Molecular Structural Characteristics of Estrogen Receptor Modulators as Determinants of Estrogen Receptor Selectivity

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Abstract: This review will discuss the structural determinants and requirements necessary for estrogen receptors alpha and beta selectivity and ligand-receptor binding affinity. In addition, strategies likely to result in the development of a pharmacophore model that account for the differences in estrogenic effects between different ligands will be discussed.

Key Words: Computer aided drug design, molecular modeling, structure-activity relationship, hormones, drug design, structure, QSAR, phytoestrogens.

INTRODUCTION

Estrogens, the sex steroid hormones, are important for a variety of physiologic processes in both men and women. They play key roles in the development and maintenance of normal sexual and reproductive function and exert a vast range of biological effects in the cardiovascular, musculoskeletal, immune, and central nervous systems [1-3]. A large number of different pathological conditions are associated with changes in the production of estrogen and the cellular response to estrogenic stimuli. Thus, compounds with estrogenic activity are of considerable interest as targets for the development of therapeutic agents. There are, in addition to this, concerns that environmental chemicals and man-made chemicals may mimic endogenous hormones [4].

The action of estrogen is mediated through the estrogen receptors (ER) alpha (ERα) and beta (ERβ) [5]. The two subtypes have distinct functions and differential distribution in certain tissues and cell types [2]. For instance, reproductive cells, especially those of the uterus and breast, are abundant in ERα, whereas bone has greater amounts of ERβ.This has stimulated the search for subtype-specific ligands that can elicit tissue- or cell-specific estrogenic activity. Such activity can be achieved in different ways, for example by selective modulation or by selective binding, and has led to increased activity in the drug discovery arena. Therefore, given the complexity of estrogen activity, ER subtype-selective ligands may potentially possess significant clinical utility. For example, ERβ selective modulators would have minimal effect on tissues that contain ERα, and thus exhibit different side-effect profiles than non-selective ligands.

The falling level of estrogen in post-menopausal women has been considered the main reason for increased osteoporosis and heart disease in ageing women [6]. Hormone replacement therapy (HRT) is used for the relief of estrogenic deficiency symptoms in post-menopausal women. While HRT is associated with a variety of clinical benefits (such as bone protection, prevention of hot flushes and decreased risks of Type 2 diabetes mellitus and colorectal cancer), such therapy also has adverse effects due to the stimulation of breast and uterine tissues leading to associated increases in rates of breast and endometrial carcinoma. Some of these adverse effects are believed to be mediated by ERα or ERβ specific mechanisms [7-9].

It follows that ERβ antagonists or agonists would display different therapeutic profiles than ERα antagonists or agonists, and would be beneficial in tissues expressing high levels of ERβ [10, 11]. Since ERα is the dominant subtype in the breast and uterus, this suggests that ERβ selective ligands may be used as HRT without increasing the risk of breast or uterine cancer.

ERs belong to a large family of nuclear receptors, structurally similar ligand-inducible transcription factors. While having in common a structural organization, they are activated by distinct lipophilic small molecules such as glucocorticoids, progesterone, estrogens, retinoids, and fatty acid derivatives. All nuclear receptors have a hydrophobic ligand binding domain, with helix-12 (H12) being the key response element. They contain three independent but interacting functional domains: NH_2 terminal or A/B domain, DNA-binding or C domain, and ligand-binding or D/E/F domain [12]. Agonists and antagonists differentially position the C-terminal helix of the ligand-binding domain (helix-12) and the F domain (carboxyl terminal domain) [13].

ERα and ERβ differ markedly in the N-terminal A/B domains, with only about 20% amino acid identity, and also in the ligand-binding domain. The differences in the A/B domain suggest that the transcriptional activation by ERα and ERβ may play different roles in carcinogenesis. The ratio of ERα/ERβ differs between normal and carcinomatous tissues such that a higher ratio has been observed in breast and endometrial carcinoma [14-16]. ERβ mRNA was detected in 36% of endometrial carcinoma cases, whereas ERα mRNA hybridisation signals were detected in 80% of those cases. Since ERβ is co-expressed with ERα, the estrogenic

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effects are considered to occur mainly through ERα in endometrial carcinomas [15].

Furthermore, some environmental chemicals and phytoestrogens may preferentially interact with ERβ by disrupting the normal function of the endocrine system of humans and wildlife [17]. As such, compounds that antagonize ERβ may also be important in regulating interactions with endocrine disrupting chemicals as well as having their own effect on human physiology [18].

NONSELECTIVE ESTROGENIC ACTIVITY

ERs can bind a remarkable number of structurally different compounds. The natural ligand for the ERs is estradiol (E2), an endogenous ligand (Fig. **1**). A number of other endogenous and synthetically derived steroids also activate the receptor providing evidence for the flexible nature of the receptor ligand binding pocket. In addition to steroidal derivatives, ERs also bind large variety of non-steroidal ligands. Nonsteroidal molecules, such as diethylstilbestrol (DES), Fig. (**1**), can potentially have similar activity to that of estradiol.

While some of these ligands are quite different, receptor binding seems to be strongly dependent upon the presence of an aromatic hydroxyl group and the conformation of the hydrophobic portion of the molecule [19]. By overlaying the crystal structures of four ligand–ER complexes (estradiol–ER, 4-hydroxytamoxifen–ER, raloxifene–ER, and DES–ER complexes) based on their common protein residues at the binding site, it was found that the phenolic rings of all four ligands are closely positioned at the same location to allow hydrogen bond interactions with Glu353, Arg394 of the receptor, and a water molecule [20].

All natural estrogens have a planar aromatic six-member ring A. The presence of phenolic ring imparts unique chemical properties to estrogens and is considered more significant than any other structural feature [21], hence a phenolic ring is often associated with estrogenic activity [19]. Aromatization of A-ring in natural estrogens is the final step in estrogen formation from its precursor androgen. Aromatization alters the overall shape of the molecule. The relative spatial orientation of the A-steroidal ring with respect to the B-ring may be considered important structural characteristics for receptor recognition. A rigid ring structure as present in estradiol favors ER binding. Steroids lacking an aromatic ring have low binding affinity. The majority of phytoestrogens (estrogens derived from plants), belong to a large group of polyphenolic compounds known as flavonoids. Flavanoids are low molecular weight hydrophobic compounds with molecular weights and structures similar to those of steroids. Recently, the prenylated flavanone, 8-prenylnaringenin (8-PN) derived from hops (Humulus lupulus L.), has been identified as a potent estrogen showing the highest *in vitro* estrogenic activity to date among all phytoestrogens known [22].

