. J. Med.* **1995**, 98 (Suppl. 2A), 3S−8S.
(13) (a) Melton, L. J., III. Epidemiology of spinal osteoporosis. *Spine* **1997**, *22* (Suppl. 24S), 2s−11s. (b) Nevitt, M. C.; Ettinger, B.; Black, D. M.; Stone, K.; Jamal, S. A.; Ensrud, K.; Segal, M.; Genant, H. K.; Cummings, S. R. The association of radiographically detected vertebral fractures with back pain and function: a prospective study. *Ann. Intern. Med.* **1998**, *128*, 793−800.
(14) Cooper, C.; Campion, G.; Melton, L. J. Hip fractures in the elderly: A worldwide projection. *Osteoporosis Int.* **1992**, *2*, 285−289.
(15) Kanis, J. A.; McCloskey, E. V. Evaluation of the risk of hip fracture. *Bone* **1996**, *18* (Suppl. 3), 127s−132s.
(16) Schneider, E. L.; Guralnik, J. M. The aging of America. *JAMA, J. Am. Med. Assoc.* **1990**, *263*, 2335−40.
(17) Cummings, S. R., Rubin, S. M., Black, D. The future of hip fractures in the United States: Numbers, costs, and potential effects of postmenopausal estrogen. *Clin. Orthop.* **1990**, *252*, 163−6.
(18) Kimmel, D. Animal models for in vivo experimentation in osteoporosis research. *Osteoporosis*; Marcus, R., Feldman, D., Kelsey, J., Ed.; Academic Press: San Diego, 1996; pp 671−690.
(19) Mitlak, B. H.; Sato, M. Bone mineral measurements by DXA in animals. In *Methods in Bone Biology*; Arnett,T. R., Henderson, B., Eds.; Chapman & Hall: London, 1998; pp 273−289.
(20) Turner, C. H.; Burr, D. B. Basic biomechanical measurements of bone: a tutorial. *Bone* **1993**, *14*, 595−608.
(21) Turner, R. T.; Vandersteenhoven, J. J.; Bell, N. H.; The effects of ovariectomy and 17β estradiol on cortical bone histomorphometry in growing rats. *J. Bone Miner. Res.* **1987**, *2*, 115−122.
(22) Wronski, T. J.; Cintron, M.; Doherty, A. L.; Dann, L. M. Estrogen treatment prevents osteopenia and depresses bone turnover in ovariectomized rats. *Endocrinology* **1988**, *123*, 681−686.
(23) Durbridge, T. C.; Morris, H. A.; Parsons, A. M.; Parkinson, I. H.; Moore, R. J.; Porter, S.; Need, A. G.; Nordin, B. E. C.; Vern-Roberts, B. Progressive cancellous bone loss in rats after adrenalectomy and oophorectomy. *Calcif. Tissue Int.* **1990**, *47*, 383−387.
(24) Wronski, T. H.; Yen, C. F.; Burton, K. W.; Mehta, R. C.; Newman, P. S.; Soltis, E. E.; DeLuca, P. P. Skeletal effects of calcitonin on ovariectomized rats. *Endocrinology* **1991**, *129*, 2246−2250.
(25) Seedor, J. G.; Quartuccio, H. A.; Thompson, D. D. The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. *J. Bone Miner. Res.* **1991**, *6*, 339−346.
(26) Toolan, B. C.; Shea, M.; Myers, E. R.; Borchers, R. E.; Seedor, J. G.; Quartuccio, H.; Rodan, G.; Hayes, W. C. Effects of ABP on bone biomechanics in rats. *J. Bone Miner. Res.* **1992**, *12*, 1399−1406.
(27) Turner, R. T.; Wakley, G. K.; Hannon, K. S.; Bell, N. H. Tamoxifen inhibits osteoclast mediated resorption of trabecular bone in ovarian hormone deficient rats. *Endocrinology* **1988**, *122*, 1146−1150.
(28) Kalu, D. N.; Salerno, E.; Liu, C. C.; Echon, R.; Ray, M.; Garza-Zapata, M.; Hollis, B. W. A comparative study of the actions of tamoxifen, estrogen and progesterone in the ovariectomized rat. *Bone Miner.* **1991**, *15*, 109−124.
(29) Moon, L. Y.; Wakley, G. K.; Turner, R. T. Dose-dependent effects of tamoxifen on long bones in growing rats: Influence of ovarian status. *Endocrinology* **1991**, *129*, 1568−1574.
(30) Black, L. J.; Sato, M.; Rowley, E. R.; Magee, D. E.; Bekele, A.; Williams, D. C.; Cullinan, G. J.; Bendele, R.; Kauffman, R. F.; Bensch, W. R.; Frolik, C. A.; Termine, J. D.; Bryant, H. U. Raloxifene (LY139481 HCl) prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats. *J. Clin. Invest.* **1994**, *72*, 63−69.
(31) Sato, M.; McClintock, C.; Kim, J.; Turner, C.; Bryant, H.; Magee, D.; Slemenda, C. Dual-energy X-ray absorptiometry of raloxifene effects on the lumbar vertebrae and femora of ovariectomized rats. *J. Bone Miner. Res.* **1994**, *9*, 715−724.
(32) Sato, M.; Kim, J.; Short, L. L.; Slemenda, C. W.; Bryant, H. U. Longitudinal and cross-sectional analysis of raloxifene effects on tibiae from ovariectomized aged rats. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 1252−1259.
(33) Sato, M.; Rippy, M. K.; Bryant, H. U. Raloxifene, tamoxifen, nafoxidine, or estrogen effects on reproductive and nonreproductive tissues in ovariectomized rats. *FASEB J.* **1996**, *10*, 905−912.
(34) Sato, M.; Bryant, H.; Iversen, P.; Helterbrand, J.; Smietana, F.; Bemis, K.; Higgs, R.; Owan, I.; Takano, T.; Burr, D. Advantages of raloxifene over alendronate or estrogen on Nonreproductive and reproductive tissues in the long-term dosing of ovariectomized rats. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 298−305.
(35) Turner, C. H.; Akhter, M. P.; Heaney, R. P. The effects of fluoridated water on bone strength. *J. Orthop. Res.* **1992**, *10*, 581−587.
(36) Sogaard, C. H.; Mosekile, L.; Schwartz, W.; Leidig, G.; Minne, H. W.; Ziegler, R. Effects of fluoride on rat vertebral body biomechanical competence and bone mass. *Bone* **1995**, *16*, 163−169.
(37) Jerome, C. P.; Turner, C. H.; Lees, C. J. Decreased bone mass and strength in ovariectomized Cynomolgus monkeys (Macaca fascicularis). *Calcif. Tissue Int.* **1997**, *60*, 265−270.
(38) Thompson, D. D.; Seedor, J. G.; Quartuccio, H.; Solomon, H.; Fioravanti, C.; Davidson, J.; Klein, H.; Jackson, R.; Clair, J.; Frankenfield, D.; Brown, E.; Simmons, H. A.; Rodan, G. A. The bisphosphonate, alendronate, prevents bone loss in ovariectomized baboons. *J. Bone Miner. Res.* **1992**, *7*, 951−960.
(39) Balena, R.; Toolan, B. C.; Shea, M.; Markatos, A.; Myers, E. R.; Lee, S. C.; Opas, E. E.; Seedor, J. G.; Klein, H.; Frankenfield, D.; Quartuccio, H.; Fioravanti, C.; Clair, J.; Brown, E.; Hayes, W. C.; Rodan, G. A. The effects of 2-year treatment with the aminobisphosphonate alendronate on bone metabolism, bone histomorphometry, and bone strength in ovariectomized nonhuman primates. *J. Clin. Invest.* **1993**, *92*, 2577−2586.
(40) Kasra, M.; Grynpas, M. Effect of long-term ovariectomy on bone mechanical properties in young female cynomolgus monkeys. *Bone* **1994**, *15*, 557−561.
(41) Lundon, K.; Dumitriu, M.; Grynpas, M. The long-term effect of ovariectomy on the quality and quantity of cancellous bone in young macaques. *Bone Miner.* **1994**, *24*, 135−149.
(42) Jerome, C. P.; Lees, C. J.; Weaver, D. S. Development of osteopenia in ovariectomized cynomolgus monkeys (Macaca fascicularis). *Bone* **1995**, *17* (Suppl. 4), 403S−408S.
(43) Burr, D. B. Estimated intracortical bone turnover in the femur of growing macaques: implications for the use as models in skeletal pathology. *Anat. Rec.* **1992,** *232*, 180−189.
(44) Parfitt, A. M.; Drezner, M. K.; Glorieux, F. H.; Kanis, J. A.; Malluche, H.; Meunier, P. J.; Ott, S. M.; Recker, R. R. Bone histomorphometry: standardization of nomenclature, symbols, and units. *J. Bone Miner. Res.* **1987**, *2*, 595−610.
(45) Hagiwara, S.; Yang, S. O.; Gluer, C. C.; Bendavid, E.; Genant, H. K. Noninvasive bone mineral density measurement in the evaluation of osteoporosis. *Osteoporosis* **1994**, *20*, 651−669.
(46) Genant, H. K.; Lang, T. F.; Engelke, K.; Fuerst, T.; Gluer, C. C.; Majumdar, S.; Jergas, M. Advances in the noninvasive assessment of bone density, quality, and structure. *Calcif. Tissue Int.* **1996**, *59* (Suppl. 1), S10−S15.
(47) Turner, C. H.; Peacock, M.; Timmerman, L.; Neal, J. M.; Johnston, C. C. Calcaneal ultrasonic measurements discriminate hip fracture independently of bone mass. *Osteoporosis Int.* **1995**, *5*, 130−135.
(48) Bouxsein, M. L.; Courtney, A. C.; Hayes, W. C. Ultrasound and densitometry of the calcaneus correlate with the failure loads of cadaveric femurs. *Calcif. Tissue Int.* **1995**, *56*, 99−103.
(49) Heaney, R. P.; Kanis, J. A. The interpretation and utility of ultrasound measurements of bone. *Bone* **1996**, *18*, 491−2.
(50) Lane, N. E.; Thompson, J. M.; Strewler, G. J.; Kinney, J. H. Intermittent treatment with human parathyroid hormone (hPTH-[1-34]) increased trabecular bone volume but not connectivity in osteopenic rats. *J. Bone Miner. Res.* **1995**, *10*, 1470−1477.
(51) Ruegsegger, P.; Koller, S.; Muller, R. A microtomographic system for the nondestructive evaluation of bone architecture. *Calcif. Tissue Int.* **1996**, *58*, 24−29.

