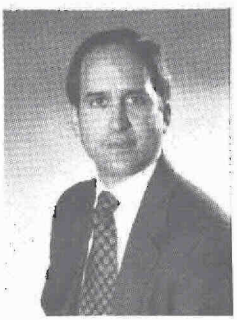
Selective Estrogen Receptor Modulators (SERMs)



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Abstract: Naturally occurring estrogens, such as 17β-estradiol and estrone, have traditionally been thought to play a central role in the development and maintenance of the female reproductive system and secondary sexual characteristics. In recent years, their beneficial effects on the skeleton, the cardiovascular system, and the central nervous system, as well as the cancer risks associated with long term exposure have also been recognized. The widespread use of " antiestrogens " such as tamoxifen for the prevention and treatment of breast cancer has revealed that such compounds, while functioning as estrogen antagonists in mammary tissue, actually mimic the effects of estrogen in other tissues. The search for more selective agents has led to the development of raloxifene, a Selective Estrogen Receptor Modulator, which functions as an estrogen antagonist in the breast and uterus and as an estrogen agonist in the skeleton and cardiovascular system. Recent progress in the development of SERMs is the subject of this review, with an emphasis on structure activity relationships and on their effects in non-traditional target tissues.

Introduction

The central role played by endogenous estrogens, such as 17β-estradiol, **1**, and estrone, **2**, in the development and maintenance of the female sex organs, mammary glands, and other sexual characteristics has long been recognized [1,2]. Recently, their involvement in the growth and function of a number of other tissues, such as the skeleton, the cardiovascular system, and the central nervous system, in both males and females has been recognized [3,4].

The primary site of estrogen biosynthesis in the adult female is the ovary. After the menopause, the ovarian production of estrogens declines dramatically producing a wide range of primary and secondary physiological effects [5,6]. The decline in levels of circulating estrogens has also been linked to a number of pathological conditions including osteoporosis [7,8], coronary artery disease [9,10], depression [11,12], and Alzheimer's disease [12,13]. Estrogen replacement therapy (ERT) has proven effective in reducing the frequency and severity of these pathologies, but the increased risk of endometrial cancer observed with ERT has necessitated the development of therapeutic regimens in which the uterine effects of estrogen are opposed by progestin treatment (hormone replacement therapy or HRT) [14,15]. Side-effects of progestin treatment, such as resumption of menses, central nervous system disturbances, and the possibility of attenuated cardiovascular benefits, have unfortunately resulted in decreased patient compliance [16,17]. Furthermore, recent studies which confirm the increased risk of breast and endometrial cancer associated with long term ERT or HRT have led to the search for treatment alternatives [18,19].

The importance of estrogen in the development and maintenance of the female reproductive system has led to the pharmaceutical development of a variety of steroidal and non-steroidal compounds which interact with the estrogen receptor (ER) as contraceptives and for the treatment of breast cancer, uterine dysfunction, and other reproductive disorders. Several reviews of ER-modulators, with a particular emphasis on their utility in the treatment of breast cancer, have been recently published [20,21]. Early synthetic estrogens such as diethylstilbestrol (DES), **3**, and hexestrol, **4**, were once widely utilized as estrogen substitutes, but due to concerns similar to those encountered with the natural hormones and other side effects their utility has diminished. The discovery that compounds such as MER-25, **5**, antagonize the action of estrogen in breast tissue led to intensive pharmaceutical research, culminating in the development of tamoxifen, **6**, which has found great utility in the treatment of breast cancer [22]. Early concerns that the long-term use of these "antiestrogens" would lead to increased risks of osteoporosis and cardiovascular disease have been dispelled by the paradoxical finding that some compounds (i.e. tamoxifen and raloxifene, **7**) actually mimic the effects of estrogen in skeletal and cardiovascular tissues, although others (i.e. ICI 182780, **8b**) do not [23]. Findings such as these have led to a

Table 1. Classification of Estrogen Receptor Modulators

Classification	Uterine Stimulation	Bone/Cardiovascular	Example
Estrogen Agonists	yes	agonist	17β-estradiol, **1**
Partial Agonists (1st Generation SERMs)	yes	agonist	tamoxifen, **6**
2nd Generation SERMs	no	agonist	raloxifene, **7**
Pure Antiestrogens	no	antagonist/neutral	ICI 182780, **8b**

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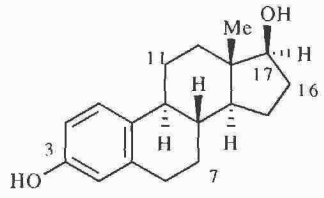
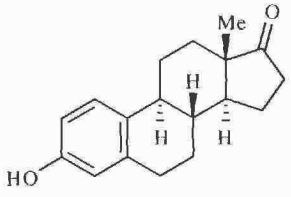
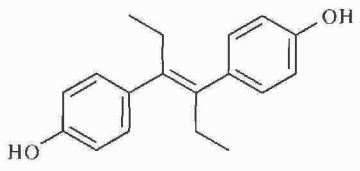


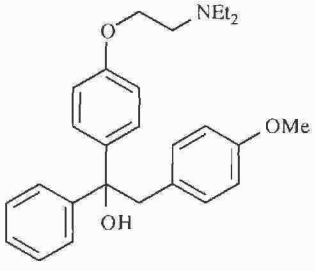
17β-estradiol, **1**



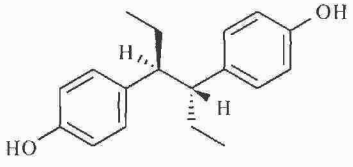
estrone, **2**



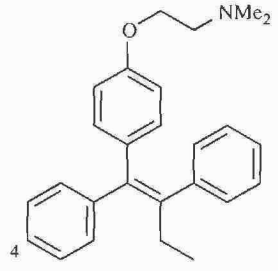
DES, **3**



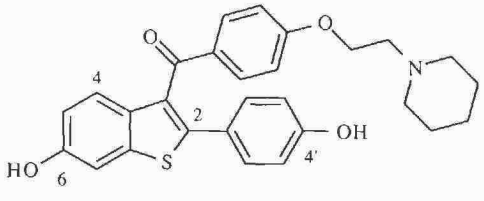
MER-25, **5**



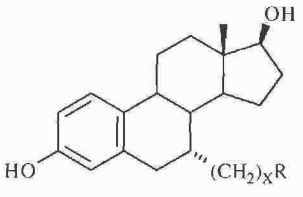
hexestrol, **4**



tamoxifen, **6**



raloxifene, **7**



ICI 164384, **8a** X = 10, R = CON(Me)*n*-Bu
ICI 182780, **8b** X = 9, R = $SO(CH_2)_3CF_2CF_3$

Fig. (1). Representative estrogen receptor modulators.

reclassification of estrogen receptor ligands (Table 1) [24] on the basis of their effects in various traditional and non-traditional target tissues. An explosion of research to understand the molecular basis for this specificity [25] and a race to develop these "designer estrogens" or Selective Estrogen Receptor Modulators (SERMs) as pharmaceutical products has also taken place [26]. The prototypical 2nd generation SERM, raloxifene, **7**, is currently undergoing clinical evaluation for the prevention and treatment of postmenopausal osteoporosis [27]. Nevertheless, it should be noted these distinctions may be somewhat arbitrary, since there is likely to be a continuum of activities from full agonist to full antagonist and the relative activity of an individual compound may be different for each tissue or animal species examined.

In this review, we will discuss the known pharmacology of various structural classes of estrogen receptor modulators, particularly with respect to their effects in non-traditional tissues. We will describe the structure-activity relationships of these compounds, where such data is available, concentrating upon how elements of structure contribute to their tissue-specific actions. Finally, we will provide a brief overview of the current theories which have been developed to account for tissue-specificity of ER-modulators.

Steroidal ER Modulators and the Estrogen Pharmacophore

Natural and synthetic steroidal estrogens have shown great utility and significant therapeutic benefits in the replacement of endogenous hormones in postmenopausal women [7-10,14-18]. Although most studies have focused on the efficacy of ERT or HRT in the prevention of osteoporosis, cardiovascular disease, and disorders of the urogenital tract [28], recent reports have also described benefits in the central nervous system, including improvements in cognitive function, and palliation of Alzheimer's disease and postmenopausal depression [11-13,29,30].

ERT and/or HRT have been demonstrated to provide a variety of cardiovascular benefits, resulting in a 40-50% reduction in the relative risk of coronary disease and atherosclerosis [31,32]. The effects of estrogens on cardiovascular risk factors include raising serum levels of high-density lipoprotein (HDL) cholesterol and apolipoprotein A-1, and lowering levels of low-density lipoprotein (LDL) cholesterol, lipoprotein (a), endothelin-1, and apolipoprotein B [33,34]. Estrogen has also been demonstrated to have direct and indirect effects on blood vessel walls including increased nitric oxide synthesis,

inhibition of vascular smooth muscle cell proliferation, and increased vasodilation [35]. Currently it is felt that the combination of these effects on serum lipids and on vascular tone are responsible for the overall cardioprotective effects of estrogen therapy.

In the prevention of osteoporosis, estrogens function primarily as antiresorptive agents, leading to decreased turnover of both cortical and cancellous bone [36,37]. As with other antiresorptive agents, this benefit is partially offset by a subsequent decrease in bone formation, however the overall result of ERT or HRT is a substantial increase in bone mineral density and a decrease in fracture incidence [38,39]. Although ERs have been detected in both osteoblasts and osteoclasts, it is currently unclear if the effects of estrogens on bone metabolism are direct or indirect [40].