The length and breadth of both the steroidal and nonsteroidal molecules fits well into the receptor binding pocket. Effective binding requires the presence of the two polar hydroxyl groups at each end of the molecule. Molecules with a distance limitation between the oxygen atoms of the hydroxyl groups on a large inert skeleton have optimal estrogenic activity [23]. Estradiol binds to the receptor using hydrogen bonding as a key interaction. The phenolic A-ring alcohol group makes hydrogen bond contacts with two specific residues of the ER ligand binding domain, Glu353(305) and Arg394(346) of ERα(ERβ) [24, 25]. The D-ring hydroxyl group binds with His524. One study analyzing the hydrogen bonding mode of phenolic hydroxyl group of 17β-estradiol [19] suggested a donor function of the phenol of 17β-estradiol and a high expectation for the 17β-hydroxyl group to be a better hydrogen bond acceptor than donor. These predictions were confirmed by subsequent x-ray crystallographic analysis of the complex of the estrogen receptor ligand-binding domain and 17β-estradiol [24]. It was found that the phenol of 17β-estradiol binds to the Glu353 of the receptor as a hydrogen donor, while the 17β-hydroxyl group in the D-ring binds to His524 as a hydrogen acceptor, either directly or via a water molecule. In addition to hydrogen bonding to Glu353, the phenolic-hydroxyl group makes direct hydrogen bonds to the guanidium group of Arg394 and a water molecule. One further study clearly indicated that alkylphenols interact with estrogen receptors only when phenol is conjugated with hydrophobic alkyl groups and that the

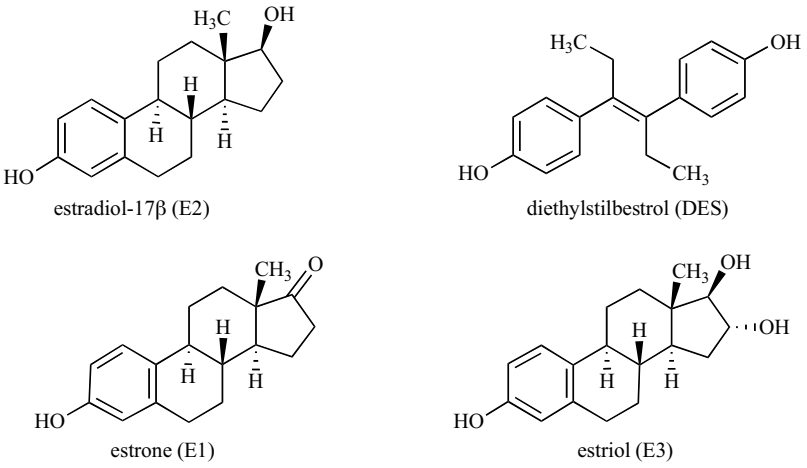

Fig. (1). Nonselective ER ligands.

hydrophobic interfaces of alkylphenols are insufficient to sustain a conformation of estradiol for full receptor binding [26].

The relative binding affinity (RBA) of substituted estradiols is more susceptible to steric and/or electrostatic effects when the substitution is on C2 rather than on C4. Earlier studies have suggested that substitution of small functional groups at the estradiol positions C2 and C4 are tolerated, whereas larger groups may reduce binding affinity due to the formation of an intra-molecular hydrogen bond with the C3 hydroxyl group [19]. Depending on size and polarity, the substituents on C1, by contrast, should cause skeletal alterations of rings B, C, and D, mainly by steric hindrance and through space electronic induction on C11 and its attached hydrogens. Substitution on C4 does not create major changes in conformation of the alicyclic system since *ortho* inductive effects are normally minimal [27]. Hydroxylation at specific sites of the estratrien-17β-ol aromatic A-ring is critical. Hydroxylation at the 2 or 3 positions promoted high affinity of a ligand for the ER, while hydroxylation at the 1 or 4 positions attenuated binding affinity. It has been hypothesized that the hydroxyl groups at positions 2 and 3 may share, via hydrogen bonding, a common H-acceptor/-donor site in the receptor cavity [28]. Electron-withdrawing groups such as chlorine or fluorine on the phenyl ring produce a roughly 4-fold improvement in RBA at both receptor subtypes. However, substitution of bromine at the C2 position of estradiol drastically reduces the RBA at ERβ and also ERα to a relatively lesser degree (<0.5% and 4% of estradiol respectively). The position of the substituent appears to have little effect on potency or selectivity as does the addition of a second electron-withdrawing group on the ring. Cyano and trifluoromethyl substituents appear to be slightly less effective in increasing ER affinity than simple halogens. Electron-donating substituents such as methyl are generally equipotent to the unsubstituted phenyl derivatives. It was also suggested that saturation of the phenyl ring leads to a loss in affinity at both receptor subtypes [28].

Although estradiol (E2) is the most potent endogenous estrogen with the almost equal binding affinity for both human ERs, it is not the major circulating estrogen. Estrone (E1) and estriol (E3), two major metabolites of estradiol (Fig. **1**), are quantitatively the main circulating estrogens in women under different physiological conditions [29]. Although their binding affinities for ERα and ERβ are much lower than that of estradiol, they may serve unique physiological functions by providing a differential activation of the ERα or ERβ signalling system. It is therefore believed that the metabolic conversion of estradiol to estrone or estriol may represent an important mechanism for achieving differential activation of the ERα or ERβ signaling system under different physiological conditions. Weak agonists, like estriol, can activate some but not all of ER responses. This selective regulation of receptor activity by estriol is not correlated with its ability to activate transcription [30]. It is associated with the inability of estriol-bound receptor to sustain tight nuclear interactions [31]. D-ring substitution of estradiol, particularly at the C16 and C17 positions, results in differential binding affinity for ERα and ERβ. Most of the D-ring metabolites have considerable binding affinity for both ERα and ERβ, and several of them (16α-OH-E2, 16β-OH-E2-17α, and 16-keto-E1) have a distinct, preferential binding affinity for ERβ over ERα (up to 18-fold). Estriol (16α-OH-estradiol) is one of the major metabolites formed during human pregnancy, has a markedly lower binding affinity for ERα compared with estradiol but retains a relatively high binding affinity for ERβ (RBA 11% and 35% of E2 respectively). Furthermore, while 16β-OH-E2-17α (16,17-epiestriol) has a very low binding affinity for ERα, it has a preferential affinity for ERβ. The difference between these binding affinities is 18-fold [32]. It appears that both ERs are sensitive to the steric hindrance in the vicinity of the 17α position on the steroid ring. The domain near the 17α position of estradiol is larger for ERα than ERβ suggesting that increasing steric bulk in this region will enhance the binding affinity more for the ERα than for ERβ. Moxestrol (RBA = 43 and 5) and norethynodrel (RBA = 0.7 and 0.22), both with 17α-ethynil substituent show higher binding affinity to ERα than to ERβ. On the other hand, most of the polar D-ring metabolites (16β-OH-E2-17α, 16α-OH-E2-17α, 16-keto-E1, 16α-OH-E2, and 16α-OH-E1) have markedly increased binding affinity for human ERβ over ERα compared with their respective precursors.

Although the steroid estrogen molecule is a rigid structure, its interactions with the receptor and general modulation of activity are highly susceptible to minor skeletal modifications. The volume available in the receptor binding pocket exceeds the size of natural ligand leaving a bit of empty space in the binding pocket below the C7α and above the C11β position of the estradiol B- and C-rings that are not filled by the ligand [29]. Compounds with lipophilic substituents at C11β substitution show high affinities. The preference for C11 substitution is based on its close proximity to the aromatic C1 which can be influenced *via* steric interaction and through space electronic induction. Due to the conformation of the B-ring, substitutions on the α-face on C11 have a more dramatic structural effect than those on the β-face [33]. However, addition of a hydrophilic group at C11 position of estradiol or estrone (such as a hydroxyl or keto group) almost completely eliminates binding affinities for both receptor subtypes. These data indicate that the drastic decrease in binding affinities of 11α-OH-estradiol, 11β-OH-estradiol, or 11-keto-estradiol for human ERα and ERβ is not due to steric hindrance caused by the C11 position substitutions, but is mainly due to alterations of the lipophilicity near the C11 position [29]. A similar situation is seen with non-steroidal estrogens. In the ER-DES structure, these pockets are filled by the two ethyl groups that extend upward and downward from the ligand.