(52) Schiessl, H.; Ferretti, J. L.; Tysarczyk-Niemeyer, G.; Willnecker, J. Noninvasive bone strength index as analyzed by peripheral quantitative computed tomography (pQCT). In *Paediatric osteology: new developments in diagnostics and therapy*; Schonau, E., Ed.; Elsevier Science B.V.: Amsterdam, 1996; pp 141−146.
(53) Helterbrand, J. D.; Higgs, R. E.; Iversen, P. W.; Tysarczyk-Niemeyer, G.; Sato, M. Application of autormatic image segmentation to tibiae and vertebrae from ovariectomized rats. *Bone* **1997**, *21*, 401−409.
(54) Westerlind, K. C.; Wronski, T. J.; Ritman, E. L.; Luo, Z. P.; An, K. N.; Bell, N. H.; Turner, R. T. Estrogen regulates the rate of bone turnover but bone balance in ovariectomized rats is modulated by prevailing mechanical strain. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 4199−4204.
(55) Einhorn, T. Bone stength: The bottom line. *Calcif. Tissue Int.* **1992**, *51*, 333−339.
(56) Martin, R. B. Aging and strength of bone as a structural material. *Calcif. Tissue Int.* **1993**, *53* (Suppl. 1), S34−S40.
(57) Longcope, C.; Franz, C.; Morello, C.; Baker, R.; Johnston, C. C. Steroid and gonadotropin levels in women during the perimenopausal years. *Maturitas* **1986**, *8*, 189−196.
(58) Turner, R. T.; Riggs, B. L.; Spelsberg, T. C. Skeletal effects of estrogen. *Endocr. Rev.* **1994**, *15*, 275−300.
(59) Kuiper, G. G.; Enmark, E.; Pelto-Huikko, M.; Nilsson, S.; Gustafsson, J. A. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, 93, 5925−5930. Kuiper, G. G.; Carlsson, B.; Grandien, K.; Enmark, E.; Haggblad, J.; Nilsson, S.; Gustafsson, J. A. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. *Endocrinology* **1997**, *138*, 863−870.
(60) Anderson, J. M.; Peck, E. J.; Clark, J. Nuclear receptor estrogen complex: relationship between concentration and early uterotrophic responses. *Endocrinology* **1973**, *92*, 1488−1495.
(61) Higgins, S. J.; Rousseau, G.; Baxter, J.; Tompkins, G. Nature of nuclear acceptor sites for glucocorticoid and estrogen receptor complexes. *J. Biol. Chem.* **1973**, *248*, 5873−5881.
(62) Komm, B. S.; Terpening, C.; Benz, D.; Graeme, K.; Gallegos, A.; Korc, M.; Greene, G. L.; O'Malley, B. W.; Haussler, M. R. Estrogen binding, receptor mRNA, and biologic response in osteoblast-like osteosarcoma cells. *Science* **1988**, *241*, 81−84.
(63) Eriksen, E.; Colvard, D.; Berg, N.; Graham, M.; Mann, K.; Spelsberg, T. C.; Riggs, B. L. Evidence of estrogen receptors in normal human osteoblast-like cells. *Science* **1988**, *241*, 84−87.
(64) Etienne, M.; Fischel, J. L.; Milano, G.; Formento, P.; Formento, J. L.; Faucoual, M.; Fenoy, M.; Maner, M. Steroid receptors in human osteoblast-like cells. *Eur. J. Cancer* **1990**, *26*, 807−810.
(65) Ikegami, A.; Inoue, S.; Hosoi, T.; Mizuno, Y.; Nakamura, T.; Ouchi, Y.; Orimo, H. Immunohistochemical detection and northern blot analysis of estrogen receptor in osteoblastic cells. *J. Bone Miner. Res.* **1993**, *8*, 1103−1109.
(66) Ciocca, D. R.; Roig, L. M. V. Estrogen receptors in human nontarget tissues: biological and clinical implications. *Endocr. Rev.* **1995**, *16*, 35−62.
(67) Onoe, Y.; Miyaura, C.; Ohta, H.; Nozawa, S.; Suda, T. Expression of estrogen receptor β in rat bone. *Endocrinology* **1997**, *138*, 4509−4512.
(68) Burch, J. C.; Byrd, B. F.; Vaughn, W. K. The effects of long-term estrogen on hysterectomized women. *Am. J. Obstet. Gynecol.* **1974**, *118*, 778−782.
(69) Lindsay, R.; Hart, D. M.; Aitken, J. M.; MacDonald, E. B.; Anderson, J. B.; Clark, A. C. Long-term prevention of postmenopausal osteoporosis by estrogen: evidence for an increased bone mass after delayed onset of estrogen treatment. *Lancet* **1976**, *1*, 1038−41.
(70) Weiss, N. S.; Ure, C. L.; Ballard, J. H.; Williams, A. R.; Daling, J. R. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. *N. Engl. J. Med.* **1980**, *303*, 1195−1198.
(71) Lindsay, R; Hart, D. M.; Forrest, C.; Baird, C. Prevention of spinal osteoporosis in oopherectomized women. *Lancet* **1980**, *2*, 1151−1154.
(72) Quigley, M. E. T.; Martin, P. L.; Curnier, A. M.; Brooks, P. Estrogen therapy arrests bone loss in elderly women. *Am. J. Obstet. Gynecol.* **1987**, *156*, 1516−1523.
(73) Moore, M.; Bracker, M.; Sartoris, D. Long-term estrogen replacement therapy in postmenopausal women sustains vertebral bone mineral density. *J. Bone Miner. Res.* **1990**, *5*, 659−664.
(74) Lindsay, R.; Tohme, J. F. Estrogen treatment of patients with established postmenopausal osteoporosis. *Obstet. Gynecol.* **1990**, *76*, 290−295.
(75) Cauley, J. A.; Seeley, D. G.; Ensrud, K.; Ettinger, B.; Black, D.; Cummings, S. R. Estrogen replacement therapy and fractures in older women. *Ann. Intern. Med.* **1995**, *122*, 9−16.
(76) Bush, T. L.; Wells, H. B.; James, M. K.; Barrett, Connor E.; Marcus, R.; Greendale, G. Effects of hormone therapy on bone mineral density: results from the postmenopausal estrogen/progestin interventions (PEPI) trial. *JAMA, J. Am. Med. Assoc.* **1996**, *276*, 1389−1396.
(77) Schneider, K. L.; Barrett-Connor, E. L.; Morton, M. A. Timing of postmenopausal estrogen for optimal bone mineral density. *JAMA, J. Am. Med. Assoc.* **1997**, *277*, 543−547.
(78) Hammond, C. B.; Jelovsek, F. R.; Lee, K. L.; Creasman, W. T.; Parker, R. T. Effects of long-term estrogen therapy. I. Metabolic effects. *Am. J. Obstet. Gynecol.* **1979**, *133*, 525−536.
(79) Stampfer, M. J.; Colditz, G. A.; Willet, W. C.; Manson, J. E.; Rosner, B.; Speizer, F. E.; Hennekens, C. H. Postmenopausal estrogen therapy and cardiovascular disease: Ten-year follow-up from the nurses' health study. *N. Engl. J. Med.* **1991**, *325*, 756−762. Grady, D., Rubin, S. M., Petitti, D. B. Hormone therapy to prevent disease and prolong life in postmenopausal women. *Ann. Intern. Med.* **1992**, *117*, 1016−1037.
(80) Devor, M.; Barrett-Conner, E.; Renvall, M.; Feigal, D.; Ramsdell, J. Estrogen replacement therapy and the risk of venous thrombosis. *Am. J. Med.* **1992**, *92*, 275−282.
(81) (a) Lip, F. Y. H.; Beevers, G.; Zarifis, J. Hormone replacement therapy and cardiovascular risk: the cardiovascular physicians viewpoint. *J. Intern. Med.* **1995**, *238*, 389−399. (b) The Writing Group for the PEPI trial. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. *JAMA, J. Am. Med. Assoc.* **1995**, *273*, 199−208.
(82) Grodstein, F.; Stampfer, M. J.; Colditz, G. A.; Willett, W. C.; Manson, J. E.; Joffe, M.; Rosner, B.; Fuchs, C.; Hankinson, S. E.; Hunter, D. J.; Hennekens, C. H.; Speizer, F. E. Postmenopausal hormone therapy and mortality. *New Eng. J. Med.* **1997**, *336*, 1769−1822.
(83) (a) Hulley, S.; Grady, D.; Bush, T.; Furberg, C.; Herrington, D.; Riggs, B.; Vittinhoff, E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *JAMA, J. Am. Med. Assoc.* **1998**, *280*, 605−613. (b) Petitti, D. B. Hormone replacement therapy and heart disease prevention: experimentation trumps observation. *JAMA, J. Am. Med. Assoc.* **1998**, *280*, 650−652.
(84) Ziel, H. K.; Finkle, W. D. Increased risk of endometrial carcinoma among users of conjugated estrogens. *N. Engl. J. Med.* **1975**, *293*, 1167−1170.
(85) Smith, D. C.; Prentice, R.; Thompson, D. J.; Herrmann, W. L. Association of exogenous estrogen and endometrial carcinoma. *N. Engl. J. Med.* **1975**, *293*, 1164−1167.
(86) Vesey, M. P. Exogenous hormones in the aetiology of cancer in women. *J. R. Soc. Med.* **1984**, *77*, 542−549.
(87) Steinberg, K. K.; Thacker, S. B.; Smith, S. C. A meta-analysis of the effect of estrogen replacement therapy on the risk of breast cancer. *JAMA, J. Am. Med. Assoc.* **1991**, *265*, 1985−1990.
(88) Dupont, W. D.; Page, D. L. Menopausal estrogen replacement therapy and breast cancer. *Arch. Int. Med.* **1991**, *151*, 67−72.
(89) Stanford, J. L. The benefits of hormone replacement therapy outweigh the breast-cancer risks for some women. *J. Natl. Inst. Health Res.* **1996**, *8*, 40−44.
(90) Colditz, G. A. The benefits of hormone replacement therapy do not outweigh the increased risk of breast cancer. *J. Natl. Inst. Health. Res.* **1996**, *8*, 41−44.
(91) Gibaldi, M. Prevention and treatment of osteoporosis: does the future belong to hormone replacement therapy? *J. Clin. Pharmacol.* **1997**, *37*, 1087−1099.
(92) Genant, H. K.; Lucas, J.; Weiss, S.; Akin, M.; Emkey, R. Low-dose esterified estrogen therapy: Effects on bone, plasma estradiol concentrations, endometrium and lipid levels. *Arch. Intern. Med.* **1997**, *157*, 2609−2615.
(93) Lindsay, R.; Hart, D. M.; Clark, B. M. The minimum effective dose of estrogen for prevention of postmenopausal bone loss. *Obstet. Gynecol.* **1984**, *63*, 759−763.
(94) Lufkin, E. G.; Wahner, H. W.; O'Fallon, W. M.; Hodgson, S. F.; Kotowicz, M. A.; Lane, A. W. *Ann. Intern. Med.* **1992**, *117*, 1−9.
(95) Connell, E. B. Transdermal estrogen therapy. *Postgrad. Med.* **1997**, *101* (6), 115−116, 122, 128−130.
(96) Bhavnani, B. R.; Woolever, C. A. Interaction of ring B unsaturated estrogens with estrogen receptors of human endometrium and rat uterus. *Steroids* **1991**, *56*, 201.
(97) Washburn, S. A.; Adams, M. R.; Clarkson, T. C.; Adelman, S. J. A conjugated equine estrogen with differential effects on uterine weight and plasma cholesterol in the rat. *Am. J. Obstet. Gynecol.* **1993**, *169*, 251.
(98) Washburn, S. A.; Lewis, C. E.; Johnson, J. E.; Voytko, M. L.; Shively, C. A. 17α-dihydroequilenin increases hippocampal dendritic spine density of ovariectomized rats. *Brain Res.* **1997**, *758*, 241.
(99) Dodge, J. A.; Magee, D. E.; Shetler, P.; Cole, H.; Adrian, D.; Bryant, H. U. Premarin components: uterine, cholesterol lowering and bone metabolic effects. *10th Int. Congr. Endocrinol.* **1996**, P1−249.
(100) Wilcox, J. G.; Hwang, J.; Hodis, H. N.; Sevanian, A.; Stanczyk, F. Z.; Lobo RA. Cardioprotective effects of individual conjugated equine estrogens through their possible modulation of insulin resistance and oxidation of low-density lipoprotein. *Fertil. Steril.* **1997**, *67*, 57−62.