Steroidal Estrogens

Early efforts to identify selective estrogens focused on changes in the parent steroid to elicit tissue specific biological responses. For example, the estrogen metabolites estriol, **9**, and 17α-estradiol, **10**, were found to be time-dependent mixed agonist-antagonists of estrogen, which stimulate early uterotrophic responses but have little effect on true uterine hypertrophy and hyperplasia unless administered chronically at high doses [41,42]. Estriol causes significantly less uterine hyperplasia than 17β-estradiol and inhibits the development of breast cancer in rodents [43]. In addition, 17α-estradiol has been shown to exert a neuroprotective effect in a human neuroblastoma cell line (SK-N-SH) [44]. The estrogen metabolite, 2-methoxyestradiol, **11**, has been implicated in the angiogenesis of vascular tissue and a number of analogs have been reported which potently inhibit tubulin polymerization [45,46]. The estrogen analog 17α-ethynylestradiol (EE_2), **12**, has been extensively studied for its bone, uterine, and lipid effects due, in large part, to an enhanced oral activity profile relative to 17β-estradiol.

Improvements in tissue selectivity have been observed with a family of D-ring halogenated estrones (such as **13**) which have demonstrated potent lipid lowering yet diminished uterine hypertrophy relative to estrone [47]. Other attempts to attenuate the estrogenic activity of steroids via opening of the steroid nucleus, such as 9,11-seco steroids, **14**, have met with limited success [48,49].

Recently, the components of Premarin® (the most prescribed form of ERT) have been evaluated for their lipid lowering effects. These conjugated equine estrogens contain sulfate esters of two distinct estrogen structural classes; (1) ring B saturated steroids

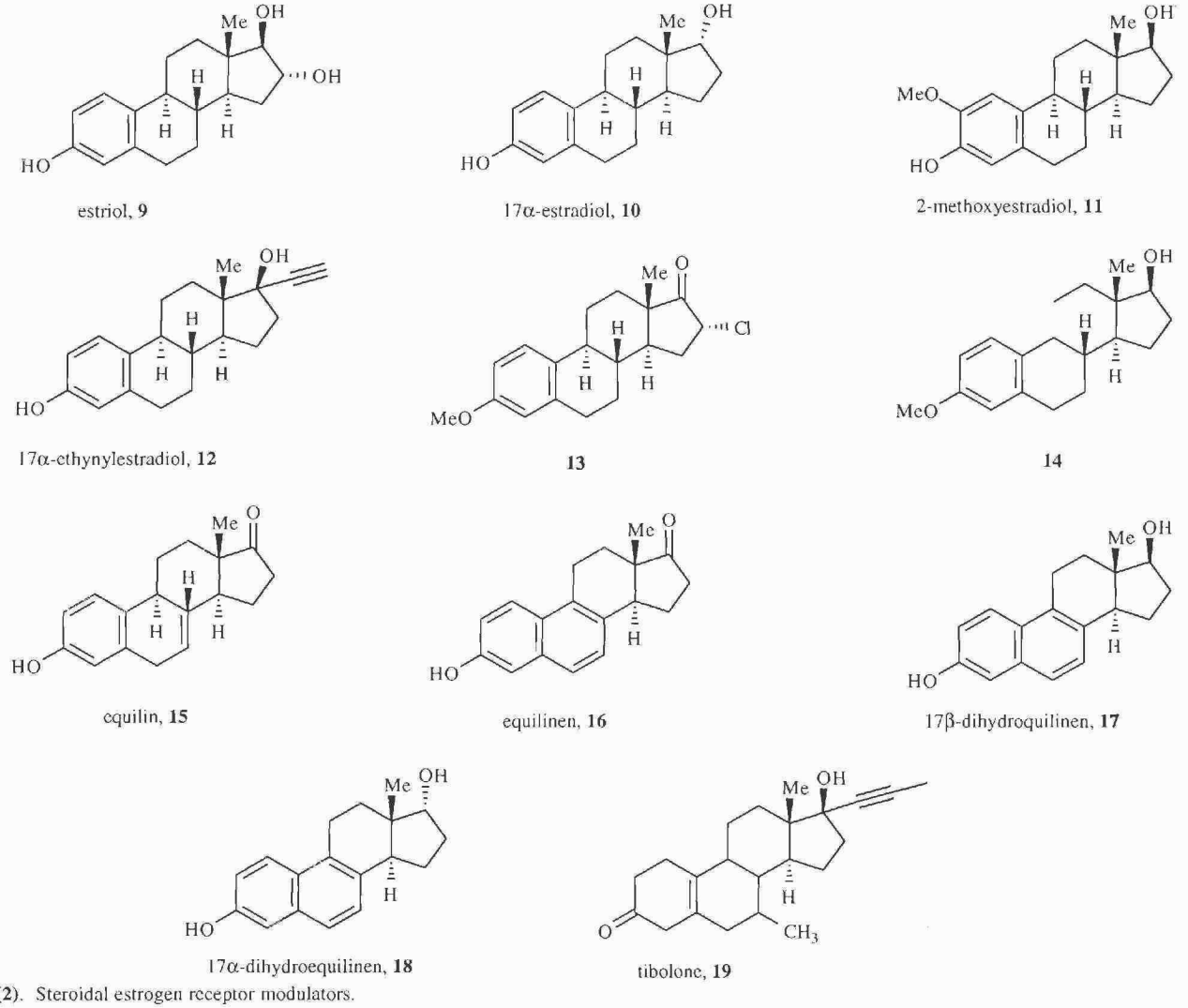


Fig. (2). Steroidal estrogen receptor modulators.

including traditional sex steroid hormones such as estrone, 17β-estradiol, and 17α-estradiol, and (2) ring B unsaturated estrogens such as equilin (Eq), **15**, equilenin (Eqn), **16**, 17β-dihydroequilenin (17β-DHEqn), **17**, 17β-dihydroequilin (17β-DHEq), 17α-dihydroequilenin (17α-DHEqn), **18**, and 17α-dihydroequilin (17α-DHEq). In 1991, Bhavnani and co-workers 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 [50]. In this study, the majority of equine components mimicked estrogen 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 and increase hippocampal dendritic spine density in rats, and improve arterial vasomotor function in macaques [51].

Work from our own laboratories on the relative effects of conjugated equine estrogens on bone versus uterus has shown that 17α-DHEqn is a partial estrogen agonist [52]. In this study, uterotrophic effects were observed after 4 days of oral dosing for Eq, **15**, Eqn, **16**, 17β-DHEqn, **17**, and 17α-DHEqn, **18**. Increases in uterine wet weight relative to ovariectomized (OVX) controls ranged from 263% for Eq to 100% for 17α-DHEqn. Serum cholesterol levels were lowered with similar potencies for all equine estrogens [52]. Bone mineral density measurements indicated that 17α-DHEqn effectively prevented osteopenia in a dose-dependent fashion after 5-weeks of oral administration (59.9% of ovariectomy-induced bone loss was prevented at 1 mg/kg, 119 % at 10 mg/kg). In addition, an average uterine weight gain of 100.4% relative to OVX controls was observed at the 1 mg/kg dose [52]. These data demonstrate that 17α-DHEqn is a full estrogen agonist on bone, but a partial agonist on the uterus in the OVX rat and further highlight the structural significance of both the stereochemistry at the 17-position and unsaturation in the B-ring.

Tibolone (OD-14), **19**, is a unique steroid that possesses estrogenic, progestenic and androgenic properties. At doses of less than 2.5 mg/day, OD-14 appears to reduce skeletal remodeling without producing concomitant endometrial stimulation [53]. However, because of its estrogenic activity, endometrial hypertrophy over the long term remains a possibility.

Pure Antiestrogens

While estrogen agonists, partial agonists, and SERMs can mimic the pharmacology of the natural hormone, pure antiestrogens (e. g., **8a,b**) represent a class of therapeutic agents which are devoid of estrogen agonism regardless of the target tissue. Initially introduced by Wakeling in 1988, these compounds demonstrate an absence of estrogenic activity on the rat uterus, vagina, and hypothalamic-pituitary axis as well as effectively antagonizing the stimulatory effects of estrogen [54]. In non-reproductive tract tissue, pure antiestrogens behave like estrogen antagonists as well. For example, ICI 164,384, **8a**, and ICI 182,790, **8b**, exhibited no capacity for lowering serum cholesterol or sparing bone loss in the OVX rat model [55]. Recent data suggests that ICI 182,780 has significantly complex effects on rat skeletal tissue [56]. For example, loss of cancellous bone is observed in intact rats after administration of the compound whereas no bone loss is observed in OVX rats.

Estradiol Pharmacophore

Recently, Katzenellenbogen, et. al. have combined literature ER binding affinity data for a large number of steroidal estrogen analogs with molecular modeling and receptor sequence analysis to develop a detailed picture of the estradiol pharmacophore [57]. Their study recognizes the important contributions of the two hydroxy groups of estradiol to receptor binding, with the 3-hydroxy acting primarily as a hydrogen bond donor and contributing approximately 1.9 kcal/mol to the binding free energy, while the 17β-hydroxy functions primarily as a hydrogen bond acceptor and contributes approximately 0.6 kcal/mol [58]. The preferred distance between the hydroxy functionalities appears to be somewhat flexible, possibly due to the inclusion of water molecules in the binding cavity [57]. Large, preformed, hydrophobic pockets apparently exist within the ligand binding domain which are able to accommodate large substituents at the 11β- and 7α-positions [59]. Smaller pockets appear to exist at the 16β- and 17β-positions, while the 16α-position and the aromatic A-ring are relatively intolerant of substitution [57]. These properties of the ER ligand binding cavity, which were primarily determined empirically, appear to be supported by the recently reported X-ray crystal structure of the ER ligand binding domain complexed with estradiol [60].