RECEPTOR SELECTIVITY

Individual ligands may differ in their affinity for ERα and ERβ. For example 17-β-estradiol binds equally well to both receptors, while estrone and raloxifene bind preferentially to ERα, and estriol and genistein bind preferentially to ERβ. Drugs that target the ER can exhibit a variety of effects in different target tissues. Tamoxifen is an estrogen antagonist in breast tissue [34] but an estrogen agonist in bone [35] and uterine tissue [36]. Raloxifene is also an estrogen antagonist in breast tissue, but exhibits estrogen agonistic activity in bone yet not uterine tissue [37].

Selectivity of a ligand for the ER subtypes can be explained on the basis of differences in ligand-binding affinity, ligand potency, or ligand efficacy. Thus, there are three types of ligands: ligands with potency, efficacy and antagonist selectivity. A ligand with potency selectivity is an agonist on both receptor subtypes, but stimulates transcription of one type at a lower concentration. Efficacy selectivity refers to the differences in the level of activity of a ligand. Antagonist selectivity refers to a ligand which is an antagonist on one receptor subtype and has either antagonist or no activity at the other subtype. In addition to specific potency, efficacy and antagonist selectivity, ligands may posses a combination of these three types of selectivities.

ANTIESTROGEN CHARACTER

Structure activity relationship (SAR) studies have been conducted to develop molecular models that explain estrogen and antiestrogen action. A "crocodile model" involved sealing of a ligand within the ligand binding domain to transform the receptor-ligand complex into its active state [38]. Planar estrogens able to be sealed within the ligand binding domain transform the receptor-ligand complex into its active state so that gene transcription can be initiated. In the case of antiestrogens, a large bulky side chain locks into the ligand binding domain and prevents full receptor activation by keeping the jaws open. This bulky side chain interacts with an antiestrogenic region of the receptor protein. Alteration in the antiestrogenic side chains will modulate the estrogenic and antiestrogenic actions of the ligand-receptor complex.

X-ray crystal structures for the ligand binding pockets of ERα bound to agonists and antagonists and ERβ bound to a partial agonist and an antagonist showed that the overall structures of both ERα and ERβ are similar [24, 25, 39]. The most striking difference in the receptor structures is the positioning of helix-12, the AF-2 region. In the ERα complexes with agonists, helix-12 is positioned over the ligand binding pocket. The agonist-ERα structure revealed that a portion of a coactivator protein is bound to a hydrophobic channel formed by helices 3,4, 5, and 12 in an α-helical conformation. This appears to be the mode by which ligand activated ERs transfer their activity.

The molecular basis of agonism and antagonism for ER has been further revised through x-ray crystallography. It has been found that liganded ERα binding domain structures support this theory for agonism and also indicate the mechanism for antagonism [40]. The bulky side chains present in estrogen antagonists exit the binding pocket. Steric hindrance between the basic side chains of these antiestrogens and helix-12 displaces helix-12 from the agonist position to a new position that occludes the coactivator recognition channel. This disorder of the coactivator binding surface is responsible for the antagonism character in both ERa and ERβ ligand binding domain complexes [17]. Binding of the ligand stabilizes specific conformations reflecting the size and shape of the ligand. The rigidified external surface features of the ligand-receptor complex then serves as specific docking sites for coregulators, thereby altering the rate of target gene transcription. When agonists bind, the C-terminal helix-12 folds over the ligand to form an hydrophobic channel in which coactivators may dock. By contrast, antagonist binding reorients helix-12 so that it will interfere with coactivator binding. Without a ligand, helix-12 sticks out from the ligand-bonding pocket; in the presence of ligand it folds back to form a scaled ligand binding pocket. Due to the overall homology in the ligand binding domains of all the nuclear receptors, it is believed that the realignment of helix-12, which forms a new interaction surface with coactivators, is the structural basis for the ligand-dependent transactivation [39].

In contrast to the two structures of ER-agonist complexes, complexes with antiestrogens such as raloxifene and hydroxytamoxifen have an altered helical topology at the C-terminal region of the ligand binding domain [24, 25]. Due to the presence of a large basic side chain, these ligands do not fit into a fully closed ligand binding pocket and the C-terminal helix-12 of the ligand binding domain becomes relocated. Another structural study of the ligand binding domain complex of the pure antiestrogen ICI 164.3S4 bound to ERβ revealed that the bulky polar side chain protrudes from the binding pocket and itself occupies the coactivator binding site [41]. Consequently there is steric prevention of helix-12 from adopting either an agonist or antagonist orientation. Such studies emphasize the role of bulky side chains in achieving antagonism on ER [42].

The recent discovery of *R,R*-tetrahydrochrysene (*R,R*-THC) as an ERβ specific antagonist (Fig. **2**) breaks the dogma that a bulky side chain is required for ER antagonists, at least for the antagonists on ERβ [43]. This has led to the theory of passive antagonism [25]. When a receptor binds to a ligand without a bulky side chain the interaction between the ligand and the receptor still could destabilize helix-12 for its agonist position even though there are no steric contacts physically preventing it [25]. It is interesting that the antagonism on ERβ can be more easily achieved than on ERα. It is speculated that the agonist position for helix-12 in ERβ is intrinsically less stable than it is in ERα [24]. In addition, the ERβ binding pocket is smaller (390Å^3 versus 490Å^3 for ERα) and more polar, due to the replacement of Met 336 in ERα with the smaller and more polar Leu 384 in ERβ. [39].

ERα SELECTIVITY

Katzenellenbogen and co-workers have developed several series of nonsteroidal compounds based on substituted furans, pyrazoles and tetrahydrochrysenes [44-46] which have been shown to exhibit unprecedented ER subtype selectivity compared with the classical steroidal compounds.

A variety of 5-membered heterocyclic analogs including imidazoles, oxazoles, thiophenes, pyrroles, and furans have been studied. Large differences in binding affinity, up to 50-fold, were found for ligands that had identical peripheral substitution patterns but different core structures (eg pyrazoles *vs* imidazoles, thiazole or isoxazole). Pyrazole-based ligands with basic side chain substituents have been shown to be selective for ERα in terms of binding affinity as well as its potency [47]. Several triaryl-substituted five-membered heterocycles show exceptionally large potency and efficacy preferences for ERα [43, 45, 48, 49]. The best of these are triaryl-alkyl-substituted pyrazoles and furans which function as complete ERα agonists but are almost completely inactive