(101) Kneifel, M. A.; Leytus, S. P.; Fletcher, E.; Weber, T.; Mangel, W. F.; Katzenellenbogen, B. A. Uterine plasminogen activator activity: modulation by steroid hormones. *Endocrinology* **1982**, *111*, 493.
(102) Lemon, L. H. Pathophysiologic considerations in the treatment of menopausal patients with oestrogen: the role of oestriol in the prevention of mammery carcinoma. *Acta Endocrinol. Supp.* **1980**, *233*, 17−27.
(103) Fotsis, T; Zhang, Y.; Pepper, M. S.; Adlercreutz, H.; Montesano, R.; Nawroth, P. P.; Schweigerer, L. The endogenous estrogen metabolite 2-methoxyestradiol inhibits tubulin angiogenesis and suppresses tumor growth. *Nature* **1994**, *268*, 237.
(104) Cushman, M.; He, H−M.; Katzenellenbogen, J. A.; Varma, R. K.; Hamel, E.; Lin, C, M.; Ram, S.; Sachdeva, Y. P. Synthesis of analogues of 2-methoxyestradiol and enhanced inhibitory effects of tubulin polymerization and cancer cell growth. *J. Med. Chem.* **1997**, *40*, 2323.
(105) Mueller, G. P.; Johns, W. F.; Cook, D. L.; Edgren, R. A. 16α-chloro and 16α-iodoestrone methyl ether, new and potent lipid-shifting agents. *J. Am. Chem. Soc.* **1958**, *80*, 1769−1770.
(106) Chinn, L. J.; Dygos, J. H.; Mares, S. E.; Aspinall, R. L.; Ranney, R. E. 9,11-Seco Steroids. An attempt to separate biological activities via ring cleavage. *J. Med. Chem.* **1974**, *17*, 351.
(107) Dygos, J. H.; Chinn, L. J. Synthesis of 9,11-secoestradiol 3-methyl ether. *J. Org. Chem.* **1975**, *40*, 685.
(108) Lippuner, K.; Haenggi, W.; Birkhaeuser, M. H. Casez, J. P.; Jaeger, P. Prevention of postmenopausal bone loss using tibolone or conventional peroral or transdermal hormone replacement therapy with 17beta-estradiol and dydrogesterone. *J. Bone Miner. Res.* **1997**, *12* (5), 806−12.
(109) Tax, L.; Goorissen, E. M.; Kicovic, P. M. Clinical profile of Org OD 14. *Maturitas* **1987**, Suppl. 1, 3−13.
(110) Fuse, H.; Fukumoto, S.; Sone, H.; Miyata, Y.; Saito, T.; Nakayama, K.; Takahashi, H.; Matsumoto, T.; Ogata, E. A new synthetic steroid, osaterone acetate (TZP-4238), increases cortical bone mass and strength by enhancing bone formation in ovariectomized rats. *J. Bone Miner. Res.* **1997**, *12*, 590−597.
(111) Wagner, J. D.; Cefalu, W. T.; Anthony, M. S.; Litwak, K. N.; Zhang, L.; Clarkson, T. B. Dietary Soy Protein and Estrogen Replacement Therapy Improve Cardiovascular Risk Factors and Decrease Aortic cholesteryl ester content in ovariectomized cynomologus monkeys. *Metabolism* **1997**, *46*, 698−705.
(112) Goodman, M. T.; Wilkens, L. R.; Hankin, J. H.; Lyu, L. C.; Wu, A. H.; Kolonel, L. N. Association of soy and fiber consumption with the risk of endometrial cancer *Am. J. Epidemiol.* **1997**, *146*, 294−305.
(113) Dodge, J. A.; Glasebrook, A. L.; Magee, D. E.; Phillips, D. L.; Sato, M.; Short, L. L.; Bryant, H. U. Environmental estrogens: effects on cholesterol lowering and bone in the ovx rat. *J. Steroid. Biochem. Molec. Biol.* **1996**, *59*, 155−161.
(114) Tsutsumi, N. The effect of coumestrol on bone metabolism in organ culture. *Biol. Pharm. Bull.* **1995**, *18*, 1012−1015.
(115) Gradishar, W. J.; Jordan, V. C. Clinical potential of new antiestrogens. *J. Clin. Oncol.* **1997**, *15*, 840−852.
(116) Jordan, V. C.; MacGregor, J. I.; Tonetti, D. A. Tamoxifen: from breast cancer therapy to the design of a postmenopausal prevention maintenance therapy. *Osteoporosis Int.* **1997**, *7* (Suppl. 1), S52−7.
(117) Grese, T. A.; Dodge, J. A. Selective estrogen receptor modulators (SERMs). *J. Med. Chem.*, in press.
(118) Willson, T. M.; Norris, J. D.; Wagner, B. L.; Asplin, I.; Baer, P.; Brown, H. R.; Jones, S. A.; Henke, B.; Sauls, H.; Wolfe, S.; Morris, D. C.; McDonnell, D. P. Dissection of the molecular mechanism of action of GW5638, a novel estrogen receptor ligand, provides insights into the role of estrogen receptor in bone. *Endocrinology* **1997**, *138* (9), 3901−3911.
(119) Love, R. R.; Mazess, R. B.; Barden, H. S.; Epstein, S.; Newcomb, P. A.; Jordan, V. C.; Carbone, P. P.; DeMets, D. L. Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. *N. Engl. J. Med.* **1992**, *326*, 852−856.
(120) Sunderland, M. C.; Osborne, C. K. Tamoxifen in premenopausal patients with metastatic breast cancer: a review. *J. Clin. Oncol.* **1991**, *9*, 1283−1297.
(121) Love, R. R.; Cameron, L.; Cornell, B. L.; Leventhal, H. Symptoms associated with tamoxifen treatment in postmenopausal women. *Arch. Int. Med.* **1991,** *151*, 1842−1847.
(122) Chander, S. K.; Sahota, S. S.; Evans, T. R. J.; Luqmani, Y. A. The biological evaluation of novel anti-estrogens for the treatment of breast cancer. *Crit. Rev. Oncol. Hematol.* **1993**, *15*, 243−269.
(123) Magarian, R. A.; Overacre, L. B.; Singh, S.; Meyer, K. L. The medicinal chemistry of nonsteroidal antiestrogens: a review. *Curr. Med. Chem.* **1994**, *1*, 61−104.
(124) Fisher, B.; Dignam, J.; Bryant, J.; DeCillis, A.; Wickerham, D. L.; Wolmark, N.; Costantino, J.; Redmond, C.; Fisher, E. R.; Bowman, D. M.; Deschenes, L.; Dimitrov, N. V.; Margolese, R. G.; Robidoux, A.; Shibata, H.; Terz, J.; Paterson, A. H. G.; Feldman, M. I.; Farrar, W.; Evans, J.; Lickley, H. L. Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. *J. Natl. Cancer Inst.* **1996**, *88*, 1529−1542.
(125) Kedar, R. P.; Bourne, T. H.; Powles, T. J.; Collins, W. P.; Ashley, S. E.; Cosgrove, D. O.; Cambell, S. Effects of tamoxifen on uterus and ovaries of postmenopausal women in a randomized breast cancer prevention trial. *Lancet* **1994**, *343*, 1318−1321.
(126) Kuo, D. Y. S.; Runowicz, C. D. Gynecologic effects of tamoxifen. *Med. Oncol.* **1995**, *12*, 87−94.
(127) Robinson, D. C.; Bloss, J. D.; Schiano, M. A. A retrospective study of tamoxifen and endometrial cancer in breast cancer patients. *Gynecol. Oncol.* **1995**, *59*, 186−190.
(128) Assikis, V. J.; Neven, P.; Jordan, V. C.; Vergote, I. A realistic clinical perspective of tamoxifen and endometrial carcinogenesis. *Eur. J. Cancer* **1996**, 32*A*, 1464−1476.
(129) Osborne, M. R.; Hewer, A.; Hardcastle, I. R.; Carmichael, P. L.; Phillips, D. H. Identification of the major tamoxifen-deoxyguanosine adduct formed in the liver DNA of rats treated with tamoxifen. *Cancer Res.* **1996**, *56*, 66−71.
(130) Hemminki, K.; Rajaniemi, H.; Lindahl, B.; Moberger, B. Tamoxifen-induced DNA adducts in endometrial samples from breast cancer patients. *Cancer Res.* **1996**, *56*, 4374−4377.
(131) Williams, G. M.; Iatropoulos, M. J.; Djordjevic, M. V.; Kaltenberg, O. The triphenylethylene drug tamoxifen is a strong liver carcinogen in the rat. *Carcinogenesis* **1993**, *14*, 315−317.
(132) Di Salle, E.; Zaccheo, T.; Ornati, G. Antiestrogenic and antitumor properties of the new triphenylethylene derivative toremifene in the rat. *J Steroid Biochem.* **1990**, *36*, 203−206.
(133) White, I. N. H.; de Matteis, F.; Davies, A.; Smith, L. L.; Crofton-Sleigh, C.; Venitt, S.; Hower, A.; Phillips, D. H. I. Genotoxic potential of tamoxifen and analogues in female Fischer F344/n rats, DBA/2 and C57BL/6 mice and in human MCL-5 cells. *Carcinogenesis* **1992**, *13*, 2197−2203.
(134) Dahme, E. G.; Rattel, B. *J. Cancer Res. Clin. Oncol.* **1992**, *118* (Suppl. l), R172.
(135) Wiseman, L. R.; Goa, K. L. Toremifene. A review of its pharmacological properties and clinical efficacy in the management of advanced breast cancer. *Drugs* **1997**, *54*, 141−160.
(136) Rauschning, W.; Pritchard, K. I. Droloxifene, a new antiestrogen: its role in metastatic breast cancer. *Breast Cancer Res. Treat.* **1994**, *31*, 83−94.
(137) Johnston, S. R. D.; Riddler, S.; Haynes, B. P.; Hern, R. A.; Smith, I. E.; Jarman, M.; Dowsett, M. The novel anti-oestrogen idoxifene inhibits the growth of human MCF-7 breast cancer xenografts and reduces the frequency of acquired anti-oestrogen resistance. *Br. J. Cancer* **1997**, *75*, 804−809.
(138) Toko, T.; Shibata, J.; Nukatsuka, M.; Yamada, Y. Antiestrogenic activity of DP-TAT-59, an active metabolite of TAT-59 against human breast cancer. *Cancer Chemother. Pharmacol.* **1997**, *39*, 390−398.
(139) Brewster, M. E.; Paran, Y.; Rushkin, E.; Biegon, A; Pop, E.; Degani, H. Evaluation of the anticancer action of a permanently charged tamoxifen derivative, tamoxifen methiodide: an MRI study. *Int. J. Pharm.* **1997**, *153*, 147−157.
(140) Niikura, K.; Nakajima, Y.; Nishio, M.; Nakayama, O.; Kojo, H.; Notsu, Y.; Ono, T. Effects of droloxifene on bone mineral content in rat models. *Jpn. J. Pharmacol.* **1992**, *58* (Suppl. 1), 361.
(141) Ke, H. Z.; Chen, H. K.; Simmons, H. A.; Qi, H.; Crawford, D. T.; Pirie, C. M.; Chidsey-Frink, K. L.; Ma, Y. F.; Jee, W. S. S.; Thompson, D. D. Comparative effects of droloxifene, tamoxifen, and estrogen on bone, serum cholesterol, and uterine histology in the ovariectomized rat model. *Bone* **1997**, *20*, 31−39.
(142) Tomas, E.; Kauppila, A.; Blanco, G.; Apaja-Sarkkinen, M.; Laatikainen, T. Comparison between the effects of tamoxifen and toremifene on the uterus in postmenopausal breast cancer patients. *Gynecol. Oncol.* **1995**, *59*, 261−266.
(143) Hayes, D. F.; Van Zyl, J. A.; Hacking, A.; Goedhals, L.; Bezwoda, W. R.; Mailliard, J. A.; Jones, S. E.; Vogel, C. L.; Berris, R. F.; Shemano, I.; et al. Randomized comparison of tamoxifen and two separate doses of toremifene in postmenopausal patients with metastatic breast cancer. *J. Clin. Oncol.* **1995**, *13*, 2556−66.
(144) Pyrhonen, S.; Valavaara, R.; Modig, H.; Pawlicki, M.; Pienkowski, T.; Gundersen, S.; Bauer, J.; Westman, G.; Lundgren, S.; Blanco, G.; Mella, O.; Nilsson, I.; Hietanen, T.; Hindy, I.; Vuorinen, J.; Hajba, A. Comparison of toremifene and tamoxifen in postmenopausal patients with advanced breast cancer: a randomized double-blind, the ′nordic′ phase III study. *Br. J. Cancer* **1997**, *76*, 270−7.
(145) Gershanovich, M.; Garin, A.; Baltina, D.; Kurvet, A.; Kangas, L.; Ellmen, J. A phase III comparison of two toremifene doses to tamoxifen in postmenopausal women with advanced breast cancer. Eastern European Study Group. *Breast Cancer Res. Treat.* **1997**, *45*, 251−62.

(146) Chen, H. K.; Ke, H. Z.; Lin, C. H.; Ma, Y. F.; Qi, H.; Crawford, D. T.; Pirie, C. M.; Simmons, H. A.; Jee, W. S. S.; Thompson, D. D. Droloxifene inhibits cortical bone turnover associated with estrogen deficiency in rats. *Bone* **1995**, *17*, 175S−179S.

(147) Chen, H. K.; Ke, H. Z.; Jee, W. S. S.; Ma, Y. F.; Pirie, C. M.; Simmons, H. A.; Thompson, D. D. Droloxifene prevents ovariectomy-induced bone loss in tibiae and femora of aged female rats: a dual-energy X-ray absorptiometric and histomorphometric study. *J. Bone Miner. Res.* **1995**, *10*, 1256−1262.

(148) Ke, H. Z.; Simmons, H. A.; Pirie, C. M.; Crawford, D. T.; Thompson, D. D. Droloxifene, a new estrogen antagonist/agonist, prevents bone loss in ovariectomized rats. *Endocrinology* **1995**, *136*, 2435−2441.

(149) Buzdar, A. U.; Marcus, C.; Holmes, F.; Hug, V.; Hortobagyi, G. Phase II evaluation of Ly156758 in metastatic breast cancer. *Oncology* **1988**, *45*, 344−5.

(150) (a) Delmas, P. D.; Bjarnason, N. H.; Mitlak, B. H.; Ravoux, A. C.; Shah, A. S.; Huster, W. J.; Draper, M.; Christiansen, C. Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. *N. Engl. J. Med.* **1997**, *337*, 1641. (b) Ettinger, B.; Black, D.; Cummings, S.; Genant, H.; Gluer, C.; Lips, P.; Knickerbocker, R.; Eckert, S.; Nickelsen, T.; Mitlak, B. Raloxifene reduces the risk of incident vertebral fractures: 24-month interim analyses. *Osteoporosis Int.* **1998**, *8* (Suppl. 3).