Triphenylethylenes

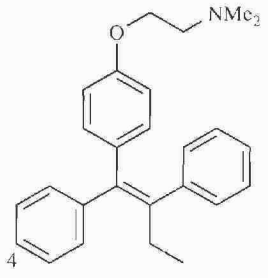
The most thoroughly investigated class of non-steroidal ER modulators are the triphenylethylenes (TPE's), such as tamoxifen, **6**, and clomiphene, **20**. A common structural motif which is incorporated in many classes of molecules with estrogen antagonist activity involves the attachment of a sidechain containing a hydrogen bond acceptor to an ER binding core unit. This theme is illustrated for the triphenylethylenes via the progression from DES to MER-25 and tamoxifen. Originally investigated for contraceptive activity, the strong estrogen antagonist activity observed with many of these compounds in mammary tissue, has led to their development for treatment of breast cancer [20d]. The success of tamoxifen in this arena, has led to the investigation and development of a wide variety of analogs. To date, SAR work in this series has been confined primarily to the investigation of antagonist effects in mammary and uterine tissue [20]. Recently, reports of estrogen agonist effects of some of these compounds in the skeletal and cardiovascular system have begun to appear [26]. In general, although they partially antagonize the effects of estrogen on the uterus, the members of this structural class tend to induce some level of uterine stimulation in the absence of endogenous estrogen, therefore they have been classified as partial agonists or first generation SERMs [61].

One of the first TPE's to be utilized clinically was clomiphene, **20**. Although it was originally developed as a contraceptive, clomiphene has been mainly utilized for the induction of ovulation in anovulatory women [62]. Its effects on uterine tissue are complex, and are at least partly complicated by its availability as a mixture of double-bond isomers (zuclomiphene and enclomiphene), but it appears to cause significant stimulation of uterine epithelia in the rat [63,64].

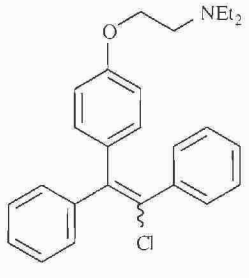
Clomiphene has been reported to reduce serum cholesterol in rats, similar to estrogen, however this may not be an ER-mediated effect [63]. Clomiphene has also been reported to inhibit bone resorption *in vitro* [65] and to protect against bone loss in both OVX rats [66] and in postmenopausal women [67]. Interestingly, the individual isomers of clomiphene have been reported to have similar effects on bone metabolism, while the uterine effects are primarily induced by zuclomiphene [68]. Recently a clomiphene analog, MDL-103,323, **21**, with antiproliferative activity in breast cancer assays has been reported to protect against bone loss in OVX rats with minimal uterine stimulation [69].

Consistent with the importance of the hydroxyl moieties of estradiol for receptor binding, tamoxifen, **6**, binds only weakly to the ER, however evidence suggests that the primary biologically active species may be its 4-hydroxy metabolite [70,71]. Estrogen antagonist effects in mammary tissue have been demonstrated in a variety of cell lines and animal models [20d]. Tissue specific estrogen agonist effects have been demonstrated in the OVX rat model of estrogen deficiency, where tamoxifen reduced serum cholesterol by 50% at doses of 0.1-10 mg/kg and protected against bone loss with an ED_{50} of 0.1 mg/kg [61,72,73]. *In vitro* effects on bone resorption and osteoclast viability have also been demonstrated [64,74]. In a primate model, tamoxifen was shown to significantly inhibit the progression of coronary artery atherosclerosis [75].

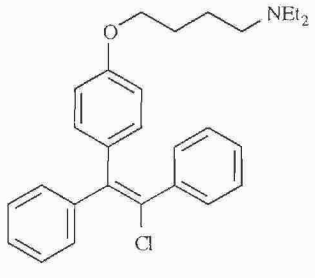
Due to the widespread use of tamoxifen in the treatment of breast cancer, a large body of clinical evidence with respect to its effects in other tissues has also accumulated [22]. In the cardiovascular system, tamoxifen has been shown to significantly reduce risk factors of disease including LDL cholesterol, lipoprotein (a), and fibrinogen in postmenopausal women with little or no effect on triglycerides or HDL cholesterol [76,77]. A corresponding decrease in mortality due to cardiovascular disease has also been reported [78]. Clinical effects on the skeleton have included the preservation of bone mineral density at the lumbar spine, femoral neck, and forearm in postmenopausal women [77,79] as well as an estrogen-like reduction in serum markers of bone turnover [79b-d,80]. Interestingly, in premenopausal women decreases in bone



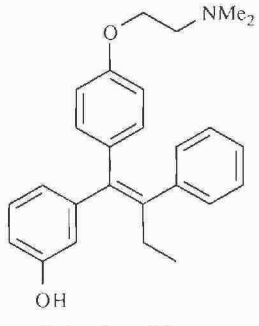
tamoxifen, **6**



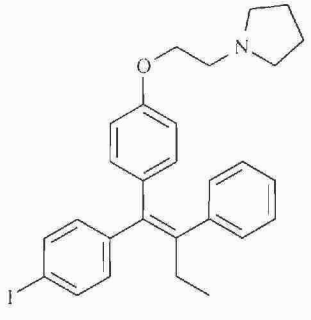
clomifene, **20**



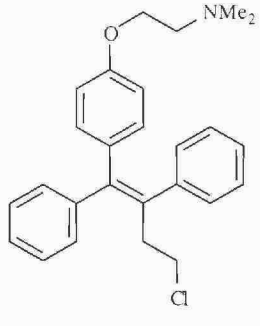
MDL-103,323, **21**



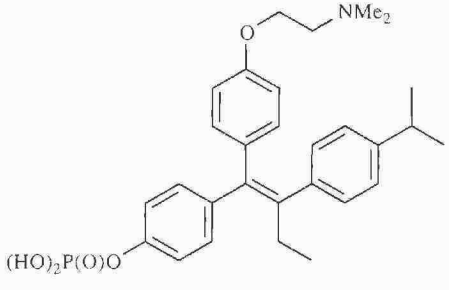
droloxifene, **22**



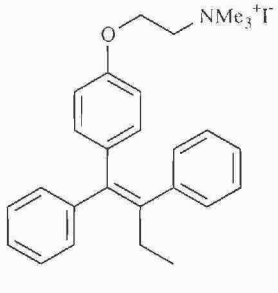
idoxifene, **23**



toremifene, **24**



TAT-59, **25**



TMI, **26**

Fig. (3). Triphenylethylene estrogen receptor modulators.

mineral density have been observed with tamoxifen treatment [79a,e].

Notwithstanding the positive effects described above for tamoxifen, there continues to be considerable concern about the increased risk of endometrial cancer which has been associated with tamoxifen use [81,82]. Estimations of the magnitude of this risk vary, however the average value of about a five-fold increase is similar to that observed with ERT [83]. Significant stimulation of uterine endometrial tissue is also observed in the OVX rat model [61,84] and DNA adduct formation has been observed in both rats and humans [85,86]. Tamoxifen has also been shown to induce liver cancer in rats [87].

The growing concern over the potential cancer causing or cancer promoting effects of tamoxifen has recently led to the development of a number of tamoxifen analogs. The hypothesis that metabolic hydroxylation of tamoxifen at the 4-position is in some way responsible for these effects has led to the investigation of agents in which this metabolic pathway is blocked [20d]. Examples of this strategy include droloxifene, **22**, and idoxifene, **23**, which have been reported to show decreased levels of DNA adduct formation and hepatocarcinogenicity [88,89]. In toremifene, **24**, chlorination of the aliphatic substituent of tamoxifen also appears to reduce DNA-adduct formation [90]. Alternative strategies for modifying the metabolic fate and/or tissue distribution observed with tamoxifen are represented by TAT-59, **25**, and tamoxifen methiodide or TMI, **26**. To date these compounds have been most extensively evaluated for the treatment of breast cancer [90,91], however recently reports on their effects in non-traditional target tissues have begun to appear.

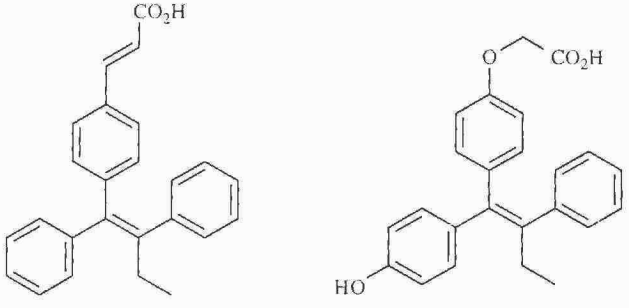
In droloxifene, **22**, hydroxylation at the 3-position of the TPE core leads to an altered metabolic profile and decreased estrogen agonist activity relative to tamoxifen [92,93]. In OVX rats, droloxifene has been reported to reduce serum cholesterol 40-46% and to protect against loss of bone mineral density, similar to tamoxifen but with reduced uterine stimulation [94]. In a head-to-head comparison with tamoxifen, droloxifene was found to be at least 10-fold less potent in terms of its effects on serum cholesterol and bone density, even though it has a tenfold higher binding affinity to the ER [95]. Estrogenic effects in the skeleton have also been observed by histomorphometry at both cancellous and cortical bone sites [96]. Recently, droloxifene has been observed to induce apoptosis of both MCF-7 cells and osteoclasts in culture, while estrogen has similar effects on osteoclasts but is mitogenic to MCF-7 cells [97]. This tissue-specific difference has led to the hypothesis that a common mechanism may account for both the estrogen agonist and antagonist activities of droloxifene. Although droloxifene has been evaluated clinically for efficacy in breast cancer treatment [91a], its effects on other estrogen target tissues in humans have not yet been reported.

Idoxifene, **23**, was designed to reduce both metabolic oxidation and *N*-demethylation, via iodination of the 4'-position and replacement of the dimethylamino group of tamoxifen with a pyrrolidine ring, respectively [98]. As with droloxifene, idoxifene has been evaluated clinically for breast cancer treatment [91b], and a preliminary report describing its effects on serum cholesterol and bone density in the OVX rat has also appeared [99]. It has also been reported to be less uterotrophic than tamoxifen [100].