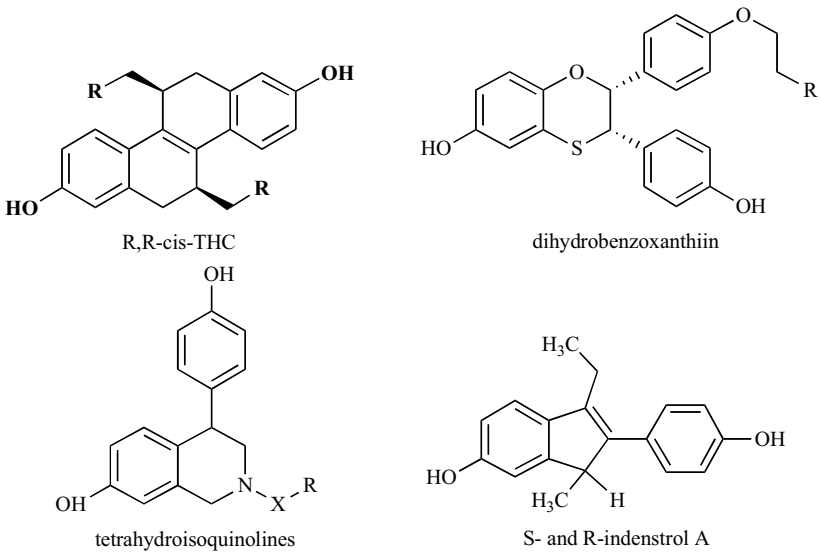

Fig. (2). Mixed agonist/antagonist ERα/ERβ ligands.

at ERβ. The most selective heterocycle, propyl pyrazole triol (PPT), Fig. (**3**), is approximately 10,000-fold more potent on ERα than on ERβ and shows ERα-selectivity *in vivo* [49, 50]. However, the molecular basis for its ERα selectivity is not fully understood. Moreover, other larger ring heterocycles such as tetrasubstituted pyrimidines and pyrazines retain greater potency and efficacy on ERα than on ERβ [51].

Interesting structure-activity relationships were seen for the tetra-substituted furans (Fig. **3**). They proved to be ERα selective agents, in both RBA (3-fold to 70-fold selectivity) and transcriptional activation assays (10-fold selectivity) [52]. The furans with the highest subtype selectivity were those of 3-alkyl-2,4,5-triaryl substitution, particularly those with all three aryl groups as para-phenols. The highest subtype binding affinity selectivity (71-fold) was observed for 2,3,5-tis(4-hydroxyphenyI)-4-methyl-furan. Both experimental evidence and molecular modeling have been used to help determine the binding mode for the furan series of ligands.

Specific chromane analogues were identified as ERα selective ligands. X-ray studies revealed that the origin of ERα selectivity was from a C4 trans methyl substitution to the cis-2,3-diphenyl-chromane platform [53].

Certain triaryl amides (Fig. **3**) show potency preferences as agonists for ERα that can be as great as 500 fold. They

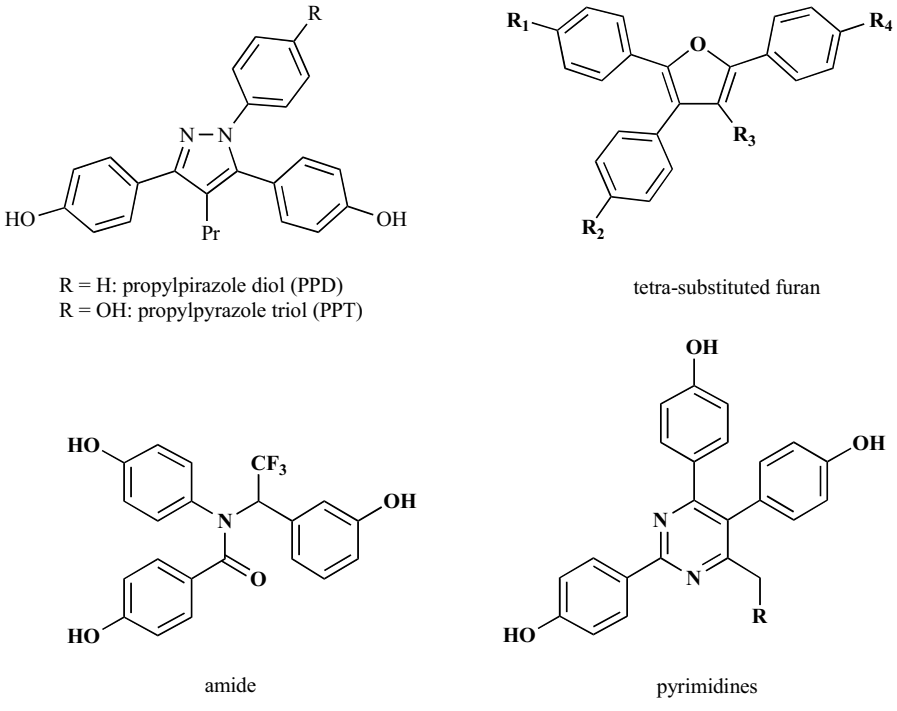

Fig. (3). ERα selective agonist ligands.

function as agonists on both ERα and ERβ, but in cell-based assays of gene transcription, they activate ERα at much lower concentrations [46]. Bisphenolic amides mimic bibenzyl and homobibenzyl motifs commonly found as substructures in ligands for the ER. ER ligands that have simple amide core structures can be readily prepared. Representative members were prepared from three classes: N- phenyl benzamides, N- phenyl acetamides, and N-benzyl benzamides. Of these three classes, the N-phenylbenzamides had the highest affinity for ER, the N-phenylacetamides had lower, and the N-benzylbenzamides were prone to fragmentation *via* a quinone methide intermediate. Therefore, high affinity binding requires an appropriate distribution of bulk, polarity, and functionality. The strong conformational preference of the core anilide function in all of these ligands defines a rather rigid geometry for further structural and functional expansion of these series.

Substituted THC ligands (Fig. **2**) are potent agonists on ERα but also potent antagonists on ERβ [54]. This character is a function of substituent size and stereochemistry. THCs can be regarded as ring-fused derivatives of diethylstilbestrol, containing an electron-donating hydroxyl group at C8 and a rigid four-ring structure reminiscent of steroidal estrogens. *RR* and *SS* enantiomers of THC exhibit different activities at ERα and ERβ. ER selective antagonists reside completely in the *RR* enantiomer. *SS* enantiomers have similar agonist activity to ERα and ERβ. The difference in efficacy of *R,R*-THC on the two ER subtypes appears to arise from its optimal fit in the ERα ligand-binding pocket and its suboptimal fit in the slightly smaller ERβ pocket [25]. Evaluation of both the RBA and agonist/antagonist selectivity of trans- and cis-THCs suggests that the induction of an antagonist conformation in ERβ can be achieved with these ligands with less steric perturbation than in ERα. Nearly all examined THCs were found to be agonists on ERα, while THCs with small substituents were agonists on both ERα and ERβ. As substituent size was increased, ERβ-selective antagonism was developed first in the *R,R*-cis enantiomer series and finally in the trans diastereomer and *S,S*-cis enantiomer series. The most potent and selective ligand was identified as *R,R*-cis-diethyl THC.

Dihydrobenzoxathiins (Fig. **2**) were recently synthesized as a novel class of selective ERα modulators (SERAMs) [53, 55-58]. Selectivity of the dihydrobenzoxathiins is highly dependent on the size, location and stereochemistry of side chain substituents. Although the magnitude of receptor subtype selectivity (ERβ/ERα ratio) varied considerably, all of the novel analogs remained ERα selective. Dihydrobenzoxathiins with alkyl substituted pyrrolidine side chains are ERα selective ligands with antagonist activity. Addition of a methyl group to the side chain at the appropriate position and with the right orientation generates substantial steric effects, causing the pyrrolidine ring to twist. Stereochemistry substantially increased estrogen antagonist activity in uterine tissue.