(151) Adrian, M. D.; Cole, H. W.; Shetler, P. K.; Rowley, E. R.; Magee, D. E.; Pell, T.; Zeng, G. Q.; Sato, M.; Bryant, H. U. Comparative pharmacology of a series of selective estrogen receptor modulators. *J. Bone Miner. Res.* **1996**, *11* (Suppl. 1), T590.

(152) Ashby, J.; Odum, J.; Foster, J. R. Activity of raloxifene in immature and ovariectomized rat uterotrophic assays. *Reg. Toxicol. Pharmacol.* **1997**, *25*, 226−31.

(153) Grese, T. A.; Sluka, J. P.; Bryant, H. U.; Cullinan, G. J.; Glasebrook, A. L.; Jones, C. D.; Matsumoto, K.; Palkowitz, A. D.; Sato, M.; Termine, J. D.; Winter, M. A.; Yang, N. N.; Dodge, J. A. Molecular determinants of selectivity in estrogen receptor modulators. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 14105−14110.

(154) McCague, R.; Jarman, M.; Leung, O. T.; Foster, A. B.; Leclercq, G.; Stoessel, S. Nonisomeisable antiestrogens related to tamoxifen. *J. Steroid Biochem.* **1988**, *31*, 545−548.

(155) Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Monn, T.; Engström, O.; Öhman, L.; Greene, G. L.; Gustafsson, J.-Å.; Carlquist, M. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **1997**, *389*, 753−757.

(156) Grese, T. A.; Sluka, J. P.; Bryant, H. U.; Cole, H. W.; Kim, J. R.; Magee, D. E.; Rowley, E. R.; Sato, M. Benzopyran selective estrogen receptor modulators (SERMs): pharmacological effects and structural correlation with raloxifene. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 903−908.

(157) Rosati, R. L.; Da Silva Jardine, P.; Cameron, K. O.; Thompson, D. D.; Ke, H. Z.; Toler, S. M.; Brown, T. A.; Pan, L. C.; Ebbinghaus, C. F.; Reinhold, A. R.; Elliot, N. C.; Newhouse, B. N.; Tjoa, C. M.; Sweetnam, P. M.; Cole, M. J.; Arriola, M. W.; Gauthier, J. W.; Crawford, D. T.; Nickerson, D. F.; Pirie, C.; Qi, H.; Simmons, H. A.; Tkalcevic, G. T. Discovery and SAR of a potent nonsteroidal estrogen agonist CP-336,156. *J. Bone Miner. Res.* **1996**, *11* (Suppl. 1), S447.

(158) Sato, M.; Turner, C. H.; Wang, T.; Adrian, M. D.; Bryant, H. U. LY353381.HCl: A novel raloxifene analogue with improved SERM potency and efficacy in vivo. *J. Pharmacol. Exp. Ther.*, in press.

(159) Lednicer, D.; Babcock, J. C.; Lyster, S. C.; Duncan, W. G. Derivatives of 1,2-diphenyl-3,4-dihydronaphthalene as antifertility agents. *Chem. Ind. (London)* **1963**, 408−409.

(160) Dukes, M.; Chester, R.; Yarwood, L.; Wakeling, A. E. Effects of a nonsteroidal pure antioestrogen, ZM 189,154, on oestrogen target organs of the rat including bones. *J. Endocrinol.* **1994**, *141*, 335−341.

(161) Jones, C. D.; Suarez, T.; Massey, E. H.; Black, L. J.; Tinsley, F. C. Synthesis and antiestrogenic activity of [3,4-dihydro-2-(4-methoxyphenyl)-1-naphthalenyl][4-[2-(1-pyrrolidinyl)ethoxy]-phenyl]methanone, methanesulfonic acid salt. *J. Med. Chem.* **1979**, *22*, 962−966.

(162) Ke, H. Z.; Paralkar, V. M.; Grasser, W. A.; Crawford, D. T.; Qi, H.; Simmons, H. A.; Pirie, C. M.; Chidsey-Fink, K. L.; Owen, T. A.; Smock, S. L.; Chen, H. K.; Jee, W. S. S.; Cameron, K. O.; Rosati, R. L.; Brown, T. A.; Di Silva, Jardine, P.; Thompson, D. D. Effects of CP-336,156, a new, nonsteroidal estrogen agonist/antagonist, on bone, serum cholesterol, uterus and body composition in rat models. *Endocrinology* **1998**, *139*, 2068−2076.

(163) Bain, S. D.; Celino, D. L.; Baily, M. C.; Strachan, M. J.; Piggott, J. R.; Labroo, V. M. Centchroman, a nonsteroidal antiestrogen, is a bone-specific estrogen agonist in the ovariectomized rat. *Calcif. Tissue Int.* **1995**, *54*, 338.

(164) Trivedi, R. N.; Chauhan, S. C.; Dwivedi, A.; Kamboj, V. P.; Singh, M. M. Time-related effects of a triphenylethylene antiestrogen on estrogen-induced changes in uterine weight, estrogen receptors, and endometrial sensitivity in rats. *Contraception* **1995**, *51*, 367−379.

(165) Nowak, J.; Festersen, U.; Andersen, A.; Christensen, N. D. Effect of levormeloxifene, a partial estrogen receptor agonist, on serum cholesterol, osteocalcin and bone in the ovariectomized rat. *J. Bone Miner. Res.* **1997**, *12* (Suppl. 1), S347.

(166) Nowak, J.; Sjogren, I.; Festersen, U.; Christensen, N. D. Effect of levormeloxifene, a partial estrogen receptor agonist, on body weight, food conversion efficacy, and uterus in the ovariectomized rat. *J. Bone Miner. Res.* **1997**, *12* (Suppl. 1), S346.

(167) Bain, S. D.; Grennspan, D.; Kurman, R.; Shalmi, M.; Guldhammer, B.; Korsgaard, N. Levormeloxifene, a nonsteroidal, partial estrogen agonist, prevents bone loss, reduces serum cholesterol and exerts a nonproliferative action on uterine tissues in the ovariectomized rat. *J. Bone Miner. Res.* **1997**, *12* (Suppl. 1), S347.

(168) Bjarnason, K.; Skrumsager, B. K.; Kiehr, B. Levormeloxifene, a new partial estrogen receptor agonist demonstrates antiresorptive and antiatherogenic properties in postmenopausal women. *J. Bone Miner. Res.* **1997**, *12* (Suppl. 1), S346.

(169) Gauthier, S.; Caron, B.; Cloutier, J.; Dory, Y. L.; Favre, A.; Larouche, D.; Mailhot, J.; Ouellet, C.; Schwerdtfeger, A.; Leblanc, G. EM800: a highly potent, specific, and orally active nonsteroidal antiestrogen. *J. Med. Chem.* **1997**, *40*, 2117−2122. Martel, L. S.; Sourla, C.; Gauthier, S.; Merand, Y.; Belanger, A.; Labrie, C.; Labrie, F. Comparative effects of 28-day treatment with a new anti-estrogen EM-800 and tamoxifen on estrogen-sensitive parameters in intact mice. *Int. J. Cancer* **1997**, *73*, 381−391.

(170) Simard, J.; Labrie, C.; Belanger, A.; Gauthier, S.; Singh, S. M.; Merand, Y.; Labrie, F. Characterization of the effects of the novel nonsteroidal antiestrogen EM-800 on basal and estrogen-induced proliferation of T-47D, ZR-75-1 and MCF-7 human breast cancer cells in vitro. *Int. J. Cancer* **1997**, *73*, 104−112. Simard, J.; Sanchez, R.; Poirier, D.; Gauthier, S.; Singh, S. M.; Merand, Y.; Belanger, A.; Labrie, C.; Labrie, F. Blockade of the stimulatory effect of estrogens, OH-tamoxifen, OH-toremifene, droloxifene, and raloxifene on alkaline phosphatase activity by the antiestrogen EM-800 in human endometrial adenocarcinoma Ishikawa cells. *Cancer Res.* **1997**, *57*, 3494−3497.

(171) McDonnell, D. P.; Clemm, D. L.; Hermann, T.; Goldman, M. E.; Pike, J. W. Analysis of estrogen receptor function in vitro reveals three distinct classes of antiestrogens. *Mol. Endocrinol.* **1995**, *9*, 659−669.

(172) Yang, N. N.; Bryant, H. U.; Hardikar, S.; Sato, M.; Galvin, R. J. S.; Glasebrook, A.; Termine, J. D. Estrogen and raloxifene stimulate transforming growth factor-β3 gene expression in rat bone: a potential mechanism for estrogen- or raloxifene-mediated bone maintenance. *Endocrinology* **1996**, *137*, 2075−2084.

(173) Yang, N. N.; Venugopalan, M.; Hardikar, S.; Glasebrook, A. Identification of an estrogen response element activated by metabolites of 17 β estradiol and raloxifene. *Science* **1996**, *273*, 1222−1225. Yang, N. N.; Venugopalan, M.; Hardikar, S.; Glasebrook, A. Correction: raloxifene response needs more than an element. *Science* **1997**, *275*, 1249.

(174) Caldwell, B. M.; Watson, R. I. An evaluation of psychologic effects of sex hormone administration in aged women. Results of therapy after six months. *J. Gerentol.* **1952**, *7*, 228−244.

(175) Paganini-Hill, A.; Henderson, V. S. Estrogen replacement therapy and risk of Alzheimer's disease. *Arch. Intern. Med.* **1996**, *156*, 2213−2217.

(176) Morrison, A.; Resnick, S.; Corrada, M.; Zonderman, A.; Kawas, C. A. prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease in the Baltimore longitudinal study of aging. *Neurology* **1996**, *46* (Suppl. 2), A435−436.

(177) Tang, M. X.; Jacobs, D.; Stern, Y. Effect of oestrogen during menopause on risk and age at onset of Alzheimers disease. *Lancet* **1996**, *348*, 429−432.

(178) Brenner, D. E.; Kukull, W. A.; Sterrgachis, A. Postemenopausal estrogen replacement therapy and the risk of Alzheimer's disease: A population-based case-controll study. *Am. J. Epidemiol.* **1994**, *140*, 262−267.

(179) Sherwin, B. B.; Phillips, S. Estrogen and cognitive function in surgically menopausal women. *Ann. N.Y. Acad. Sci.* **1990**, *592*, 474−475.

(180) Wickelgren, I. Estrogen stakes claim to cognition. *Science* **1997**, *276*, 675−678.

(181) Li, X.; Schwartz, P. E.; Rissman, E. F. Distribution of estrogen receptor-beta-like immunoreactivity in rat forebrain. *Neuroendocrinology* **1997**, *66*, 63−67.

(182) Merchenthaler, I.; Lane, L. V.; Shughrue, P. J. The comparative distribution of estrogen receptor (α and β) mRNA-expressing neurons in the rat central nervous system: An in situ hybridization study. *Soc. Neurosci. Abs.* **1997**, *23*, 1683.