Toremifene, **24**, has recently been approved for the treatment of breast cancer and has demonstrated clinical effects on serum cholesterol and bone mineral density which are similar to those of tamoxifen in postmenopausal breast cancer patients [101,102]. Although it has been reported to be less uterotrophic in the rat [103], its estrogenic effects on the uterus in postmenopausal women have been reported to be comparable to those of tamoxifen [104].

Tamoxifen methiodide, **26**, was designed to inhibit crossing of the blood-brain barrier, in order to avoid possible estrogen antagonist effects in the central nervous system [91d]. Interestingly, this compound has recently been reported to selectively stimulate creatinine kinase activity in bone cells but not uterine cell lines, while tamoxifen and estrogen stimulate this activity in both [105]. Similar effects have been described *in vivo*, although correlation of these effects with bone density and uterine stimulation have not yet been reported [106].

Two new TPE's which contain carboxylic acid functionality in place of the amine side chain moiety have also been reported. GW5638, **27**, has been described as a bone-selective estrogen agonist, and has demonstrated decreased uterine stimulation, relative to tamoxifen, in OVX rats [107,108]. Interestingly, amide analogs of **27** showed an increased tendency toward uterine stimulation both *in vivo* and *in vitro* [107]. In OVX rats, **27** was observed to maintain bone mineral density at both the lumbar spine and the proximal tibia with an efficacy similar to that of 17β-estradiol or tamoxifen at doses of 1-10 mg/kg [107,108]. It has also been shown to reduce serum cholesterol in OVX rats with a maximal efficacy of 20-30% [108,109]. The magnitude of this effect, although similar to that observed with 17β-estradiol, appears to be somewhat muted in comparison to the more bioavailable 17α-ethynyl estradiol and other TPE's, implying perhaps that multiple mechanisms may be involved in the regulation of serum lipid concentrations by these compounds [110]. Hydroxytamoxifen acid, **28**, a tamoxifen metabolite, has also been reported to have bone-selective effects in the OVX rat [111].



GW5638, **27** **28**

Fig. (4). Acidic triphenylethylenes.

Several groups have recently reported the application of parallel synthesis techniques for the preparation of TPE libraries [112]. It is expected that the ready availability of more structurally diverse members of this class, together with the development of molecular biological assays predictive of *in vivo* tissue selectivity, will lead to greater understanding of the SAR of these compounds in multiple tissues.

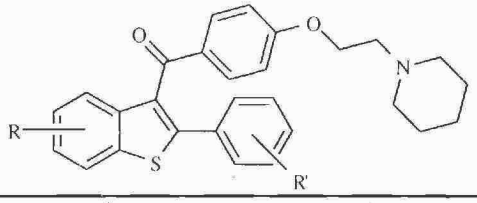
Benzothiophenes

In order to avoid the problems associated with double bond isomerization of the TPE's, a variety of cyclic frameworks have been investigated for their ER modulating properties. Out of these structure-activity studies, raloxifene, **7**, a benzothiophene-containing compound with a unique profile of biological activity emerged [26b,c].

Raloxifene has been shown to bind the estrogen receptor with high affinity and to function as a potent estrogen antagonist in mammary tumor cells and in rat models of mammary cancer [113,114]. In contrast, in the cardiovascular system, raloxifene functions primarily as an estrogen agonist. In cell culture, raloxifene has demonstrated estrogen-like effects on vascular smooth muscle cells and on the inhibition of LDL oxidation [115,116]. In the OVX rat model, raloxifene has been shown to reduce serum cholesterol by 50-75% after 1-5 weeks of daily dosing [117,118]. Most importantly, in postmenopausal women treated daily with raloxifene, significant reductions in total serum cholesterol and LDL cholesterol have been observed after both eight weeks and two years of treatment [27].

Similarly, the effects of raloxifene on the skeleton seem to parallel those observed with estrogen. *In vitro* studies have shown similar effects of raloxifene and 17β-estradiol on

Table 2. ER Binding and Inhibition of MCF-7 Cell Proliferation by 2-Aryl Raloxifene Analogs [127a]



no.	R	R'	ER RBA[a,b]	MCF-7 Inhib. IC_{50} (nM)[c]
estradiol			1.00	NA[d]
4-OH-tam[e]			0.36	0.5
raloxifene, **7**	6-OH	4-OH	0.34	0.2
7a	none	none	<0.002	300
7b	6-OMe	4-OMe	<0.002	300
7c	6-OH	4-OMe	0.073	1000
7d	6-OMe	4-OH	0.008	250
7e	none	4-OH	0.003	35
7f	6-OH	none	0.062	2.5
7g	6-OH	4-Cl	0.046	1
7h	6-Cl	4-OH	0.006	1000
7i	6-OH	4-Me	0.07	50
7j	6-Me	4-OH	NA[d]	300
7k	6-OH	4-F	0.19	2.3
7l	7-OH	4-OH	0.02	300
7m	4-OH	4-OH	<0.002	190
7n	5-OH	4-OH	0.10	100
7o	6-OH	2-OH	0.057	10
7p	6-OH	3-OH	0.16	3.2
7q	5-F,6-OH	4-OH	0.098	3
7r	5-Me,6-OH	4-OH	0.07	ND[f]
7s	5,7-di(Me),6-OH	4-OH	0.005	500
7t	6-OH	2-Me,4-OH	0.41	2
7u	6-OH	3-Me,4-OH	0.13	1
7v	6-OH	3-Cl,4-OH	0.12	2.3
7w	6-OH	3-F,4-OH	0.20	0.3
7x	6-OH	3,5-di(Me),4-OH	0.12	100

[a]RBA = relative binding affinity by competition with ^{3}H-17β-estradiol. [b]Average of at least 2 determinations. Values are ± 10%. [c]Dose required to give 50% inhibition of a maximally effective (10^{-11}) dose of 17β-estradiol. Average of at least 3 determinations. Values are ± 10%. [d]NA = not active at the doses tested. [e]4-Hydroxytamoxifen, the primary biologically active metabolite of tamoxifen. [f]ND = not determined.

osteoclastogenesis and on creatinine kinase activity in human osteoblast cells [119,120]. In rats, several studies have demonstrated a protective effect against ovariectomy-induced osteopenia at doses as low as 0.1 mg/kg [117,121,122]. Positive effects on bone mineral density at both cortical and cancellous bone sites have been reported, as have positive effects on bone strength [121,123]. Interestingly, although raloxifene suppresses bone resorption in the rat with efficacy which is approximately equal to that of estrogen, bone formation appears to be suppressed to a lesser degree, resulting in a net gain in bone mass with raloxifene [121,123b]. These positive results in animal studies, have now been confirmed with clinical studies in postmenopausal women [27]. Significant positive effects on histomorphometric parameters, bone markers, and on bone mineral density at both the lumbar spine and hip were observed after two years of raloxifene treatment [27,124].

In terms of its pharmacology, raloxifene is distinguished from the TPE's primarily on the basis of its effects on the uterus, where a qualitative difference has been observed [61,125]. In a direct comparison with tamoxifen, droloxifene, and idoxifene, raloxifene was a significantly more effective antagonist of estrogen action in the immature female rat uterus [61a]. In this assay the TPE's functioned as partial agonists, inhibiting the effects of estrogen on uterine weight gain only to the level of their own intrinsic agonist activity, while raloxifene functioned essentially as a complete antagonist. Similarly, in OVX rats, the TPE's have been found to induce a larger maximal stimulation of uterine weight and to induce uterine eosinophilia while raloxifene did not [61]. Although raloxifene has also been reported to stimulate a modest increase in uterine wet weight, this increase is not dose related and is not coincident with increases in other measures of uterine hypertrophy, and has therefore been attributed to water retention [117]. In postmenopausal women, raloxifene has been reported to show no stimulatory effects on the uterus, even after 2 years of treatment [27,126].

SAR studies in the raloxifene series have centered on modifications of the 2-arylbenzothiophene [127], the amine-containing side chain [113,128], and the carbonyl hinge [129]. The 2-arylbenzothiophene unit appears to be the primary site of ER binding, mimicking the interactions of 17β-estradiol with the receptor. On the basis of binding and *in vitro* activation data (Table 2) the 6-hydroxy of raloxifene is believed to imitate the 3-hydroxy of estradiol, while the 4'-hydroxy roughly approximates the 17β-hydroxy [127a]. The recently published crystal structures of raloxifene and 17β-estradiol bound to the ER confirm this interpretation [60]. Consistent with the estradiol pharmacophore (*vide supra*), the 6-hydroxy appears to be more important and any modification thereof or additional substitution of the benzothiophene portion of raloxifene significantly inhibited receptor binding and *in vitro* biological activity (Table 2) [125,127a]. The 4'-hydroxy substituent showed considerably more flexibility with respect to the effects of substitution (Table 2). Surprisingly, significant binding activity was observed for a number of derivatives in which the entire 2-phenyl substituent had been replaced (Table 3) [127].

Glucuronidation of the 6- and 4'-hydroxy moieties have been shown to be the primary metabolic pathways exhibited by raloxifene in rats, dogs, monkeys, and humans [130]. Although these metabolites show significantly reduced ER binding and activation *in vitro*, conversion to the parent molecule via deconjugation has been shown to occur at the tissue level [131]. Interestingly, no significant differences in conversion at various target organs such as uterus, bone, and liver have been observed.