Although specific biological responses have been attributed to the activation of ERα or ERβ, it is also clear that in cells where both receptors are expressed, ERβ functions reduces ERα transcriptional activity [59]. Thus, the pharmacological response of target cells to estrogens and antiestrogens represents the composite activities of both receptors. From studies aimed at developing new classes of ER agonists and antagonists the Selective Estrogen Receptor Modulators (SERMs) have emerged, compounds whose relative agonist/antagonist activities are manifest in a cell and promoter selective manner. The molecular basis of SERM activity has been attributed to the ability of these molecules to induce different changes in receptor architecture, an event that engenders the recruitment of functionally distinct cofactors [60].

Considering importance of developing SERMs, selectivity requirements of tetrahydroisoquinoline derivatives (Fig. **2**) for binding with ERα versus ERβ were investigated [61]. Since the binding pocket of the ER is rather lipophilic, increasing the lipophilicity of the tetrahydroisoquinoline by adding an alkyl substituent at the 1-position of the tetrahydroisoquinoline nucleus lead to development of a pure antiestrogen with high affinity for ERα [62]. Tetrahydroisoquinolines bind to ER with high affinity. Studies have shown that modification of the substitution pattern of the N-phenyl ring has a modest impact on potency in most instances but significantly influences the selectivity. The pyrrolidine and piperidine were found to exhibit up to 50-fold specificities for ERα over ERβ. From the analysis it appeared that the nitrogen atom of the aminoethoxyphenyl substituent and 6-hydroxy substituent of the tetrahydroisoquinoline nucleus play important roles in ERα/ERβ selectivity in addition to R1 and R2 substituents.

To gain insight into the ligand-receptor interaction, the x-ray crystallographic structure of the 1-H tetrahydroisoquinoline derivative – ER complex was solved. An overlay of this x-ray crystal structure with that reported for the complex of ER and raloxifene showed that both compounds bind to the same cleft of the receptor and display comparable binding modes, with differences being observed in the conformation of their phenyl groups corresponding to the D-ring of estradiol.

Furthermore, ERα exhibits stereo-selective ligand binding and transactivation for several structural derivatives and metabolites of the synthetic estrogen diethylstilbestrol. DES (Fig. **1**) is a known carcinogen which is oxidatively metabolized to a variety of metabolites with varying degrees of hormonal activity [63]. Indenestrol A (IA) is a metabolite with high binding affinity for ERα but with weak biological activity [64]. It exists as a racemic mixture of the enantiomers *S*-indenestrol A and *R*-indenestrol A [65], which have a methyl substitution on the chiral carbon (Fig. **2**).

Both enantiomers have agonistic properties. *S*-IA is a strong agonist, whereas *R*-IA displayed only weak agonistic activity for ERα and is a potency-selective agonist for ERβ in a cell-type specific manner. One single residue in the ligand binding domain of ERα and ERβ modulates their transcriptional activity in a cell type-independent fashion. These demonstrates that a single residue in the ligand binding domain determines the stereoselectivity of ERα and ERβ for indenestrol ligands, and that *R*-IA shows cell-type selectivity through ERβ [66].

ERβ SELECTIVITY

The literature demonstrates that it is more difficult to develop ligands that stimulate ERβ to a greater extent than

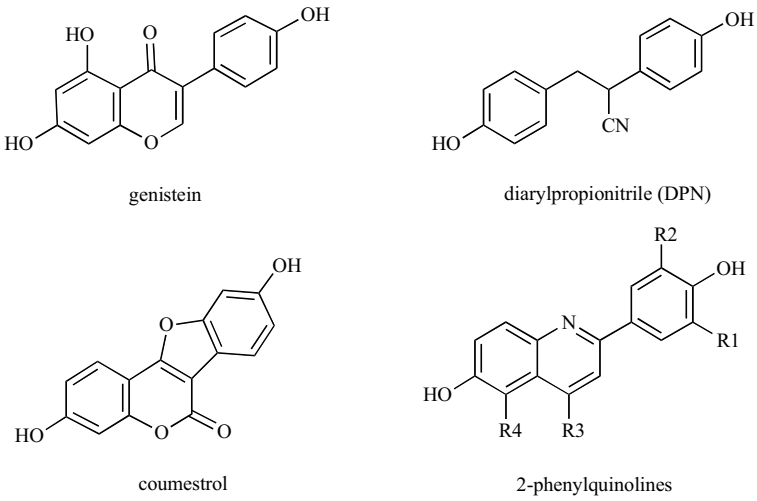

Fig. (4). ERβ selective agonist ligands.

ERα. There are a number of estrogens with good selectivity for ERα but fewer compounds with good selectivity for ERβ. All environmental estrogenic chemicals compete with E2 for binding to both ER subtypes with a similar preference and degree. In most instances the relative binding affinities (RBA) are at least 1000-fold lower than that of E2. Some phytoestrogens such as coumestrol, genistein (Fig. **4**), apigenin, naringenin, and kaempferol compete stronger with E2 for binding to ERβ than to ERα. While certain isoflavone phytoestrogens, such as genistein and coumestrol, have higher affinity for ERβ than ERα [47], this does not translate into significant difference in potency in cell-based transcription assays [67]. Metabolism of isoflavones may also be responsible and active isoflavone metabolites may show receptor selectivity [68]. The isoflavone genistein is a relatively potent agonist for the ERβ with approximately equal affinity as natural estrogen, 17β-estradiol. The relative selective binding of genistein to the ERβ indicates that the isoflavones may produce different clinical effects from that of estrogens by selectively triggering ERβ-mediated transcriptional pathways or differentially triggering transcriptional activation or repression pathways by ER [67]. Similar selectivity is reported for some aryl benzothiophene derivatives [69] and 4-hydroxy-N-phenylubstituted phthalimides [70].

Some simple diarylethane systems do show considerable affinity and potency preference as agonists at ERβ [71]. The best of these, diarylpropionitrile (DPN), Fig. (**4**), will activate ERβ at 100-fold lower concentrations than ERα. The ERβ selectivity of DPN seems to result from its preferential dynamic interaction of the nitrile moiety with a key methionine residue (M336) that is present only in the ERβ ligand binding pocket [72]. In addition, some differences in helix-3 constrain a portion of the ER ligand binding pocket, which can improve interactions between receptor and this rather small ligand [72].

New synthetic bis-benzylnitriles and related compounds have up to 170-fold potency selectivity at ERβ [71]. Recently a number of diarylpropionitriles, diarylsuccinonitriles as well as acetylene and polar analogues of these nitriles were also found to be ERβ selective agonists. The acetylene analogues have higher binding affinities but lower selectivities than their nitrile analogues. This study suggests that the nitrile functionality is critical to ER selectivity. It provides the optimal combination of linear geometry and polarity. Furthermore, the addition of a second nitrile group to the nitrile in DPN, or the addition of a methyl substitutent at an *ortho* position on the aromatic ring increases the affinity and selectivity of these compounds for ERβ. This study suggests that the nitrile functionality is critical to ER selectivity in this series of ligands. These ligands have been shown to have the highest known receptor selectivity and considerable ER binding affinity. Some of these compounds have affinities for the ER that are almost the same as that of the estradiol. Structure-activity relationship results of these studies suggested that the nitrile functionality represents the optimal combination of linear *sp* geometry and local polarity, and it is the best functional group for ligands of this type in respect to ER binding affinity, more so for ERα than for ERβ. Apparently, ERα has a lesser ability to tolerate the polar nature of the nitrile functionality, while the ERβ is less affected by the polar nature of the nitrile function than by the geometric requirement of the *sp* hybridization. As a result, ligands with linear groups show high selectivity for ERβ, and the increased polarity of the nitrile group reduces the affinity of the ligand for ERα, resulting in higher ERβ binding selectivities.