(183) Mermelstein, P. G.; Becker, J. B.; Surmeier, D. J. Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. *J. Neurosci.* **1996**, *16*, 595−604.
(184) Song, J.; Standley, P. R.; Zhang, F.; Joshi, D.; Gappy, S.; Sowers, J. R.; Ram, J. L. Tamoxifen (estrogen antagonist) inhibits voltage-gated calcium current and contractility in vascular smooth muscle from rats. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 1444−53.
(185) Lomax, P.; Schonbaum, E. Postmenopausal hot flushes and their management. *Pharmacol. Ther.* **1993**, *57*, 347−358.
(186) Draper, M. W.; Flowers, D. E.; Huster, W. J.; Neild, J. A.; Harper, K. D.; Arnaud, C. A controlled trial of raloxifene (LY139481) HCl: impact on bone turnover and serum lipid profile in healthy postmenopausal women. *J. Bone Miner. Res.* **1996**, *11*, 835−842.
(187) Love, R. R.; Wiebe, D. A.; Newcomb, P. A.; Cameron, L.; Leventhal, H.; Jordan, V. C.; Feyzi, J.; DeMets, D. L. Effects of tamoxifen on cardiovascular risk factors in postmenopausal women. *Ann. Intern. Med.* **1991**, *115*, 860−864.
(188) Bryant, H. U.; Bales, K. R.; Paul, S. M.; Yang, H.; Cole, H. W.; Walker-Daniels, J.; McEwen, B.; Chow, H.; Santerre, R. E. Estrogen agonist effects of selective estrogen receptor modulators in ovariectomized rat brain. *Soc. Neurosci. Abs.* **1997**, *23*, 2377.
(189) Fink, G.; Sumner, B. E.; Rosie, R.; Grace, O. and Quinn, J. P. Estrogen control of neurotransmission: Effect on mood, mental state and memory. *Cell Molec. Neurobiol.* **1996**, *16*, 325−344.
(190) Magos, A. L.; Brewster, E.; Singh, R.; O'Dowd, T.; Brincat, M.; Studd, J. W. The effects of norethistrone in postmenopausal women on oestrogen replacement therapy: A model for the premenstrual syndrome. *Br. J. Obstet. Gynecol.* **1984**, *3*, 93−99.
(191) Nicholson, G. C.; Moseley, J. M.; Sexton, P. M.; Mendelsohn, G. A. O.; Martin, T. J. Abundant calcitonin receptors in isolated rat osteoclasts. *J. Clin. Invest.* **1986**, *64*, 355−360.
(192) Arnett, T. R.; Demptser, D. W. A comparative study of disaggregated chick and rat osteoclasts in vitro: Effects of calcitonin and prostaglandins. *Endocrinology* **1987**, *120*, 602−608.
(193) Murrills, R. J.; Shane, E.; Lindsay, R.; Dempster, D. W. Bone resorption by isolated human osteoclasts in vitro: effects of calcitonin. *J. Bone Miner. Res.* **1989**, *4*, 259−268.
(194) Raisz, L. G.; Wener, J. A.; Trummel, C. L.; Feinblatt, J. F.; Au, W. Y. W. Induction, inhibition and escape as phenomena in bone resorption. *Excerpta Med. Int. Congr. Ser.* **1972**, *243*, 446−449. Tashjian, A. H.; Wright, D. R.; Ivey, J. L.; Pont, A. Calcitonin binding sites in bone: relationships to biological response and "escape". *Recent Prog. Horm. Res.* **1978**, *34*, 285−299. Nicholson, G. C.; Moseley, J. M.; Yates, A. J. P.; Martin, T. J. Control of cAMP production in osteoclasts: calcitonin-induced persistent activation and homologous desensitization of adenylate cyclase. *Endocrinology* **1987**, *120*, 1902−1908.
(195) Kohno, T.; Murasugi, N.; Sakurai, H.; Watabe, K.; Nakamuta; Koida, M.; Sugie, Y.; Nomura, M.; Yanagawa, A. A sandwich transfer enzyme immuno assay for salmon calcitonin: Determination of the bioavailability of intranasal salmon calcitonin in human. *J. Clin. Lab Anal.* **1997**, *11*, 380−387.
(196) Overgaard, K.; Hansen, M. A.; Jensen, S. B.; Christiansen, C. Effect of salcatonin given intranasally on bone mass and fracture rates in established osteoporosis. *Br. Med. J.* **1992**, *305*, 556−561.
(197) Cardona, J. M.; Pastor, E. Calcitonin versus etidronate for the treatment of postmenopausal osteoporosis: a meta-analysis of published clinical trials. *Osteoporosis Int.* **1997**, *7* (3), 165−74.
(198) Thamsborg, G.; Jensen, J. E.; Kollerup, G.; Hauge, E. M.; Melsen, F.; Sorensen, O. H. Effect of nasal salmon calcitonin on bone remodeling and bone mass in postmenopausal osteoporosis. *Bone* **1996**, *18* (2), 207−12.
(199) Stock, J. L.; Avioli, L. V.; Baylink, D. J.; Chesnut, C.; Genant, H. K.; Maricic, M. J.; Silverman, S. L.; Schaffer, A. V.; Feinblatt, J. Calcitonin-salmon nasal spray reduces the incidence of new vertebral fractures in postmenopausal women: 3 year interim results of the proof study. *J. Bone Miner. Res.* **1997**, *12* (Suppl. 1), S149.
(200) Rodan, G.; Fleisch, HA. Bisphosphonates: mechanisms of action. *J. Clin. Invest.* **1996**, *97*, 2692−2696. Fleisch, H. *Bisphosphonates in Bone Disease: From the Laboratory to the Patient*, 3rd ed.; Parthenon Publishing: 1997.
(201) Reginster, J. Y. L.; Halkin, V.; Gosset, C.; Deroisy, R. The role of bisphosphonates in the treatment of osteoporosis. *Drugs Today* **1997**, *33*, 563−570. Jeal, W.; Barradell, L. B.; McTavish, D. Alendronate: a review of its pharmacological properties and therapeutic efficacy in postmenopausal osteoporosis. *Adis Drug Evaluation* **1997**, *53*, 415−434. Yates, J.; Rodan, G. A. Alendronate and osteoporosis. *DDT* **1998**, *3*, 69−78.
(202) Watts, N. B.; Harris, S. T.; Genant, H. K.; Wasnich, R. D.; Miller, P. D.; Jackson, R. D.; Licata, A. A.; Ross, P.; Woodson, G. C.; Yanover, M. J.; Mysiw, J. W.; Kohse, L.; Rao, M. B.; Steiger, P.; Richmond, B.; Chesnut, C. H. Intermittent cyclical etidronate treatment of postmenopausal osteoporosis. *N. Engl. J. Med.* **1990**, *323*, 73−79. Harris, S. T.; Watts, N. B.; Jackson, R. D.; Genant, H. K.; Wasnich, R. D.; Ross, P.; Miller, P. D.; Licata, A. A.; Chesnut, C. H. Four-year study of intermittent cyclic etidronate treatment of postmenopausal osteoporosis: three years of blinded therapy followed by one year of open therapy. *Am. J. Med.* **1993**, *95*, 557−567.
(203) Jowsey, J.; Riggs, B. L.; Kelly, P. J.; Hoffman, D. L.; Bordier, P. The treatment of osteoporosis with disodium ethane-1-hydroxy-1,1-diphosphonate. *J. Lab. Clin. Med.* **1971**, *78*, 574−581. Heaney, R. P.; Saville, P. D. Etidronate disodium in postmenopausal women. *Clin. Pharmacol. Ther.* **1976**, *20*, 593−604. Boyce, B. F.; Smith, L.; Fogelman, I.; Johnston, E.; Ralston, S.; Boyle, I. T. Focal osteomalacia due to low-dose diphosphonate therapy in Paget's disease. *Lancet* **1984**, 821−824. Gibbs, C. J.; Aaron, J. E.; Peacock, M. Osteomalacia in Paget's disease treated with short-term, high dose sodium etidronate. *Br. Med. J.* **1986**, *292*, 1227−1229.
(204) Devogelaer, J. P.; Broll, H.; Correa-Rotter, R.; Cumming, D. C.; De, Deuxchaisnes; C. N.; Geusens, P.; Hosking, D.; Jaeger, P.; Kaufman, J. M.; Leite, M.; Leon, J.; Liberman, U.; Menkes, C. J.; Meunier, P. J.; Reid, I.; Rodriguez, J.; Romanowicz, A.; Seeman, E.; Vermeulen, A.; Hirsch, L. J.; Lombardi, A.; Plezia, K.; Santora, A. C.; Yates, A. J.; Yuan, W. Oral alendronate induces progressive increases in bone mass of the spine, hip, and total body over 3 years in postmenopausal women with osteoporosis. *Bone* **1996**, *18*, 141−150.
(205) Black, D. M.; Cummings, S. R.; Karpf, D. B.; Cauley, J. A.; Thompson, D. E.; Nevitt, M. C.; Bauer, D. C. Genant, H. K.; Haskell, W. L.; Marcus, R.; Ott, S. M.; Torner, J. C.; Quandt, S. A.; Reiss, T. F.; Ensrud, K. E. Randomized trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. *Lancet* **1996**, *348* (9041), 1535−41.
(206) Liberman, U. A.; Weiss, S. R.; Broll, J.; Minne, H. W.; Dequeker, J.; Favus, M.; Seeman, E.; Recker, R.; Capizzi, T.; Santora, A. C.; Lombardi, A.; Shah, R.; Hirsch, L. J.; Karpf, D. B. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. *N. Engl. J. Med.* **1995**, *333*, 1437−1443.
(207) Hosking, D.; Chilvers, C.; Christiansen, C.; Ravn, P.; Wasnich, R.; Ross, P.; McClung, M.; Balske, A.; Thompson, D.; Daley, M.; Yates, A. J. Prevention of bone loss with alendronate in postmenopausal women under 60 years of age. *New Engl. J. Med.* **1998**, *338*, 485−492.
(208) Chavassieux, P. M.; Ariot, M. E.; Reda, C.; Wei, L.; Yates, A. J.; Meunier, P. J. Histomorphometric assessment of the long-term effects of alendronate on bone quality and remodeling in patients with osteoporosis. *J. Clin. Invest.* **1997**, *100*, 1475−1480.
(209) Balena, R.; Markatos, A.; Seedor, J. G.; Gentile, M.; Stark, C.; Peter, C. P.; Rodan, G. A. Long-term safety of the aminobisphosphonate alendronate in adult dogs. II. Histomorphometric analysis of the L5 vertebra. *J. Pharmacol. Exp. Ther.* **1996,** *276*, 277−283.
(210) Sato, M.; Grasser, W.; Endo, N.; Akins, R; Simmons, H.; Thompson, D. D.; Golub, E.; Rodan, G. A. Bisphosphonate action: Alendronate localization in rat bone and effects on osteoclast ultrastructure. *J. Clin. Invest.* **1991**, *69*, 2095−2105.
(211) Breuil, V.; Cosman F.; Stein, L.; Horbert, W.; Nieves, J.; Shen, V.; Lindsay, R.; Dempster, D. W. Human osteoclast formation and activity in vitro: effects of alendronate. *J. Bone Miner. Res.*, in press.
(212) Lin, J. H.; Chen, I. W.; deLuna, F. A. Nonlinear kinetics of alendronate, plasma protein binding and bone uptake. *Drug Metab. Dispos.* **1994**, *22*, 400−405. Lin, J. H. Bisphosphonates: A review of their pharmacokinetic properties. *Bone* **1996**, *18*, 75−85.
(213) Lin, J. H.; Chen, I. W.; deLuna, F. A. On the absorption of alendronate in rats. *J. Pharm. Sci.* **1994**, *83*, 1741−46.
(214) Azuma, Y.; Sato, H.; Oue, Y.; Okabe, K.; Ohta, T.; Tsuchimoto, M.; Kiyoki, M. Alendronate distributed on bone surfaces inhibits osteoclastic bone resorption in vitro and in experimental hypercalcemia models. *Bone* **1995**, *16*, 235−45.
(215) Gertz, B. J.; Holland, S. D.; Kline, W. F.; Matuszewski, B. K.; Porras, A. G. Clinical pharmacology of alendronate sodium. *Osteoporosis Int.* **1993**, (Suppl. 3), S13−S16. Fern, E. D.; Vasikaran, S. D.; Khan, S. Sustained suppression of bone resorption after intravenous alendronate in postmenopausal osteoporosis. In *Fourth International Symposium of Osteoporosis*; Hong Kong, 1993 (abstract).
(216) Sato, M.; Grasser, W. Effects of bisphosphonates on isolated rat osteoclasts as examined by reflected light microscopy. *J. Bone Miner. Res.* **1990**, *5*, 31−40.
(217) Hughes, D. E.; MacDonald, B. R.; Russell, R. G. G.; Gowen, M. Inhibition of osteoclast-like cell formation by bisphosphonates in long-term cultures of human bone marrow. *J. Clin. Invest.* **1989**, *67*, 1930−1935.