Table 3. ER Binding and Inhibition of MCF-7 Cell Proliferation by 2-Alkyl, 2-Naphthyl, and 2-Heteroaryl Raloxifene Analogs [127a]

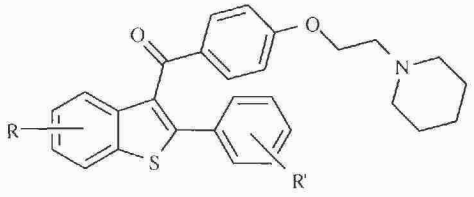
no.	R	ER RBA[a,b]	MCF-7 Inhib. IC_{50} (nM)[c]
raloxifene, 7		0.34	0.2
7aa	1'-naphthyl	0.20	0.8
7bb	2'-naphthyl	0.067	80
7cc	4'-OH-1'-naphthyl	0.16	2
7dd	2'-thienyl	0.30	20
7ee	3'-thienyl	0.20	10
7ff	methyl	0.15	35
7gg	ethyl	0.13	20
7hh	isopropyl	0.15	3
7ii	cyclopentyl	0.08	5
7jj	cyclohexyl	0.09	2.5
7kk	*t*-4'-OH-cyclohexyl	0.09	2
7ll	4'-hydroxybenzyl	0.19	5
7mm	4'-pyridyl	0.056	100
7nn	4'-pyridyl-N-oxide	0.005	100

[a]RBA = relative binding affinity by competition with ^{3}H-17β-estradiol. [b]Average of at least 2 determinations. Values are ± 10%. [c]Dose required to give 50% inhibition of a maximally effective (10^{-11}) dose of 17β-estradiol. Average of at least 3 determinations. Values are ± 10%.

In vivo data from the OVX rat assay provides a number of additional insights into the effects of structural modification on the biological activities of raloxifene analogs. Although many of the compounds caused modest increases in uterine weight similar to raloxifene, uterine eosinophil peroxidase activity provided a more consistent measure of uterine stimulation in the OVX rat [61]. Compounds in which the 6 and/or 4'-hydroxyls were absent or were capped as methyl ethers had potent *in vivo* effects on serum cholesterol lowering without significant induction of uterine eosinophilia, possibly indicating the potential for metabolic activation (Table 4) [127a]. In general, replacement of the hydroxy moieties by other substituents or increased substitution of the 2-aryl benzothiophene core resulted in reduced potency. Increased steric bulk at the 4'-position appeared to increase the uterine stimulation observed with these

analogs (Table 4)[127a]. Several analogs in which the 2-phenyl group was replaced also showed significant *in vivo* biological activity, with demonstrated effects on serum cholesterol lowering and bone mineral density (Tables 5 and 6) [127]. In general, however, the 6-hydroxy-2-(4'-hydroxyphenyl) substitution pattern of raloxifene appears to be nearly optimal for both *in vitro* and *in vivo* activity.

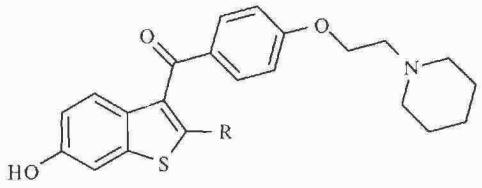
Table 4. Serum Cholesterol Lowering and Uterine EPO Activity of 2-aryl Raloxifene Analogs in the OVX Rat [127a]



no.	R	R'	Uterine EPO MED(V_{max})[a]	Serum Cholesterol (Max% decr OVX)[b]	ED_{50}[c] (mg/kg)
17α-ethinyl estradiol			0.1(281.7 ± 6)	84.5*± 1.7	0.005
tamoxifen, **6**			0.1(57.1 ± 3.3)	71.1*± 4.3	0.2
raloxifene, **7**			>10	74.0*± 2.1	0.2
7a	none	none	>10	72.6*± 6.1	<0.1
7b	6-OMe	4-OMe	>10	78.0*± 3.4	<0.1
7c	6-OH	4-OMe	>10	75.6*± 2.8	<0.1
7d	6-OMe	4-OH	>10	64.5*± 5.3	<0.1
7e	none	4-OH	>10	70.7*± 5.6	<0.1
7f	6-OH	none	>10	66.0*± 4.1	<0.1
7g	6-OH	4-Cl	>10	69.9*± 6.9	0.3
7h	6-Cl	4-OH	>10	37.6*± 7.0	>10
7i	6-OH	4-Me	>10	69.1*± 6.0	2.9
7j	6-Me	4-OH	>10	33.9*± 6.0	>10
7k	6-OH	4-F	>10	71.9*± 6.7	1.4
7l	7-OH	4-OH	>10	-3.4 ± 12.0	----
7m	4-OH	4-OH	>10	31.8*± 6.3	>10
7n	5-OH	4-OH	>10	22.6 ± 3.9	----
7o	6-OH	2-OH	>10	33.2*± 11.8	>10
7p	6-OH	3-OH	>10	52.3*± 5.5	0.8
7q	5-F, 6-OH	4-OH	>10	31.6*± 6.4	>10
7r	5-Me, 6-OH	4-OH	>10	43.7*±	5.4
7t	6-OH	2-Me, 4-OH	>10	68.5*± 3.9	0.4
7u	6-OH	3-Me, 4-OH	>10	73.9*± 3.9	1.0
7v	6-OH	3-Cl, 4-OH	>10	71.5*± 2.8	1.2
7w	6-OH	3-F, 4-OH	>10	59.2*± 5.3	0.4
7x	6-OH	3,5-Me, 4-OH	1.0(40.8 ± 20.7)	69.1*± 3.6	1.8
7y	6-OH	4-Et	1.0(39.9 ± 15.3)	57.4*± 8.3	4.3
7z	6-OH	4-Ph	1.0(25.4 ± 0.9)	68.5*± 10.6	2.4

[a]MED at which a significant (> 5-fold increase relative to OVX control and value of $V_{max} \geq 10$) increase in EPO activity was observed. Activity at the MED is expressed as V_{max} ± standard error. [b]Maximum observed percent decrease in serum cholesterol relative to OVX controls ± standard error. Statistically significant ($p \leq 0.05$) differences are denoted "*". [c]Dose required to reduce serum cholesterol by 50% relative to OVX controls.

Table 5. Serum Cholesterol Lowering and Uterine EPO Activity of 2-Alkyl, 2-Naphthyl, and 2-Heteroaryl Raloxifene Analogs in the OVX Rat [127a]



no.	R	Uterine EPO MED(V_{max})[a]	Serum Cholesterol (Max% decr OVX)[b]	ED_{50}[c] (mg/kg)
raloxifene, **7**		>10	74.0*± 2.1	0.2
7aa	-1-naphthyl	>10	67.8*± 4.1	0.5
7bb	-2-naphthyl	0.1(12.3 ± 0.3)	63.6*± 3.6	0.2
7cc	-4-hydroxy-1-naphthyl	0.1(29.4 ± 0.6)	59.9*± 4.8	1.2
7dd	-2-thienyl	>10	51.4*± 10.8	11
7ee	-3-thienyl	>10	31.8*± 5.9	>10
7ff	-Me	>10	62.9*± 7.3	1.8
7gg	-Et	>10	31.1 ± 6.9	----
7hh	-*i*Pr	>10	35.0*± 16.1	>10
7ii	-cyclopentyl	>10	69.6*±7.1	1.2
7jj	-cyclohexyl	>10	61.7*± 2.3	2.1
7kk	-*trans*-4-hydroxycyclohexyl	>10	60.4*±3.1	1.0
7ll	-4-hydroxybenzyl	>10	54.0*± 5.5[e]	4.0
7mm	-4-pyridyl	>10	47.6*± 9.9	14.6
7nn	-4-pyridyl-*N*-oxide	>10	42.6*± 6.2	>10

[a]MED at which a significant (> 5-fold increase relative to OVX control and value of $V_{max} \geq 10$) increase in EPO activity was observed. Activity at the MED is expressed as V_{max} ± standard error. [b]Maximum observed percent decrease in serum cholesterol relative to OVX controls ± standard error. Statistically significant ($p \leq 0.05$) differences are denoted "*". [c]Dose required to reduce serum cholesterol by 50% relative to OVX controls.

Table 6. Bone Protective Effects of Raloxifene Analogs in the OVX Rat [127a]

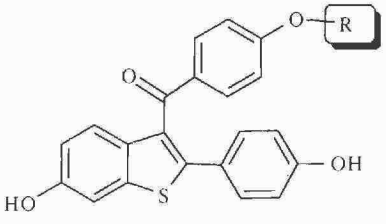
no.	Bone Mineral Density, distal femur (% protection vs. OVX)[a]			
	0.01 mg/kg/day	**0.1 mg/kg/day**	**1.0 mg/kg/day**	**10.0 mg/kg/day**
17α-ethinyl estradiol	----	69.2*± 7.7	----	----
raloxifene, **7**	9.6 ± 5.8	50.0*± 5.8	57.7*± 5.8	53.8*± 5.8
7e	-1.3 ± 12.7	-4.0 ± 9.6	41.8*± 19.0	26.7*± 14.7
7p	----	-30.9 ± 20.7[b]	79.7*± 17.1[b]	44.2*± 22.9[b]
7w	34.5 ± 36.5	34.1 ± 17.2	68.0* ± 15.1	69.8*± 20.5
7cc	35.9*± 14.0	42.3*± 13.7	82.5*± 14.0	----
7kk	----	17.0 ± 8.4	61.3*± 30.0	67.4*± 15.0

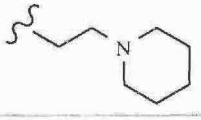
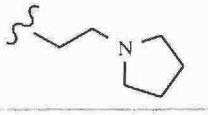
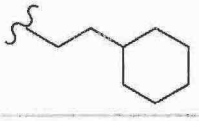
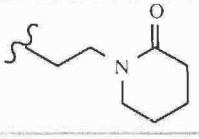
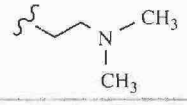
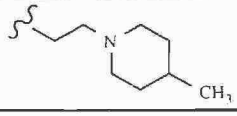
[a]Measured X-ray image analysis. Values are given as % protection relative to OVX controls ± standard error, with sham control values defined as 100% and OVX conrols defined as 0. [b]BMD determined by quantitative computed tomography at the proximal tibia.