The 2-phenylquinolines (Fig. **4**) have been identified as a new series of potential ERβ-selective agonists [73]. Substitution at the C4 position, particularly with electronegative groups, is essential for ERβ selectivity. The SAR study has shown that selectivity enhancement could be achieved by incorporating a fluoro group at the 3' position of the phenyl ring. A number of substituted 2-phenylquinolines displayed superior ERβ affinity and selectivity compared to that of genistein. The best compound of this study (compound 13b) contained both fluoro and bromo substitutions and was found to be a selective partial agonist at ERβ in a cell-based transcriptional assay. Its uterine weight bioassay showed no significant uterine stimulation, suggesting that this compound would not activate ERα *in vivo*.

Recently, other more polar heterocyclic core systems, benzothiazoles, benzimidazoles, and benzoxazoles have been

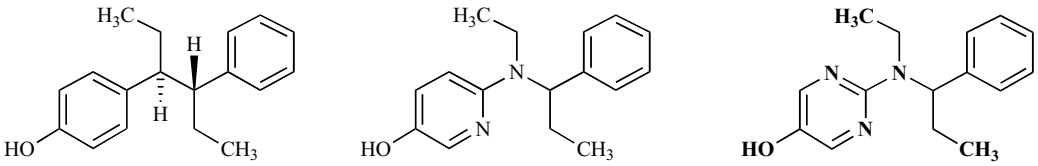

Fig. (5). Pyridine and pyrimidine analogs of the phenol in deoxyhexestrol.

described as ERβ-selective agents [74-76]. Notable in these ligands is a relatively narrow structural profile and a core system enriched in heteroatoms: characteristics that appear favor ERβ selective binding (although do not appear to be essential). Recently, deoxyhexestrol (Fig. **5**), a compound that was first examined long before the discovery of ERβ, was tested again for its binding to both ERs. It had good affinity, especially for ERβ, being in this regard more preferential than its congeners hexestrol and diethylstilbestrol. In order to develop compounds selective for ERβ, pyridine and pyrimidine analogs (Fig. **5**) of the non-steroidal estrogen deoxyhexestrol were synthesized. Their low affinity for the ER was attributed to resonance enforcement of a conformation unfavorable for binding. Resonance-enforced conformational constraint prevents optimal accommodation in the ER ligand binding pocket [77]. DES and hexestrol fit very well to the estrogen receptor: one phenol fits in the narrow A-ring binding pocket as does the A-ring of estradiol, and the two ethyl groups can nicely fill the major 7α, 11β subpockets. By contrast, because of the more pronounced amine-heterocycle resonance, the pyridine and pyrimidine analogs of deoxyhexestrol appear to be forced to adopt a conformation in which the backbone is almost coplanar with the hydroxy-containing heteroarene, with the result that the two ethyl groups are not well disposed to fill the 7α, 11β subpockets. Thus, introduction of nitrogen heteroatoms within the flexible structure of a high affinity all-carbon ligand, deoxyhexestrol, can dramatically reduce binding affinity, even without altering the overall hydrophobicity of the ligand.

CONCLUSION

As the molecular mechanisms of the action of SERMs become more completely understood, rational drug design will replace the current empirical method for the discovery of new drugs that will selectively express desirable actions and suppress undesirable actions of the various steroid hormones. A tissue-selective drug would have all the beneficial effects of estrogen, none of its side effects, and might therefore offer protection against numerous conditions including breast cancer. Current research has identified ligands with selective potency, efficacy and antagonist properties at ER subtypes. There is value in deciphering the target site and specific actions of ligands at ERα and ERβ. Advances have been made in identifying both ligand and ligand binding domain structural requirements for selectivity. As our understanding grows there is the potential for selective ER modulators to be developed as useful clinical medicines.

REFERENCES

[1] Farhat, M.Y.; Lavigne, M.C.; Ramwell, P.W. *FASEB J.,* **1996**, *10*, 615.
[2] Nilsson, S.; Gustafsson, J.A. *Crit. Rev. Biochem. Mol. Biol.,* **2002**, *37*, 1.
[3] Turner, R.T.; Riggs, B.L.; Spelsberg, T.C. *Endocr. Rev.,* **1994**, *15*, 275.
[4] Kavlock, R.J.; Daston, G.P.; DeRosa, C.; Fenner-Crisp, P.; Gray, L.E.; Kaattari, L.G.; Luster, M.; Mac, M.J.; Maczka, C.; Miller, R.; Moore, J.; Rolland, R.; Scott, G.; Sheehan, D.M.; Sinks, T.; Tilson, H.A. *Environ. Health Perspect.,* **1996**, *104*, 715.
[5] Katzenellenbogen, B.S.; Korach, K.S. *Endocrinology,* **1997**, *138*, 861.
[6] Davidson, N.E. *N. Engl. J. Med.,* **1995**, *332*, 1638.
[7] Stampfer, MJ.; Colditz, G.A.; Willett, W.C.; Manson, J.E.; Rosner, B.; Speizer, F.E.; Hennekens, C.H. *N. Engl. J. Med.,* **1991**, *325*, 756.
[8] Barrett-Connor, E.; Bush, T.L. *J. Am. Med. Soc.,* **1991**, *265*, 1861.
[9] Rice, M.M.; Graves, A.B.; McCurry, S.M.; Larson, E.B. *Am. J. Med.,* **1997**, *103*, 26S.
[10] Chang, W.Y.; Prins, G.S. *Prostate,* **1999**, *40*, 115.
[11] Nilsson, S.; Kuiper, G.; Gustafsson, J.A. *Trends Endocrinol. Metab.,* **1998**, *9*, 387.
[12] Kumar, V.; Green, S.; Stack, G.; Berry, M.; Jin, J.R.; Chambon, P. *Cell,* **1987**, *51*, 941.
[13] Kraus, W.L.; McInerney, E.M.; Katzenellenbogen, B.S. *Proc. Natl. Acad. Sci. USA,* **1995**, *92*, 12314.
[14] Sasano, H.; Suzuki, T.; Matsuzaki, Y.; Fukaya, T.; Endoh, M.; Nagura, H.; Kimura, M. *J. Clin. Endocrinol. Metab.,* **1999**, *84*, 781.
[15] Utsunomiya, H.; Suzuki, T.; Harada, N.; Ito, K.; Matsuzaki, S.; Konno, R.; Sato, S.; Yajima, A.; Sasano, H. *Int. J. Gynecol. Pathol.,* **2000**, *19*, 335.
[16] Bardin, A.; Boulle, N.; Lazennec, G.; Vignon, F.; Pujol, P. *Endocr. Relat. Cancer,* **2004**, *11*, 537.
[17] Kuiper, G.G.; Lemmen, J.G.; Carlsson, B.; Corton, J.C.; Safe, S.H.; van der Saag, P.T.; van der Burg, P.; Gustafsson, J.A. *Endocrinology,* **1998**, *139*, 4252.
[18] Tong, W.; Perkins, R.; Fang, H.; Hong, H.; Xie, Q.; Branham, W.; Sheehan, D.M.; Anson, J. *Regul. Res. Perspect.,* **2002**, *1*, 1.
[19] Anstead, G.M.; Carlson, K.E.; Katzenellenbogen, J.A. *Steroids,* **1997**, *62*, 268.
[20] Shi, L.M.; Fang, H.; Tong, W.; Wu, J.; Perkins, R.; Blair, R.M.; Branham, W.S.; Dial, S.L.; Moland, C.L.; Sheehan, D.M. *J. Chem. Inf. Comput. Sci.,* **2001**, *41*, 186.
[21] Fang, H.; Tong, W.; Shi, L.M.; Blair, R.; Perkins, R.; Branham, W.; Hass, B.S.; Xie, Q.; Dial, S.L.; Moland, C.L.; Sheehan, D.M. *Chem. Res. Toxicol.,* **2001**, *14*, 280.
[22] Fokialakis, N.; Lambrinidis, G.; Mitsiou, D.J.; Aligiannis, N.; Mitakou, S.; Skaltsounis, A.L.; Pratsinis, H.; Mikros, E.; Alexis, M.N. *Chem. Biol.,* **2004**, *11*, 397.
[23] Schueler, F.W. *Science,* **1946**, *103*, 221.
[24] Brzozowski, A.M.; Pike, A.C.; Dauter, Z.; Hubbard, R.E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G.L.; Gustafsson, J.A.; Carlquist, M. *Nature,* **1997**, *389*, 753.
[25] Shiau, A.K.; Barstad, D.; Radek, J.L.; Meyers, M.J.; Nettles, K.W.; Katzenellenbogen, B.S.; Katzenellenbogen, J.A.; Agard, D.A.; Greene, G.L. *Nat. Struct. Biol.,* **2002**, *9*, 359.
[26] Tabira, Y.; Nakai, M.; Asai, D.; Yakabe, Y.; Tahara, Y.; Shinmyozu, T.; Noguchi, M.; Takatsuki, M.; Shimohigashi, Y. *Eur. J. Biochem.,* **1999**, *262*, 240.
[27] Rozhin, J.; Huo, A.; Zemlicka, J.; Brooks, S.C. *J. Biol. Chem.,* **1977**, *252*, 7214.
[28] Bradbury, S.; Mekenyan, O.; Ankley, G. *Environ. Toxicol. Chem.,* **1996**, *15*, 1945.