(218) Schmidt, A.; Rutledge, S. U.; Endo, N.; Opas, E. E.; Tanaka, H.; Wesolowski, G.; Leu, C. T.; Huang, Z.; Ramachandaran, C.; Rodan, S. B.; Rodan, G. A. Protein-tyrosine phosphatase activity regulates osteoclast formation and function: inhibition by alendronate. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3068−3073.
(219) Hughes, D. E.; Wright, K. R.; Uly, H. L.; Sasaki, A.; Yoneda, T.; Roodman, G. D.; Mundy, G. R.; Boyce, B. F. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. *J. Bone Miner. Res.* **1995**, *10*, 1478−1487.
(220) Sahni, M.; Guenther, H. L.; Fleisch, H.; Collin, P.; Martin, T. J. Bisphosphonates act on rat bone resorption through the mediation of osteoblasts. *J. Clin. Invest.* **1993**, *71*, 2004−11.
(221) Owens, J. M.; Fuller, K.; Chambers, T. J. Osteoclast activation: potent inhibition by the bisphosphonate alendronate through a nonresorptive mechanism. *J. Cell Physiol.* **1997**, *172*, 79−86.
(222) Burr, D. B.; Forwood, M. R.; Fyhrie, D. P.; Martin, R. B.; Schaffler, M. B.; Turner, C. H. Bone microdamage and skeletal fragility in osteoporotic and stress fractures. *J. Bone Miner. Res.* **1997**, *12*, 6−15.
(223) Gertz, B. J.; Holland, S. D.; Kline, W. F.; Bogdan, K.; Matuszewski, B. K.; Freeman, A.; Porras, A. G. Studies of the oral bioavailability of alendronate. *Clin. Pharmacol. Ther.* **1995**, *58*, 288−298.
(224) Pizzani, E.; Valenzuela, G. Esophagitis associated with alendronate sodium. *Virginia Med. Q.* **1997**, *124* (3), 181−182.
(225) Levine, J.; Nelson, D. Esophageal stricture associated with alendronate therapy. *Am. J. Med.* **1997**, *102* (5), 489−91.
(226) Horton, M. A.; Rodan, G. A. Integrins as therapeutic targets in bone. In *Adhesion Receptors as Therapeutic Targets*, Horton, M. A., Ed.; CRC Press: New York, 1996; pp 223−245.
(227) Sato, M. M. K.; Sardana, W. A.; Grasser, V. M.; Garsky, J. M.; Murray, A. H.; Gould, R. J. Echistatin is a potent inhibitor of bone resorption in culture. *J. Cell Biol.* **1990**, *111*, 1713−1723.
(228) Sato, M. V.; Garsky, R. J.; Majeska, T. A.; Einhorn, J.; Murray, A. H.; Tashjian; Gould, R. J. Structure−activity studies of the s-echistatin inhibition of bone resorption: Implications for therapeutic utility. *J. Bone Miner. Res.* **1994**, *9*, 1441−1449.
(229) Fisher, J. E.; Caulfield, M. P.; Sato, M.; Quartuccio, H. A.; Gould, R. J.; Garsky, V. M.; Rodan, G. A.; Rosenblatt, M. Inhibition of osteoclastic bone resorption in vivo by echistatin, an arginyl-glycyl-asartyl (RGD)-containing protein. *Endocrinology* **1993**, *132*, 1411−1413.
(230) Yamamoto, M.; Fisher, J. E.; Gentile, M.; Seedor, J. G.; Leu, C. T.; Rodan, S. B.; Rodan, G. A. The integrin ligand echistatin prevents bone loss in ovariectomized mice and rats. *Endocrinology* **1998**, *139*, 1411−9
(231) Fisher, J. E.; Caulfield, M. P.; Sato, M.; Quartuccio, H. A.; Gould, R. J.; Garsky, V. M.; Rodan, G. A.; Rosenblatt, M. Response to letter. *Endocrinology* **1993**, *133*, 2408.
(232) Engleman, V. W.; Nickols, A. G.; Ross, F. P.; Horton, M. A.; Griggs, D. W.; Settle, S. L.; Ruminski, P. G.; Teitelbaum, S. L. A peptidomimetic antagonist of the avB3 integrin inhibits bone resorption in vitro and prevents osteoporosis in vivo. *J. Clin. Invest.* **1997**, *99*, 22284−2292.
(233) Tezuka, K.; Tezuka, Y.; Maejima, A.; Sato, T.; Nemoto, K.; Kamioka, H.; Hakeda, Y.; Kumegawa, M. Molecular cloning of a possible cysteine proteinase predominantly expressed in osteoclasts. *J. Biol. Chem.* **1994**, *269*, 1106−1109.
(234) Bossard, M. J.; Tomaszek, T. A.; Thompson, S. K.; Amegadzie, B. Y.; Hannings, C. R.; Jones, C.; Kurdla, J. T.; McNulty, D. E.; Drake, F. H.; Gowen, M.; Levy, M. A. Proteolytic activity of human osteoclast cathepsin K. *J. Biol. Chem.* **1996**, *271*, 12517−12524.
(235) Drake, F. H.; Dodds, R.; Connor, J. I.; Debouck, J.; Richardson, S.; Lee, E.; Rieman, D.; Barthlow, R.; Hastings, G.; Gowen, M. Cathepsin K, but not cathepsins B, L, or S, is abundantly expressed in human osteoclasts. *J. Biol. Chem.* **1996**, *271*, 12511−12516.
(236) Gelb, B. D.; Shi, G. P.; Chapman, H. A.; Desnick, R. J. Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. *Science* **1996**, *273*, 1236−1238.
(237) Votta, B. J.; Levy, M. A.; Badger, A.; Bradbeer, J.; Dodds, R. A.; James, I. E.; Thompson, S.; Bossard, M. J.; Carr, T.; Connor, J. R.; Tomaszek, T. A.; Szewczuk, L.; Drake, F. H.; Veber, D. F.; Gowen, M. Peptide aldehyde inhibitors of cathepsin K inhibit bone resorption both in vitro and in vivo. *J. Bone Miner. Res.* **1997**, *12*, 1396−1406.
(238) Keeling, D. J.; Herslof, M.; Ryberg, D.; Sjogren, S.; Solvell, L. Vacuolar H+-ATPases: targets for drug discovery? *Ann. N.Y. Acad. Sci.* **1996**, *834*, 600−608.
(239) Williams, S.; Wakisaka, A.; Zeng, Q. Q.; Barnes, J.; Martin, G.; Wechter, W. J.; Liang, C. T. Minocycline prevents the decrease in bone mineral density and trabecular bone in ovariectomized aged rats. *Bone* **1996**, *19* (6), 637−44.
(240) Heaney, R. P.; Baylink, D. J.; Johnston, C. C., Jr.; Melton, L. J., 3rd; Meunier, P. J.; Murray, T. M.; et al. Fluoride therapy for vertebral crush fracture syndrome. A status report. *Ann. Intern. Med.* **1989**, *111*, 678−680.
(241) Farley, J. R.; Wergedal, J. E.; Baylink, D. J. Fluoride directly stimulates proliferation and alkaline phosphatase activity of bone forming cells. *Science* **1983**, *227*, 330−332.
(242) Hall B. K. Sodium fluoride as an initiator of osteogenesis from embryonic mesenchyme in vitro. *Bone* **1987**, *8*, 111−116.
(243) Khokher, M. A.; Dandona, P. Fluoride stimulates (3H) thymidine incorporation and alkaline phosphatase production by human osteoblasts. *Metabolism* **1990**, *39*, 1118−1121.
(244) Kopp, J. B.; Robey, P. G. Sodium fluoride does not increase human bone cell proliferation or protein synthesis in vitro. *Calcif. Tissue Int.* **1990**, *47*, 221−226.
(245) Chavassieux, P.; Chenu, C.; Valentin-Opran, A.; Delmas, P. D.; Boivin, G.; Chapuy, M. C.; Meunier, P. J. In vitro exposure to sodium fluoride does not modify activity or proliferation of human osteoblastic cells in primary cultures. *J. Bone Miner. Res.* **1993**, *8*, 37−44.
(246) Einhorn, T. A.; Wakley, G. K.; Linkhart, S.; Rush, E. B.; Maloney, S.; Faierman, E. Incorporation of sodium fluoride into cortical bone does not impair the mechanical properties of the appendicular skeleton in rats. *Calcif. Tissue Int.* **1992**, *51*, 127−131.
(247) Faccini, J. M. Fluoride and bone. *Calcif. Tissue Res.* **1969**, *3*, 1−16.
(248) Wolinsky, I.; Simkin, A.; Guggenheim, K. Effects of fluoride on metabolism and mechanical properties of rat bone. *Am. J. Physiol.* **1972**, *223*, 46−50.
(249) Lafage, M. H.; Balena, R.; Battle, M. A.; Shea, M.; Seedor, J. G.; Klein, H.; Hayes, W. C.; Rodan, G. A. Comparison of alendronate and sodium fluoride effects on cancellous and cortical bone in minipigs. A one year study. *J. Clin. Invest.* **1995**, *95*, 2127−2133.
(250) Sogaard, C. H.; Mosekile, L.; Thomsen, J. S.; Richards, A.; McOsker, J. E. A comparison of the effects of two anabolic agents (Fluoride and PTH) on ash density and bone strength assessed in an osteopenic rat model. *Bone* **1997**, *20*, 439−449.
(251) Turner, C. H.; Dunipace, A. J. On fluoride and bone strength (letter). *Calcif. Tissue Int.* **1993**, *53*, 289−290.
(252) Turner, C. H.; Hasegawa, K.; Zhang, W.; Wilson, M.; Li, Y.; Dunipace, A. J. High fluoride intake causes osteomalacia and diminished bone strength in rats with renal deficiency. *Bone*, in press.
(253) Boivin, G.; Duriez, J.; Chapuy, M-C.; Flautre, B.; Hardouin, P.; Meunier, P. J. Relationship between bone fluoride content and histological evidence of calcification defects in osteoporotic women treated long term with sodium fluoride. *Osteoporosis Int.* **1993**, *3*, 204−208.
(254) Grynpas, M. D.; Holmyard, D. P.; Pritzker, K. P. H. Bone mineralization and histomorphometry in biopsies of osteoporotic patients treated with fluoride. *Cells Mater.* **1994**, *4*, 287−297.
(255) Lundy, M. W.; Stauffer, M.; Wergedal, J. E.; Baylink, D. J.; Featherstone, J. D.; Hodgson, S. F.; Riggs, B. L. Histomorphometric analysis of iliac crest bone biopsies in placebo-treated versus fluoride-treated subjects. *Osteoporosis Int.* **1995**, *5*, 115−129.
(256) Dure-Smith, B. A.; Farley, S. M.; Linkhart, S. G.; Farley, J. R.; Baylink, D. J. Calcium deficiency in fluoride-treated osteoporotic patients despite calcium supplementation. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 269−275.
(257) Grynpas, M. D.; Rey, C. The effect of fluoride treatment on bone mineral crystals in the rat. *Bone* **1992**, *13*, 423−429.
(258) Fratzl, P.; Schreiber, S.; Roschger, P.; Lafage, M. H.; Rodan, G.; Klaushofer, K. Effects of sodium fluoride and alendronate on the bone mineral in minipigs: a small-angle X-ray scattering and backscattered electron imaging study. *J. Bone Miner. Res.* **1996**, *11*, 248−253.
(259) Fratzl, P.; Roschger, P.; Eschberger, J.; Klaushofer, K. Abnormal bone mineralization after fluoride treatment in osteoporosis: a small-angle X-ray scattering study. *J. Bone Miner. Res.* **1994**, *9*, 1541−1549.
(260) Walsh, W. R.; Labrador, D. P.; Kim, H. D.; Guzelsu, N. The effect of in vitro fluoride ion treatment on the ultrasonic properties of cortical bone. *Ann. Biomed. Eng.* **1994**, *22*, 404−415.
(261) Catanese, J.; Keaveny, T. M. Role of collagen and hydroxyapatite in the mechanical behavior of bone tissue. *J. Bone Miner. Res.* **1996**, *11*, S295.
(262) Riggs, B. L.; Hodgson, S. F.; O'Fallon, W. M.; Chao, E. Y. S.; Wahner, H. W.; Muhs, J. M.; Cedel, S. L.; Melton, L. J. Effect of fluoride treatment on the fracture rate in postmenopausal women with osteoporosis. *N. Engl. J. Med.* **1990**, *322*, 802−809.
(263) Pak, C. Y.; Sakhaee, K.; Adams-Huet, B.; Piziak, V.; Peterson, R. D.; Poindexter, J. R. Treatment of postmenopausal osteoporosis with slow-release sodium fluoride: final report of a randomized controlled trial. *Ann. Intern. Med.* **1995**, *123*, 401−408.
(264) Meunier, P. J.; Sebert, J. L.; Reginster, J. Y.; et al. Fluoride salts do not better prevent new vertebral fractures than calcium-vitamin D in postmenopausal osteoporosis: the Favos study. *Osteoporosis Int.* **1998**, *1*, 4−12.
(265) Kleerekoper, M. Fluoride: the verdict is in, but the controversy lingers. *J. Bone Miner. Res.* **1996**, *11* (5), 565−7.