In TPE's such as tamoxifen, the importance of the position and nature of the amine functionality with regard to estrogen antagonist activity in the breast and uterus has been demonstrated [132]. Likewise, studies with raloxifene indicated

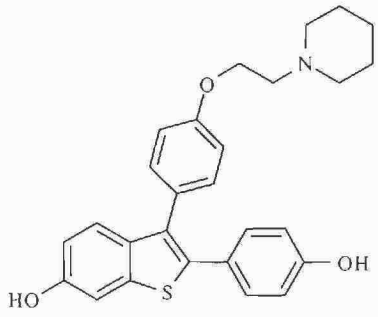
that the piperidine moiety is critical for antagonizing the effects of estrogen both *in vivo* and *in vitro* [125]. Indeed, the X-ray crystal structure of raloxifene bound to the ER confirms the hydrogen bonding interaction of the nitrogen with an aspartic acid residue and provides a structural basis for its influence on antagonist activity [60]. Replacement of the amine functionality with carbon or non-basic nitrogen resulted in a loss of antagonist activity (Table 7) [113,125]. Interestingly, modification of the substituents on nitrogen can significantly alter the profile of tissue specificity which is observed. LY117018, **7oo**, in which the piperidine moiety of raloxifene has been replaced with a pyrrolidine, demonstrates a similar profile of biological activity, however replacement with the dimethylamino group present in tamoxifen leads to a compound with reduced antagonist activity (Table 7) [113,125]. In direct contrast, however, replacement of the dimethylamino group of tamoxifen with a piperidine moiety does not improve its antiuterotrophic properties [132]. Thus, whereas the basic side chain moiety of raloxifene is critical for its' estrogen antagonist properties, it is not the primary determinant of tissue selectivity. Although in general these analogs retain the ability to reduce serum cholesterol and protect against bone loss, the reduced antagonist activities of these compounds in immature rats are paralleled by an increase in observed uterine agonist activity in OVX rats [128].

Table 7. Effects on *In vivo* Estrogen Antagonism for Various Basic Side Chain Raloxifene Analogs [125]

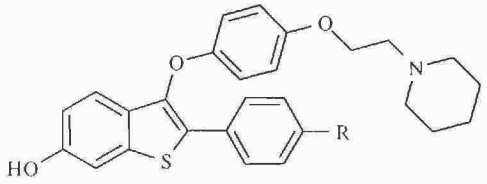


cmpd	R	*In Vivo* Estrogen Antagonism Maximal Percent Inhibition (Uterine Wt/Body Wt)
tamoxifen, **6**		48*
raloxifene, **7**		91*
LY117018, **7oo**		81*
7pp		3.8
7qq		10
7rr		38*
7ss		36*

"*" denotes statistical significance relative to ethynyl estradiol-treated control ($P \leq 0.05$).



29



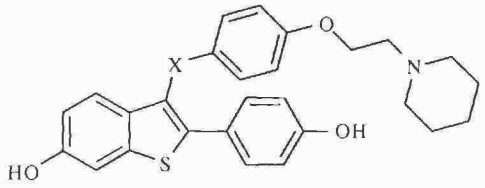
30c, R = OH
LY353381, **30e**, R = OMe

Fig. (5). Modifications to the carbonyl hinge of raloxifene.

The carbonyl hinge which attaches the side chain of raloxifene to the ER binding benzothiophene nucleus is nonexistent in the TPE's and has therefore been the focus of considerable investigation. Indeed, studies with **29**, in which the hinge has been excised, have revealed a profile of biological activity similar to that of tamoxifen [125]. Varying the electronic nature of this hinge revealed a surprising influence on *in vitro* potency, with a trend toward increased potency with more electron-donating connectors (Table 8) [129]. Thus, compound **30c** emerged as one of the most potent estrogen antagonists described to date, with an IC_{50} of 50 pM for inhibition of MCF-7 cell proliferation. Attempts to improve the bioavailability of **30c** have led to the discovery of LY353381, **30e**, a SERM with improved *in vivo* potency as an oral estrogen antagonist, which maintains tissue-specific estrogen agonist effects on serum cholesterol and bone mineral density at doses as low as 0.01 mg/kg [133]. Additional studies on the conformational effects imposed by the hinge functionality are discussed *vide infra*.

A series of hybrid compounds which incorporate the side chains of steroidal pure antiestrogens ICI 164,384 or ICI 182,780 attached to a benzothiophene nucleus have also been reported to function as pure estrogen antagonists [134]. To date, no reports concerning their effects in non-traditional target tissues have appeared.

Table 8. Effects of Hinge Substituent on *In vitro* Estrogen Antagonism [128]



Compound	X	MCF-7 Inhib. IC_{50} (nM)±SE[a]
raloxifene,7	C=O	0.4 ± 0.3
19a	CH_2	0.19 ± 0.10
19b	S	0.28 ± 0.15
19c	O	0.05 ± 0.02
19d	NH	0.10

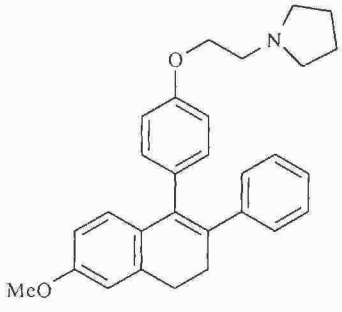
[a]Dose required to give 50% inhibition of a maximally effective (10^{-11}) dose of 17β-estradiol (111).

Naphthalenes

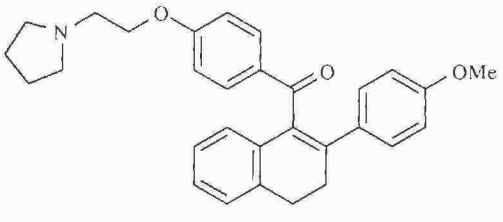
The naphthalene nucleus has provided a structural template for a variety of estrogen receptor modulators. In 1963, Lednicer reported the synthesis and anti-fertility activity of a 1,2-diphenyl-3,4-dihydronaphthalene, nafoxidene, **31**, which was subsequently evaluated as a treatment for breast cancer [135]. Recently, other workers have shown that nafoxidene significantly improves femoral bone mineral density after 5 weeks of oral dosing to OVX rats at doses of 10 mg/kg/day but not 1 mg/kg/day [61b,136]. Another 3,4-dihydronapthalene compound, trioxifene, **32**, has also been shown to effectively prevent ovariectomy-induced bone loss [137,138]. Both of these dihydronaphthalenes, however, demonstrate estrogen agonist effects on the uterus.

As nafoxidene was found to be phototoxic in humans due to the nature of the 1,2-unsaturation present in the dihydronaphthalene ring, considerable efforts have been directed at reduced nafoxidene analogs. In 1979, Katzenellenbogen reported that *cis* isomers of the nafoxidene nucleus, such as analog **33a**, showed a reduced estrogenic response in the uterus relative to the corresponding *trans* isomer [139]. Recently, the Pfizer group has identified a similar reduced nafoxidine derivative, CP-336,156, **33b**, as a potent tissue selective estrogen agonist [140]. Structure-activity studies evaluating the rate of phenolic glucuronidation revealed that this *cis*-diaryl-substituted tetrahydronaphthalene demonstrates good oral bioavailability in addition to its *in vitro* estrogen receptor binding and anti-proliferative effects. Oral administration of CP-336,156 to 5-month old OVX rats showed slight but significant increases in uterine wet weight relative to controls while providing complete protection against bone loss (trabecular and cortical) at doses as low as 100 μg/kg/day [141]. Mechanistic studies suggest that CP336,156 and estrogen inhibit osteoclastogenesis *in vitro* via a p53-related apoptotic pathway [142].

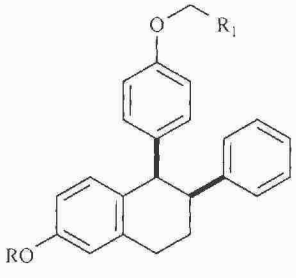
We have recently disclosed a novel 1,2-diaryl-6-hydroxynaphthalene ER modulator, LY326315, **34**, which possesses a fully differentiated agonist/antagonist profile on reproductive vs. non-reproductive tissue [143]. In MCF-7 breast cancer cells, LY326315 effectively binds to the estrogen receptor (RBA = 36 %) and is a potent inhibitor of cellular proliferation (IC_{50} = 0.1 nM). In the OVX rat this compound demonstrated a profile of activity similar to that of raloxifene, preventing bone loss (ED_{50} = 0.5 mg/kg) and lowering serum cholesterol (ED_{50} = 0.5 mg/kg) after oral exposure for 5 weeks [143]. In contrast to these agonist responses on bone and lipids, LY326315 had no estrogen-like effects on the uterus as



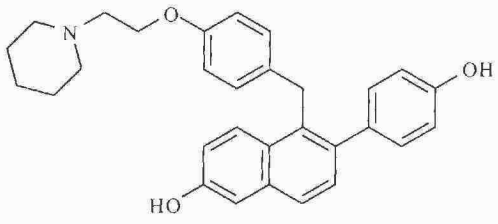
nafoxidene, **31**



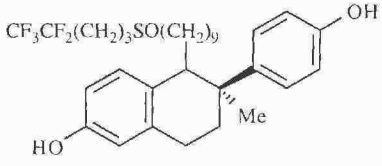
trioxifene, **32**



U23,469, **33a** , R = Me, R_1 = $CH(OH)CH_2OH$
CP-336156, **33b**, R = H, R_1 = $CH_2N(CH_2)_4$

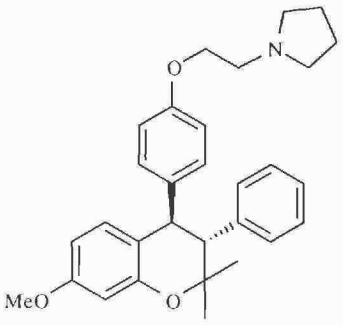


LY326315, **34**

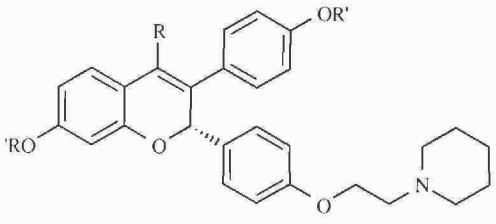


ZK 189,154, **35**

Fig. (6). Naphthalene-derived estrogen receptor modulators.



ormeloxifene (centchroman), **36** (racemic)



37 R = H, R'= H
EM 343, **38a** R = Me, R'= H (racemic)
EM 800, **38b** R = Me, R'= pivaloyl (*S*-enantiomer)

Fig. (7). Benzopyran estrogen receptor modulators.

determined by histological analysis of the endometrial epithelia [143].