[29] Zhu, B.T.; Han, G.Z.; Shim, J.Y.; Wen, Y.; Jiang, X.R. *Endocrinology,* **2006**, *147*, 4132.
[30] Melamed, M.; Castano, E.; Notides, A.C.; Sasson, S. *Mol. Endocrinol.,* **1997**, *11*, 1868.
[31] Clark, J.H.; Paszko, Z.; Peck, E.J. Jr. *Endocrinology,* **1977**, *100*, 91.
[32] Kuiper, G.G.; Carlsson, B.; Grandien, K.; Enmark, E.; Haggblad, J.; Nilsson, S.; Gustafsson, J.A. *Endocrinology,* **1997**, *138*, 863.
[33] Palomino, E.; Heeg, M.J.; Horwitz, J.P.; Polin, L.; Brooks, S.C. *J. Steroid Biochem. Mol. Biol.,* **1994**, *50*, 75.
[34] Jordan, V.C. *Cancer,* **1992**, *70*, 977.
[35] Love, R.R.; Mazess, R.B.; Barden, H.S.; Epstein, S.; Newcomb, P.A.; Jordan, V.C.; Carbone, P.P.; Demets, D.L. *N. Engl. J. Med.,* **1992**, *326*, 852.
[36] Kedar, R.P.; Bourne, T.H.; Powles, T.J.; Collins, W.P.; Ashley, S.E.; Cosgrove, D.O.; Campbell, S. *Lancet,* **1994**, *343*, 1318.
[37] 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. *J. Clin. Invest.,* **1994**, *93*, 63.
[38] Tate, A.C.; Greene, G.L.; DeSombre, E.R.; Jensen, E.V.; Jordan, V.C. *Cancer Res.,* **1984**, *44*, 1012.
[39] Pike, A.C.W.; Brzozowski, A.M.; Hubbard, R.E.; Bonn, T.; Thorsell, A.G.; Engstrom, O.; Ljunggren, J.; Gustafsson, J.K.; Carlquist, M. *EMBO J.,* **1999**, *18*, 4608.
[40] Kraichely, D.M.; Sun, J.; Katzenellenbogen, J.A.; Katzenellenbogen, B.S. *Endocrinology,* **2000**, *141*, 3534.
[41] Pike, A.C.; Brzozowski, A.M.; Walton, J.; Hubbard, R.E.; Thorsell, A.G.; Li, Y.L.; Gustafsson, J.A.; Carlquist, M. *Structure,* **2001**, *9*, 145.
[42] Heldring, N.; Pike, A.; Andersson, S.; Matthews, J.; Cheng, G.; Hartman, J.; Tujague, M.; Strom, A.; Treuter, E.; Warner, M.; Gustafsson, J.A. *Physiol. Rev.,* **2007**, *87*, 905.
[43] Sun, J.; Meyers, M.J.; Fink, B.E.; Rajendran, R.; Katzenellenbogen, J.A.; Katzenellenbogen, B.S. *Endocrinology,* **1999**, *140*, 800.
[44] Mortensen, D.S.; Rodriguez, A.L.; Sun, J.; Katzenellenbogen, B.S.; Katzenellenbogen, J.A. *Bioorg. Med. Chem. Lett.,* **2001**, *11*, 2521.
[45] Stauffer, S.R.; Huang, Y.; Coletta, C.J.; Tedesco, R.; Katzenellenbogen, J.A. *Bioorg. Med. Chem.,* **2001**, *9*, 141.
[46] Stauffer, S.R.; Sun, J.; Katzenellenbogen, B.S.; Katzenellenbogen, J.A. *Bioorg. Med. Chem.,* **2000**, *8*, 1293.
[47] Kuiper, G.G.; Lemmen, J.G.; Carlsson, B.; Corton, J.C.; Safe, S.H.; van der Saag, P.T.; van der Burg, B.; Gustafsson, J.A. *Endocrinology,* **1998**, *139*, 4252.
[48] Mortensen, D.S.; Rodriguez, A.L.; Carlson, K.E.; Sun, J.; Katzenellenbogen, B.S.; Katzenellenbogen, J.A. *J. Med. Chem.,* **2001**, *44*, 3838.
[49] Stauffer, S.R.; Coletta, C.J.; Tedesco, R.; Nishiguchi, G.; Carlson, K.; Sun, J.; Katzenellenbogen, B.S.; Katzenellenbogen, J.A. *J. Med. Chem.,* **2000**, *43*, 4934.
[50] Harris, H.A.; Katzenellenbogen, J.A.; Katzenellenbogen, B.S. *Endocrinology,* **2002**, *143*, 4172.
[51] Ghosh, U.; Ganessunker, D.; Sattigeri, V.J.; Carlson, K.E.; Mortensen, D.J.; Katzenellenbogen, B.S.; Katzenellenbogen, J.A. *Bioorg. Med. Chem.,* **2003**, *11*, 629.
[52] Shiau, A.K.; Barstad, D.; Radek, J.T.; Meyers, M.J.; Nettles, K.W.; Katzenellenbogen, B.S.; Katzenellenbogen, J.A.; Agard, D.A.; Greene, G.L. *Nat. Struct. Biol.,* **2002**, *9*, 359.
[53] Tan, Q.; Blizzard, T.A.; Morgan, J.D. 2nd; Birzin, E.T.; Chan, W.; Yang, Y.T.; Pai, L.Y.; Hayes, E.C.; DaSilva, C.A.; Warrier, S.; Yudkovitz, J.; Wilkinson, H.A.; Sharma, N.; Fitzgerald, P.M.; Li, S.; Colwell, L.; Fisher, J.E.; Adamski, S.; Reszka, A.A.; Kimmel, D.; DiNinno, F.; Rohrer, S.P.; Freedman, L.P.; Schaeffer, J.M.; Hammond, M.L. *Bioorg. Med. Chem. Lett.,* **2005**, *15*, 1675.