(266) Dempster, D. W.; Cosman, F.; Parisien, M; Shen, V.; Lindsay, R. Anabolic actions of parathyroid hormone on bone. *Endocr. Rev.* **1993**, *14*, 690−709.
(267) Ibbotson, K. J.; Orcutt, C. M.; D'Souza, S. M.; Paddock, C. L.; Arthur, J. A.; Jankowsky, M. L.; Boyce, R. W. Contrasting effects of parathyroid hormone and insulin-like growth factor I in an aged ovariectomized rat model of postmenopausal osteoporosis. *J. Bone Miner. Res.* **1992**, *7*, 425−432.
(268) Kimmel, D. B.; Bozzato, R. P.; Kronis, K. A.; Kwong, P.; Recker, R. R. The effect of recombinanat human (1-84) or synthetic human (1-34) parathyroid hormone on the skeleton of adult osteopenic ovariectomized rats. *Endocrinology* **1993**, *132*, 1577−1584.
(269) Shen, V.; Dempster, D. W.; Birchman, R.; Xu, R.; Lindsay, R. Loss of cancellous bone mass and connectivity in ovariectomized rats can be restored by combined treatment with parathyroid hormone and estradiol. *J. Clin. Invest.* **1993**, *71*, 2479−2487.
(270) Wronski, T. J.; Yen, C. F.; Dann, L. M. Parathyroid hormone is more effective than estrogen or bisphosphonates for restoration of lost bone mass in ovariectomized rats. *Endocrinology* **1993**, *132*, 823−831.
(271) Sogaard, C. H.; Wronski, T. J.; McOsker, J. E.; Mosekilde, L. The positive effect of parathyroid hormone on femoral neck bone strength in ovariectomized rats is more pronounced than that of estrogen or bisphosphonates. *Endocrinology* **1994**, *134*, 650−657.
(272) Li, M.; Wronski, T. J. Response of femoral neck to estrogen depletion and parathyroid hormone in aged rats. *Bone* **1995**, *16*, 551−557.
(273) Gunness-Hey, M.; Hock, J. M. Increased trabecular bone mass in rats treated with human synthetic parathyroid hormone. *Metab. Bone Dis. Rel. Res.* **1984**, *5*, 177−181.
(274) Hock, J. M.; Gera, I.; Fonseca, J.; Raisz, L. G. Human parathyroid hormone (1-34) increases bone mass in ovariectomized and orchidectomized rats. *Endocrinology* **1988**, *122*, 2899−2904.
(275) Kalu, D. N.; Echon, R.; Hollis, B. W. Modulation of ovariectomy-related bone loss by parathyroid hormone in rats. *Mech. Aging Develop.* **1990**, *56*, 49−62.
(276) Mosekilde, L.; Sogaard, C. H.; Danielsen, C. C.; Torring, O.; Nilsson, M. H. L. The anabolic effects of human parathyroid hormone (hPTH) on rat vertebral bone mass are also reflected in the quality of bone assessed by biomechanical testing: A comparison study between hPTH (1-34) and hPTH (1-84). *Endocrinology* **1991**, *129*, 421−428.
(277) Mosekilde, L.; Sogaard, C. H.; McOsker, J. E.; Wronski, T. J. PTH has a more pronounced effect on vertebral bone mass and biomechanical competence than anti-resorptive agents (estrogen and bisphosphonates) assessed in sexually mature, ovariectomized rats. *Bone* **1994**, *15*, 401−408.
(278) Takahashi, H. E.; Tanizawa, T.; Hori, M.; Uzawa, T. Effect of intermittent administration of human parathyroid hormone (1-34) on experimental osteopenia of rats induced by ovariectomy. *Cell Mater.* **1991**, (Suppl. 1), 113−117.
(279) Mitlak, B. H.; Williams, D. C.; Bryant, H. U.; Paul, D. C.; Neer, R. M. Intermittent administration of PTH (1-34) increases serum 1,25 dihydroxyvitamin D concentrations and spinal bone density in senile (23 month) rats. *J. Bone Miner. Res.* **1992**, *7*, 479−484.
(280) Tada, K.; Yamamura, T.; Okumura, H.; Kasai, R.; Takahashi, H. Restoration of axial and appendicular bone volumes by hPTH (1-34) in parathyroidectomized and osteopenic rats. *Bone* **1990**, *11*, 163−169.
(281) Li, M.; Mosekilde, L.; Sogaard, C. H.; Thomsen, J. S.; Wronski, T. J. Parathyroid hormone monotherapy and cotherapy with antiresorptive agents restore vertebral bone mass and strength in aged ovariectomized rats. *Bone* **1995**, *16*, 629−635.
(282) Qi, H.; Li, M.; Wronski, T. J. A comparison of the anabolic effects of parathyroid hormone at skeletal sites with moderate and severe osteopenia in aged ovariectomized rats. *J. Bone Miner. Res.* **1995**, *10*, 948−955.
(283) Sato, M.; Zeng, G. Q.; Turner, C. H. Biosynthetic human PTH (1-34) effects on bone quality in aged ovariectomized rats. *Endocrinology* **1997**, *10*, 4330−4337.
(284) Liu, C. C.; Kalu, D. N. Human parathyroid hormone (1-34) prevents bone loss and augments bone formation in sexually mature ovariectomized rats. *J. Bone Miner. Res.* **1990**, *5*, 973−981.
(285) Liu, C. C.; Kalu, D. N.; Salerno, E.; Echone, R.; Hollis, B. W.; Ray, M. Preexisting bone loss associated with ovariectomy in rats is reversed by parathyroid hormone. *J. Bone Miner. Res.* **1991**, *6*, 1071−1080.
(286) Ejersted, C.; Andreassen, T. T.; Oxlund, H.; Jorgensen, P. H.; Bak, B.; Haggblad, J.; Torring, O.; Nilsson, M. H. L. Human parathyroid hormone (1-34) and (1-84) increase the mechanical strength and thickness of cortical bone in rats. *J. Bone Miner. Res.* **1993**, *8*, 1097−1101.
(287) Gunness-Hey, M.; Hock, J. M. Anabolic effect of parathyroid hormone on cancellous and cortical bone histology. *Bone* **1993**, *14*, 447−452.
(288) Oxlund, H.; Ejersted, C.; Andreassen, T. T.; Torring, O.; Nilsson, M. H. Parathyroid hormone (1-34) and (1-84) stimulate cortical bone formation both from periosteum and endosteum. *Calcif. Tissue Int.* **1993**, *53*, 394−399.
(289) Shen, V.; Dempster, D. W.; Mellish, R. W. E.; Birchman, R.; Horbert, W.; Lindsay, R. Effects of combined and separate intermittent administration of low dose parathyroid hormone fragment (1−34) and 17b-estradiol on bone histomorphometry in ovariectomized rats with established osteopenia. *Calcif. Tissue Int.* **1992**, *50*, 214−220.
(290) Wronski, T. J.; Yen, C. F. Anabolic effects of parathyroid hormone on cortical bone in ovariectomized rats. *Bone* **1994**, *15*, 51−58.
(291) Baumann, B. D.; Wronski, T. J. Response of cortical bone to antiresorptive agents and parathyroid hormone in aged ovariectomized rats. *Bone* **1995**, *16*, 247−253.
(292) Mosekilde, L.; Danielsen, C. C.; Sogaard, C. H.; McOsker, J. E.; Wronski, T. J. The anabolic effects of parathyroid hormone on cortical bone mass, dimensions and strength-assessed in a sexually mature, ovariectomized rat model. *Bone* **1995**, *16*, 223−230.
(293) Hock, J. M.; Hummert, J. R.; Boyce, R. W.; Fonseca, J.; Raisz, L. G. Resorption is not essential for the stimulation of bone growth by hPTH (1-34) in rats in vivo. *J. Bone Miner. Res.* **1989**, *4*, 449−458.
(294) Onyia, J. E.; Bidwell, J.; Herring, J.; Hulman, J.; Hock, J. M. In vivo, human parathyroid hormone fragment (hPTH 1-34) transiently stimulates immediate early response gene expression, but not proliferation, in trabecular bone cells of young rats. *Bone* **1995**, *17*, 479−84.
(295) Schmidt, I. U.; Dobnig, H.; Turner, R. T. Intermittent parathyroid hormone treatment increases osteoblast number, steady-state mRNA levels for osteocalcin. *Endocrinology* **1995**, *136*, 5127−5134.
(296) Dobnig, H; Turner, R. T. Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. *Endocrinology* **1995**, *136*, 3632−3638.
(297) Leaffer, D.; Sweeney, M.; Kellerman, L. A.; Avnur, Z.; Krstenansky, J. L.; Vickery, B. H.; Caulfield, J. P. Modulation of osteogenic cell ultrastructure by RS-23481, an analogue of human parathyroid hormone related peptide (1−34) and bovine PTH (1-34). *Endocrinology* **1995**, *136*, 3624−3631.
(298) Dobnig, H.; Turner, R. T. The effects of programmed administration of human parathyroid hormone fragment (1−34) on bone histomorphometry and serum chemistry in rats. *Endocrinology* **1997**, *138*, 4607−4612.
(299) Heath, H., III. Primary hyperparathyroidism, hyperparathyroid bone disease, and osteoporosis. In *Osteoporosis*; Marcus, R., Feldman, D., Kelsey, J., Eds.; Academic Press: New York, 1996; pp 885−897.
(300) Reeve, J.; Hesp, R.; Williams, D.; Hulme, P.; Klenerman, L.; Zanelli, J.; Darby, A.; Tregear, G.; Parsons, J. The anabolic effect of low doses of human parathyroid hormone fragment on the skeleton in postmenopausal osteoporosis. *Lancet* **1976**, *1*, 1035−1038.
(301) Reeve, J.; Meunier, P. J.; Parsons, J. A.; Bernat, M.; Bijvoet, O. L. M.; Courpron, P.; Edouard, C.; Klenerman, L.; Neer, R. M.; Renier, J. C. Anabolic effect of human parathyroid hormone fragment on trabecular bone in involutional osteoporosis: A multicenre trial. *Br. Med. J.* **1980**, *280*, 1340−1344.
(302) Hodsman, A. B.; Fraher, L. J.; Ostbye, T.; Adachi, J. D.; Steer, B. M. An evaluation of several biochemical markers for bone formation and resorption in a protocol utilizing cyclical parathyroid hormone and calcitonin therapy for osteoporosis. *J. Clin. Invest.* **1993**, *71*, 1138−48.
(303) Finkelstein, J.; Klebanski, A.; Schaefer, E.; Hornstein, M.; Schiff, I.; Neer, R. Parathyroid hormone for the prevention of bone loss induced by estrogen deficiency. *N. Engl. J. Med.* **1994**, *331*, 1618−1623.
(304) Sone, T.; Fukunaga, M.; Ono, S.; Nishiyama, T. A small dose of human parathyroid hormone (1-34) increased bone mass in the lumbar vertebrae in patients with senile osteoporosis. *Miner. Electrolyte Metab.* **1995**, *21*, 232−235.
(305) Lindsay, R; Nieves, J. Formica, C.; Henneman, E.; Woelfert, L.; Shen, V.; Dempster, D.; Cosman, F. Randomized controlled study of effect of parathyroid hormone on vertebral-bone mass and fracture incidence among postmenopausal women on oestrogen with osteoporosis. *Lancet* **1997**, *350*, 550−555.
(306) Slovik, D. M.; Rosenthal, S. I.; Doppelt, S. H.; Potts, J. T.; Daly, M. A.; Campbell, J. A.; Neer, R. M. Restoration of spinal bone in osteoporotic men by treatment with human parathyroid hormone (1-34) and 1,25 dihydroxyvitamin D. *J. Bone Miner. Res.* **1986**, *1*, 377−381.