Recently, a naphthalene based pure antiestrogen ZM 189,154, **35**, has been reported [144]. As with the steroidal pure antiestrogens, **35** reversed the bone-sparing action of EE_2 in OVX animals while remaining neutral in intact animals.

Benzopyrans

Several classes of estrogen receptor modulators based upon a benzopyran framework have been developed. Ormeloxifene (formerly centchroman), **36**, has been reported to have tissue-selective agonist effects on bone in OVX rats and to inhibit osteoclastic bone resorption [145]. Other authors have, however, reported potent uterine stimulation with **36** in OVX rats [146]. Recently, a single enantiomer of ormeloxifene (levormeloxifene) has been reported to reduce bone loss and serum cholesterol in OVX rats with reduced uterine stimulation relative to 17β-estradiol [147]. In preliminary clinical experiments, levormeloxifene has also demonstrated the ability to reduce serum cholesterol and biochemical markers of bone remodeling in postmenopausal women [148].

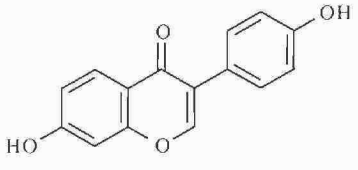
Rearrangement of the substitution pattern about the benzopyran ring system led to the discovery of **37** and EM 343, **38a** [149,150]. Originally described as pure antiestrogens, these compounds have now been shown to have a tissue-selective activity profile in OVX rats with respect to bone protection and serum cholesterol reduction [151]. A recent study of the effects of changes to the amine group in this series parallels the effects which have been described for the raloxifene series [152]. The *S*-isomer of **38b** (EM 800), in which the phenolic moieties have been masked as pivaloate esters, has been reported as an extremely potent estrogen antagonist in both *in vitro* and *in vivo* breast cancer models [153,154].

Miscellaneous

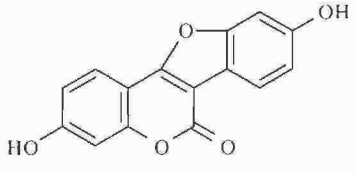
Environmental Estrogens

Whereas the consequences of human and wildlife exposure to environmental estrogens on reproductive tissue has been well-studied, remarkably little focus has been directed toward the pharmacological effects of such exposure on non-reproductive tract tissue [155]. To address this issue a representative cross-section of environmental estrogens (Fig. (**8**)) have been studied based on their relative abundance in the ecosystem and/or potential for human exposure. These included phytoestrogens (genistein, **39** [156], coumestrol **40**), detergents (*p*-octylphenol, **41** [157]), animal health products (zeranol, **42** [158]), pesticides (methoxychlor, **43** [159]), and plastics (bisphenol A, **44** [160]).

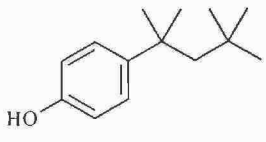
Initial *in vitro* studies on **39-44** indicated at least some degree of intrinsic estrogen agonist character in a series of hormone-dependent assays. For example, all compounds compete with ^{3}H-17β-estradiol for binding to the estrogen receptor with the exception of methoxychlor [161].



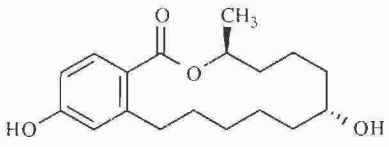
genistein, **39**



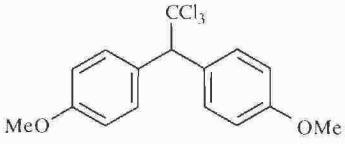
coumestrol, **40**



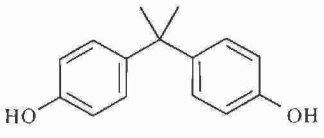
p-octylphenol, **41**



zeranol, **42**



methoxychlor, **43**



bisphenol A, **44**

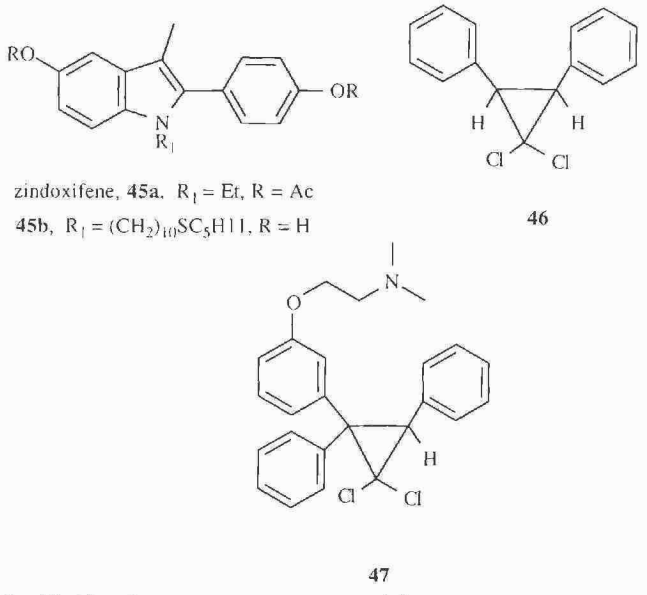
Fig. (8). Representative environmental estrogens.

Pharmacological examination of these environmental estrogens in the OVX rat model indicate that this class of compounds has the ability to act as estrogen agonists in non-reproductive tract tissue. For example, compounds **39-44** (Fig. (**8**)) lower serum cholesterol after only 4 days of oral exposure [161]. In this assay, zeranol exhibits the most potent hypocholesterolemic response attaining equivalent efficacy to EE_2. Not surprisingly, all compounds cause significant increases in uterine wet weight, results which confirm the traditional effects generally associated with environmental estrogens. Zeranol elicits the most dramatic uterine hypertrophy, a 251% increase relative to control, followed most closely in magnitude by coumestrol (186%) [161]. In addition, preliminary results on the antiestrogenic effects of zeranol indicate that this compound antagonizes the uterine effects of EE2 to its own intrinsic level of estrogenicity.

Comparison of the *in vivo* and *in vitro* results for these compounds reveals a distinct trend in which zeranol competes most effectively for receptor binding and proves the most potent at (a) stimulating MCF-7 cell proliferation (EC_{50} = 0.05 nM), (b) lowering serum cholesterol (ED_{50} = 0.2 mg/kg) and (c) causing uterine hypertrophy [161]. In addition, zeranol prevented trabecular bone loss in the OVX rat model following 5 weeks of oral administration [161]. In contrast, not all environmental estrogens exhibit similar estrogen agonist effects on bone metabolism. For example, whereas coumestrol and methoxychlor demonstrated effectiveness in this model, bisphenol A, genistein, and *p*-octylphenol appear neutral on this particular tissue [161]. Furthermore, it is evident that the pharmacological effects observed in the uterus, cholesterol, and bone responses can be divorced from each other. For example, genistein and bisphenol A mimic estrogen in causing uterine hypertrophy and lowering cholesterol, yet these agents are devoid of estrogen agonist effects on bone.

Others

A variety of other structural templates have been identified which elicit estrogen agonist and/or antagonist effects. The majority of the relevant data for these compounds focus on their pharmacological effects on traditional estrogen target tissues, particularly breast and uterus. Consequently, relatively little is known about these agents with regards to their effects on non-reproductive tissue. Representatives of this class of ER modulators (Fig (**9**)) include: (a) indoles such as zindoxifene, **45a**, and **45b**, which have been studied extensively by von Angerer and others for their antiestrogenic character [162] and (b) arylcyclopropane analogs, such as **46** and **47**, pioneered by Magarian and co-workers [20b].



zindoxifene, **45a**, R_1 = Et, R = Ac

45b, $R_1 = (CH_2)_{10}SC_5H11$, R = H

46

47

Fig. (9). Miscellanous estrogen receptor modulators.