[54] Meyers, M.J.; Sun, J.; Carlson, K.E.; Katzenellenbogen, B.S.; Katzenellenbogen, J.A. *J. Med. Chem.,* **1999**, *42*, 2456.
[55] Blizzard, T.A.; DiNinno, F.; Morgan, J.D. 2nd; Chen, H.Y.; Wu, J.Y.; Gude, C.; Kim, S.; Chan, W.; Birzin, E.T.; Yang, Y.T.; Pai, L.Y.; Zhang, Z.; Hayes, E.C.; DaSilva, C.A.; Tang, W.; Rohrer, S.P.; Schaeffer, J.M.; Hammond, M.L. *Bioorg. Med. Chem. Lett.,* **2004**, *14*, 3861.
[56] Blizzard, T.A.; DiNinno, F.; Morgan, J.D. 2nd; Chen, H.Y.; Wu, J.Y.; Kim, S.; Chan, W.; Birzin, E.T.; Yang, Y.T.; Pai, L.Y.; Fitzgerald, P.M.; Sharma, N.; Li, Y.; Zhang, Z.; Hayes, E. C.; DaSilva, C.A.; Tang, W.; Rohrer, S.P.; Schaeffer, J.M.; Hammond, M.L. *Bioorg. Med. Chem. Lett.,* **2005**, *15*, 107.
[57] Blizzard, T.A.; DiNinno, F.; Morgan, J.D. 2nd; Wu, J.Y.; Chen, H.Y.; Kim, S.; Chan, W.; Birzin, E.T.; Yang, Y.T.; Pai, L.Y.; Zhang, Z.; Hayes, E.C.; DaSilva, C.A.; Tang, W.; Rohrer, S.P.; Schaeffer, J.M.; Hammond, M.L. *Bioorg. Med. Chem. Lett.,* **2004**, *14*, 3865.
[58] Chen, H.Y.; Kim, S.; Wu, J.Y.; Birzin, E.T.; Chan, W.; Yang, Y.T.; Dahllund, J.; DiNinno, F.; Rohrer, S.P.; Schaeffer, J.M.; Hammond, M.L. *Bioorg. Med. Chem. Lett.,* **2004**, *14*, 2551.
[59] Hall, J.M.; McDonnell, D.P. *Endocrinology,* **1999**, *140*, 5566.
[60] Dusell, C.D.; Umetani, M.; Shaul, P.W.; Mangelsdorf, D.J.; McDonnell, D.P. *Mol. Endocrinol.,* **2008**, *22*, 65.
[61] Mukherjee, S.; Saha, A.; Roy, K. *Bioorg. Med. Chem. Lett.,* **2005**, *15*, 957.
[62] Renaud, J.; Bischoff, S.F.; Buhl, T.; Floersheim, P.; Fournier, B.; Halleux, C.; Kallen, J.; Keller, H.; Schlaeppi, J.M.; Stark, W. *J. Med. Chem.,* **2003**, *46*, 2945.
[63] Kohno, H.; Bocchinfuso, W.P.; Gandini, O.; Curtis, S.W.; Korach, K.S. *J. Mol. Endocrinol.,* **1996**, *16*, 277.
[64] Barkhem, T.; Carlsson, B.; Nilsson, Y.; Enmark, E.; Gustafsson, J.A.; Nilsson, S. *Mol. Pharmacol.,* **1998**, *54*, 105.
[65] Couse, J.F.; Lindzey, J.; Grandien, K.; Gustafsson, J.A.; Korach, K.S. *Endocrinology,* **1997**, *138*, 4613.
[66] Mueller, S.O.; Hall, J.M.; Swope, D.L.; Pedersen, L.C.; Korach, K.S. *J. Biol. Chem.,* **2003**, *278*, 12255.
[67] Kuiper, G.G.; Carlsson, B.; Grandien, K.; Enmark, E.; Haggblad, J.; Nilsson, S.; Gustafsson, J.A. *Endocrinology,* **1997**, *138*, 863.
[68] Barnes, S.; Kim, H.; Darley-Usmar, V.; Patel, R.; Xu, J.; Boersma, B.; Luo, M. *J. Nutr.,* **2000**, *130*, 656S.
[69] Schopfer, U.; Schoeffter, P.; Bischoff, S.F.; Nozulak, J.; Feuerbach, D.; Floersheim, P. *J. Med. Chem.,* **2002**, *45*, 1399.
[70] Ullrich, J.W.; Unwalla, R.J.; Singhaus, R.R. Jr.; Harris, H.A.; Mewshaw, R.E. *Bioorg. Med. Chem. Lett.,* **2007**, *17*, 118.
[71] Meyers, M.J.; Sun, J.; Carlson, K.E.; Marriner, G.A.; Katzenellenbogen, B.S.; Katzenellenbogen, J.A. *J. Mol. Model.,* **2001**, *44*, 4230.
[72] Sun, J.; Baudry, J.; Katzenellenbogen, J.A.; Katzenellenbogen, B.S. *Mol. Endocrinol.,* **2003**, *17*, 247.
[73] Vu, A.T.; Cohn, S.T.; Manas, E.S.; Harris, H.A.; Mewshaw, R.E. *Bioorg. Med. Chem. Lett.,* **2005**, *15*, 4520.
[74] Barlaam, B.; Bernstein, P.; Dantzman, C.; Warwick, P. Preparation of Benzoxazoles and Benzothiazoles as Selective Ligands for Human β-estrogen Receptor. WO Patent 02/051821-A1, 2002.
[75] Barlaam, B.; Dock, S.; Folmer, J. Therapeutic Benzimidazole Compounds. WO Patent 02/46168-A1, 2002.
[76] Malamas, M.A.; McDevitt, R.E.; Gunawan, I.; Manas, E.S.; Collini, M.D.; Harris, H.A.; Keith, J.C. Jr.; Albert, L.M.; Lyttle, C.R. Substituted Benzoxazoles and Analogues as Estrogenic Agents. WO Patent 03/050095-A1, 2003.
[77] De Angelis, M.; Katzenellenbogen, J.A. *Bioorg. Med. Chem. Lett.,* **2004**, *14*, 5835.

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