(307) Reeve, J. PTH: a future role in the management of osteoporosis? *J. Bone Miner. Res.* **1996**, *11*, 440−445.
(308) Mosekilde, L.; Danielsen, C. C.; Gasser, J. The effect on vertebral bone mass and strength of long-term treatment with antiresorptive agents (estrogen and calcitonin), human parathyroid hormone (1−38), and combination therapy, assessed in aged ovariectomized rats. *Endocrinology* **1994**, *134*, 2126−2134.
(309) Jerome, C. P. Anabolic effect of high doses of human parathyroid hormone (1−38) in mature intact female rats. *J. Bone Miner. Res.* **1994**, *9* (6), 933−42.
(310) Whitfield, J. F.; Morley, P.; Willick, G.; Langille, R.; Ross, V.; MacLean, S.; Barbier, J. R. Cyclization by a specific lactam increases the ability of human parathyroid hormone (hPTH)−(1−31)NH2 to stimulate bone growth in ovariectomized rats. *J. Bone Miner. Res.* **1997**, *12*, 1246−1252.
(311) Whitfield, J. F.; Morley, P.; Willick, G. E.; Ross, V.; Langille, R.; MacLean, S.; Barbier, J.; Isaacs, R. J.; Ohannessian-Barry, L. Comparison of the abilities of human parathyroid hormone-(1−31)NH2 and human parathyroid hormone-related protein-(1−31)NH2 to stimulate femoral trabecular bone growth in ovariectomized rats. *Calcif. Tissue Int.* **1997**, *61*, 322−326.
(312) Hodsman, A. B.; Steer, B. M.; Fraher, L. J.; Drost, D. J. Bone densitometric and histomorphometric responses to sequential human parathyroid hormone (1-38) and salmon calcitonin in osteoporotic patients. *Bone Miner.* **1991**, *14*, 67−83.
(313) Gasser, J. A.; Gombert, F. O.; Cardnaux, F. SDZ PTS 893, a PTH analogue with improved therapeutic window in skeletally mature rats. *J. Bone Miner. Res.* **1997**, *12* (Suppl. 1), S236.
(314) Jerome, C. P.; Obasanjo, I.; Kaplan, K.; Gamse, R. SDZ PTS 893, a novel PTH analogue, increases spinal bone mass in ovariectomized monkeys. *J. Bone Miner. Res.* **1997**, *12* (Suppl. 1), S237.
(315) Potts, J. T.; Bringhurst, F. R.; Gardella, T.; Nussbaum, S.; Segre, G.; Kronenberg, H. Parathyroid hormone: physiology, chemistry, biosynthesis, secretion, metabolism, and mode of action. In *Endocrinology*, 3rd ed., DeGroot, L. J., Ed.; WB Saunders: Philadelphia, PA, 1995; Vol. 2, pp 920−966.
(316) Hock, J. M.; Fonseca, J.; Gunness-Hey, M.; Kemp, B. E.; Martin, T. J. Comparison of the anabolic effects of synthetic parathyroid hormone-related protein (PTHrP) 1−34 and PTH 1−34 on bone in rats. *Endocrinology* **1989**, *125*, 2022−2027.
(317) Weir, E. C.; Terwilliger, G.; Sartori, L.; Insogna, K. L. Synthetic parathyroid hormone-like protein (1-74) is anabolic for bone in vivo. *Calcif. Tissue Int.* **1992**, *51*, 30−34.
(318) Vickery, B. H.; Avnur, Z.; Cheng, Y.; Chiou, S. S.; Leaffer, D.; Caulfield, J. P.; Kimmel, D. B.; Ho, T.; Krstenansky, J. L. RS-66271, a C-terminally substituted analogue of human parathyroid hormone-related protein (1-34), increases trabecular and cortical bone in ovariectomized, osteopenic rats. *J. Bone Miner. Res.* **1996**, *11*, 1943−51.
(319) Stewart, A. F. PTHrP (1-36) as a skeletal analbolic agent for the treatment of osteoporosis. *Bone* **1996**, *4*, 303−306. Wysolmerski, J. J.; Stewart, A. F. The physiology of parathyroid hormone-related protein: an emerging role as a developmental factor. *Annu. Rev. Physiol.* **1998**, *60*, 431−460.
(320) Gasser, J. A.; Gombert, F. O.; Cardinaux, F. SDZ PTS 893, a PTH-analogue with improved therapeutic window in skeletally mature rats. *J. Bone Miner. Res.* **1997**, *12* (Suppl. 1), S236.
(321) Agnusdei, D.; Crepaldi, G.; Isaia, G.; Mazzuoli, G.; Ortolani, S.; Passeri, M.; Bufalino, L.; Gennari, C.; A double blind, placebo-controlled trial of ipriflavone for prevention of postmenopausal spinal bone loss. *Calcif. Tissue Int.* **1997**, *61*, 142−7.
(322) Notoya, K.; Yoshida, K.; Taketomi, S.; Yamazaki, I.; Kumegawa, M. Inhibitory effect of ipriflavone on pit formation in mouse unfractioned bone cells. *Calcif. Tissue Int.* **1992**, *51*, S3−S6.
(323) Miyauchi, A.; Notoya, K.; Taketomi, S.; Takagi, Y.; Fujii, Y.; Jinnai, K.; Takahashi, K.; Chihara, K.; Fujita, T. Novel ipriflavone receptors coupled to calcium influx regulate osteoclast differentiation and function. *Endocrinology* **1996**, *137*, 3544−3550.
(324) Kakai, Y.; Kawase, T.; Nakano, T.; Mikuni-Takagaki, Y.; Saito, S. Effect of ipriflavone and estrogen on the differentiation and proliferation of osteogenic cells. *Calcif. Tissue Int.* **1992**, *51*, S11−S15.
(325) Adami, S.; Bufalino, L.; Cervetti, R.; Di Marco, C.; Di Munno, O.; Fantasia, L.; Isaia, G. C.; Serni, U.; Vecchiet, L.; Passeri, M. Ipriflavone prevents radial bone loss in postmenopausal women with low bone mass over 2 years. *Osteoporosis Int.* **1997**, *7*, 119−25.
(326) Frost, H. Osteoporoses: their nature and therapeutic targets. In *Anabolic Treatments for Osteoporosis*; Whitfield, J. F., Morley, P., Eds.; CRC Press: New York, 1997; pp 1−29.
(327) Frost, H. M.; Ferretti, J. L.; Jee, W. S. S. Perspectives: some roles of mechanical usage, muscle strength, and the mechanostat in skeletal physiology, disease, and research. *Calcif. Tissue Int.* **1998**, *62*, 1−7.
(328) Forwood, M. R.; Burr, D. B. Physical activity and bone mass: exercises in futility? *Bone Miner.* **1993**, *21*, 89−112.
(329) Lohman, T.; Going, S.; Pamenter, R.; Hall, M.; Boyden, T.; Houtkooper, L.; Ritenbaugh, C.; Bare, L.; Hill, A.; Aichin, M. Effects of resistance training on regional and total bone mineral density in premenopausal women: a randomized prospective study. *J. Bone Miner. Res.* **1995**, *10*, 1015−1024.
(330) Colletti, L. A.; Edwards, J.; Gordon, L.; Shary, J.; Bell, N. H. The effects of muscle building exercise on bone mineral density of the radius, spine and hip in young men. *Calcif. Tissue Int.* **1989**, *45*, 12−14.
(331) Davee, A. M.; Rosen, C.; Adler, R. A. Exercise patterns and trabecular bone density in college women. *J. Bone Miner. Res.* **1990**, *5*, 245−250.
(332) Wolman, R. L.; Faulman, L.; Clark, P.; Hesp, R.; Harries, M. G. Different training patterns and bone mineral density of the femoral shaft in elite female athletes. *Ann. Rheum. Dis.* **1991**, *50*, 487−489.
(333) Jones, H.; Priest, J.; Hayes, W.; Tichenor, C.; Nagel, D. Humeral hypertrophy in response to exercise. *J. Bone Jt. Surg.* **1977**, *59A*, 204−207.
(334) Bassey, E. J.; Ramsdale, S. J. Weight-bearing exercise and ground reaction forces: A 12-month randomized controlled trial of effects on bone mineral density in healthy postmenopausal women. *Bone* **1995**, *16*, 469−476.
(335) Friedlander, A. L.; Genant, H. K.; Sadowsky, S.; Byl, N. N.; Gluer, C-C. A two-year program of aerobics and weight training enhances bone mineral density of young women. *J. Bone Miner. Res.* **1995**, *10*, 574−585.
(336) Heinonen, A.; Kannus, P.; Sievanen, H.; Oja, P.; Pasanen, M.; Rinne, M.; Uusi-Rasi, K.; Vuori, I. Randomized controlled trial of effect of high-impact exercise on selected risk factors for osteoporotic fractures. *Lancet* **1996**, *348*, 1343−1347.
(337) Heinonen, A.; Sievanen, H.; Kannus, P.; Oja, P.; Vuori, I. Effects of unilateral strength training and detraining of bone mineral mass and estimated mechanical characteristics of the upper limb bones in young women. *J. Bone Miner. Res.* **1996**, *11*, 490−501.
(338) Snow-Harter, C.; Bouxsein, M. L.; Lewis, B. T.; Carter, D. R.; Marcus, R. Effects of resistance and endurance exercise on bone mineral status of young women: a randomized exercise intervention trial. *J. Bone Miner. Res.* **1992**, *7*, 761−769.
(339) Teegarden, D.; Lyle, R. M.; Proulx, W. R.; Kern, M. K.; McCabe, G.; Peacock, M.; Johnston, C. C.; Weaver, C. M. Effect of exercise intervention and oral contraceptive use in spine bone mineral density in young women. *J. Bone Miner. Res.* **1995**, *10*, S456.
(340) Welsh, L.; Rutherford, O. M. Hip bone mineral density is improved by high-impact aerobic exercise in postmenopausal women and men over 50 years. *Eur. J. Appl. Physiol.* **1996**, *74*, 511−517.
(341) Bertram, J. E., Swartz, S. M. The 'law of bone transformation': a case of crying Wolff? *Biol. Rev.* **1991**, *66*, 245−273.
(342) Parfitt, A. M. The two faces of growth: benefits and risks to bone integrity. *Osteoporosis Int.* **1994**, *4*, 382−398.
(343) Rubin, C. T.; Bain, S. D.; McLeod, K. J. Suppression of the osteogenic response in the aging skeleton. *Calcif. Tissue Int.* **1992**, *50*, 306−313.
(344) Turner, C. H.; Takano, Y.; Owan, I. Aging changes mechanical loading thresholds for bone formation in rats. *J. Bone Miner. Res.* **1995**, *10*, 1544−1549.
(345) Haapasalo, H.; Kannus, P.; Sievanen, H.; Pasanen, M.; Uusi-Rasi, K.; Heinonen, A.; Oja, P.; Vuori, I. Effect of long-term unilateral activity on bone mineral density of female junior tennis players. *J. Bone Miner. Res.* **1998**, *13*, 310−319.
(346) Kannus, P.; Haapasalo, H.; Sankelo, M.; Sievanen, H.; Pasanen, M.; Heinonen, A.; Oja, P.; Vuori, I. Effect of starting age of physical activity on bone mass in the dominant arm of tennis and squash players. *Ann. Intern. Med.* **1995**, *123*, 27−31.
(347) Cavanaugh, D. J., Cann, C. E. Brisk walking does not stop bone loss in postmenopausal women. *Bone* **1988**, *9*, 201−204.
(348) Nelson, M. E.; Fisher, E. C.; Dilmanian, F. A.; Dallal, G. E.; Evans, W. J. A 1-yr walking program and increased dietary calcium in postmenopausal women: effects on bone. *Am. J. Clin. Nutr.* **1991**, *53*, 1305−1311.
(349) Sandler, R. B.; Cauley, J. A.; Hom, D. L.; Sashin, D.; Kriska, A. M. The effects of walking on the cross-sectional dimensions of the radius in postmenopausal women. *J. Bone Miner. Res.* **1987**, *41*, 65−69.
(350) Turner, C. H.; Owan, I.; Takano, Y. Mechanotransduction in bone: role of strain rate. *Am. J. Physiol.* **1995**, 269, E438−E442.
(351) O'Connor, J. A.; Lanyon, L. E.; MacFie, J. The influence of strain rate on adaptive bone remodeling. J. *Biomechanics* **1982**, *15*, 767−781.
(352) Fehling, P. C.; Alekel, L.; Clasey, J.; Rector, A.; Stillman, R. J. A comparison of bone mineral densities among female athletes in impact loading and active loading sports. *Bone* **1995**, *17*, 205−210.
(353) Taaffe, D. R.; Robinson, T. L.; Snow, C. M.; Marcus, R. High-impact exercise promotes bone gain in well-trained female athletes. *J. Bone Miner. Res.* **1997**, *12*, 255−260.

(354) Radin, E. L.; Paul, I. L.; Rose, R. M. Role of mechanical factors in pathogenesis of primary osteoarthritis. *Lancet* **1972**, *1*, 519−522.
(355) Felson, D. T.; Zhang, Y.; Hannan, M. T.; Naimark, A.; Weissman, B.; Aliabadi, P.; Levy, D. Risk factors for incident radiographic knee osteoarthritis in the elderly. *Arthritis Rheum.* **1997**, *40*, 728−733.
(356) Hayes, W. C.; Myers, E. R.; Robinovitch, S. N.; Van Den, Kroonenberg, A.; Courtney, A. C.; McMahon, T. A. Etiology and prevention of age-related hip fractures. *Bone* **1996**, *18*, 77S−86S.
(357) Rooks, D. S.; Kiel, D. P.; Parsons, C.; Hayes, W. C. Self-paced resistance training and walking exercise in community-dwelling older adults: effects on neuromotor performance. *J. Gerontol.* **1997**, *52*, M161−168.
(358) Nakamura, T. Vitamin D for the treatment of osteoporosis. *Osteoporosis Int.* **1997**, *7* (Suppl. 3), S155−S158.
(359) Nakamura, T. The importance of genetic and nutritional factors in responses to vitamin D and its analogues in osteoporotic patients. *Calcif. Tissue Int.* **1997**, *60*, 119−123.
(360) Francis, R. M. Is there a differential response to alfacalcidol and vitamin D in the treatment of osteoporosis? *Calcif. Tissue Int.* **1997**, *60*, 111−114.
(361) Kanis, J. A.; McCloskey, E. V.; Beneton, M. N. Vitamin D and analogues in renal bone disease and implications for osteoporosis. *Osteoporosis Int.* **1997**, *7* (Suppl. 3), S179−S183.

JM980344O