Conformational Effects on Tissue Selectivity

Recently, a structural basis for the differences in tissue selectivity demonstrated by raloxifene and TPE-like molecules has been hypothesized [125]. Comparison of the low energy conformations of raloxifene and tamoxifen demonstrates that the amine-containing side chains occupy dramatically different regions of space when the molecules are overlaid in the stilbene plane (Fig. (**10**)). Compound **29**, in which the carbonyl hinge of raloxifene has been excised, adopts a conformation similar to that of tamoxifen in which the side chain is forced to be coplanar with the rest of the molecule. Conversely, **48**, in which the side chain has been constrained in an orthogonal orientation relative to the stilbene plane, resembles the low energy conformation of raloxifene [163]. Parallel studies of these four compounds in OVX and immature rats demonstrated that while all four compounds had similar effects on serum cholesterol and bone mineral density, tamoxifen and **29** were partial agonists with respect to uterine effects while raloxifene and **48** were full antagonists [125]. Presumably, the distinct molecular shapes of these compounds are in some way reflected by changes in the

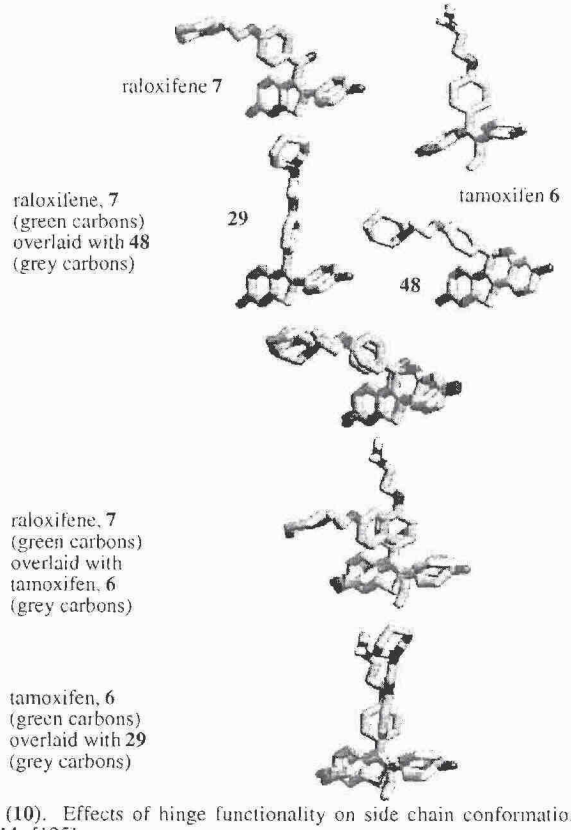
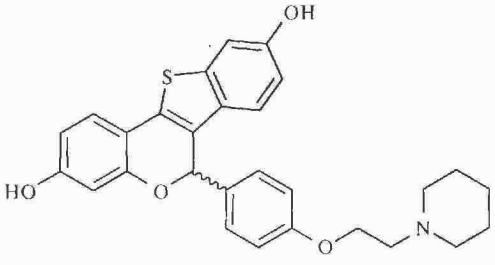


Fig. (10). Effects of hinge functionality on side chain conformation of SERMs [125].



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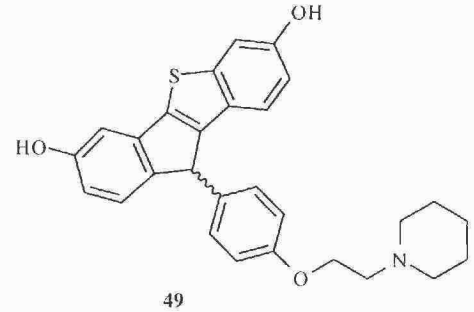

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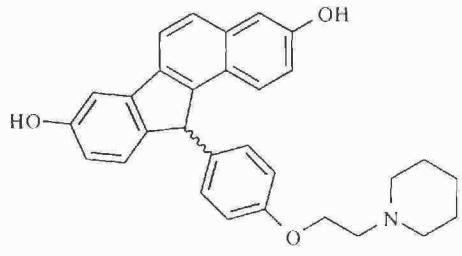

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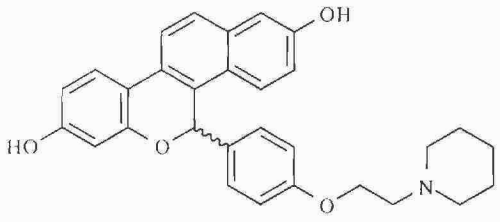

LY357489, 51

Fig. (11). Conformationally restricted SERMs.

conformation of the ER-ligand complex, depending upon which ligand is involved. The crystal structure of raloxifene bound to the estrogen receptor lends additional support to this hypothesis [60].

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 [140,151]. In addition, recent reports have detailed the incorporation of this feature into the design of several new series of SERMs including **49**, **50**, and **51** [164,165]. In particular, LY357489, **51** has been shown to maintain estrogen agonist effects on serum cholesterol and bone mineral density in OVX rats at oral doses of 0.01 mg/kg without evidence of uterine stimulation [165].

Mechanisms of Tissue Selectivity

Although a variety of mechanisms which are independent of the ER have been advanced to explain the actions of estrogen agonists and antagonists [166], it is generally accepted that the majority of their biological effects are mediated via interaction with this receptor. The sequence of events which allows individual ligands to manifest profiles of gene activation which are distinct from the natural ligand and are dependent upon cell and tissue-specific factors is incompletely understood and, consequently, the subject of much research and debate [167,168]. A brief discussion of the current understanding of this phenomenon follows [25].

Interaction of a ligand with the ER induces a conformational change in the protein which causes dissociation of heat shock proteins and allows for formation of ER homodimers [168]. These dimeric receptor complexes then interact with a specific DNA sequence or hormone response element (HRE) and initiate the transcription of HRE-containing genes (Fig. (**12**), Effector System One)[25]. The ER contains two transcriptional activation functions AF-1 and AF-2, but only AF-2 is dependent upon ligand binding [169,170]. Some compounds, such as tamoxifen, inhibit AF-2 mediated gene activation selectively and are therefore unable to block the activation of genes which are mediated by AF-1 [170]. Whether or not AF-1 is sufficient to activate transcription is dependent upon the cell type, the individual gene promoter, and the presence or absence of various adapter proteins which may function as coactivators or corepressors (Fig. (**12**), Effector System 2) [25,171,172]. A third transcriptional activation function has also recently been hypothesized [173].

This mechanistic picture is further complicated by the discovery that non-HRE containing genes can also respond to the ER-ligand complex (Fig. (**12**), Effector System 3) [25]. For example, genes which contain an AP-1 site have been shown to be activated by estrogen agonists [174]. Once again, activation by a particular ligand is dependent upon cell type, as tamoxifen has shown agonist activity at AP-1 sites in uterine but not breast tissue [175]. Recently, the interaction of the ER-raloxifene complex with a novel response element within the TGF-β3 gene has been proposed as a potential mechanism for tissue selective estrogen agonist effects [176].

The recent discovery of a second ER, ERβ, adds a further layer of complexity [177]. Differential tissue distribution of the two ERs has been reported and differential responses to various ligands have been observed *in vitro* [178]. Although present in non-traditional estrogen responsive tissues such as bone and brain [179,180], the pharmacological relevance of ERβ has not yet been established [181]. The role of ERβ in determining tissue selectivity together with the possibility of additional ER isoforms are areas of continuing research.

It has been hypothesized that the various profiles of tissue-specificity observed with different ER ligands are the result of different conformations of the receptor-ligand complex [24,182]. Furthermore, features of molecular structure which

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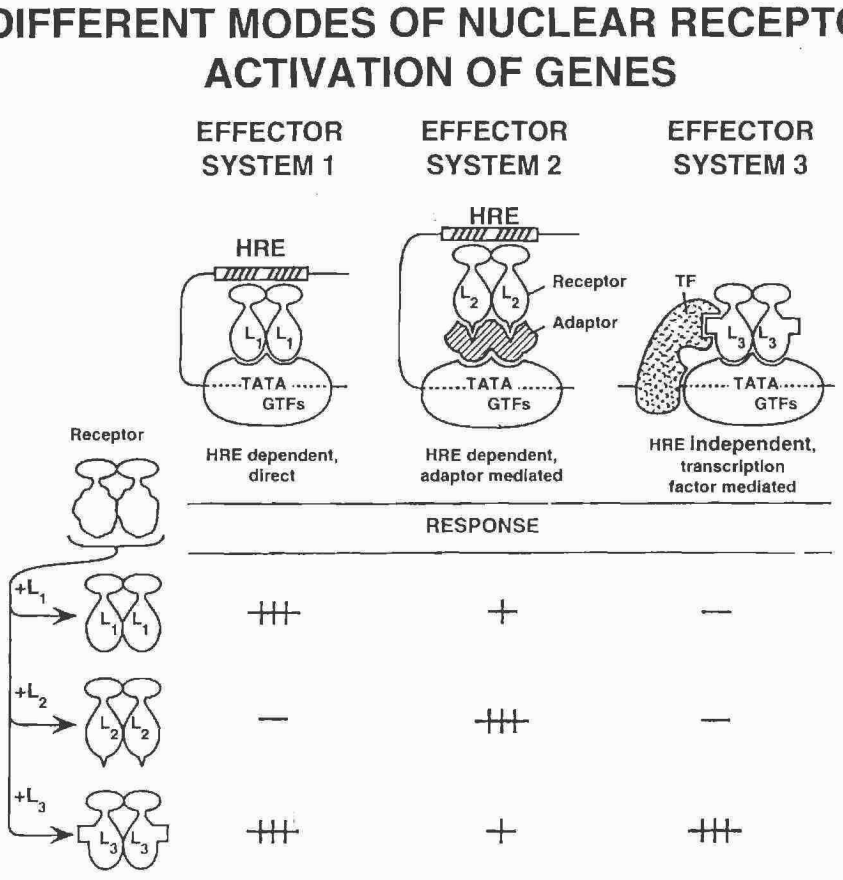

Fig. (12). Tripartite (ligand-receptor-effector) pharmacology. With permission from Katzenellenbogen et. al. [25], ©The Endocrine Society.

effect the profile of tissue-specificity have been described [125]. To date, unique transcriptional profiles have been observed for 17β-estradiol, tamoxifen, raloxifene, and ICI 164384, therefore four distinct classes of ER modulators have been identified (Table 1) [24,183]. It is anticipated that the continuing exploration of new ER isoforms, new estrogen-responsive genes, and additional target tissues will result in the discovery of new classes of ER modulators with great potential for the prevention and treatment of estrogen related disorders.

Abbreviations

ERT	=	Estrogen replacement therapy
HRT	=	Hormone replacement therapy
ER	=	Estrogen receptor
DES	=	Diethyl stilbestrol
SERM	=	Selective estrogen receptor modulator
HDL	=	High density lipoprotein
LDL	=	Low density lipoprotein
Eq	=	Equilin
Eqn	=	Equilenin
DHEq	=	Dihydroequilin
DHEqn	=	Dihydroequilenin
OVX	=	Ovariectomized
TPE	=	Triphenylethylene
EE2	=	Ethynyl estradiol

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