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PERSPECTIVE

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The 2010 Philip S. Portoghese Medicinal Chemistry Lectureship: Addressing the "Core Issue" in the Design of Estrogen Receptor Ligands†

Award Address

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■ ESTROGEN RECEPTOR, AN IMPORTANT PHARMACEUTICAL TARGET WITH DIVERSE BIOLOGICAL FUNCTIONS

The estrogen receptors ERα and ERβ are ligand-regulated transcription factors and also initiators of extranuclear signaling cascades in target cells. As such, they function as mediators of estrogen actions in reproductive tissues as well as in many other organs, such as brain, cardiovasculature, bone, and liver. ERα is a well established target for pharmaceuticals in fertility enhancement, contraception, menopausal hormonal therapy, and endocrine therapies for breast cancer and for radiopharmaceuticals for functional imaging of breast cancer by positron emission tomography (PET).[1] ERs are also likely the targets of some of the disruptive effects of certain hormone mimics found in the environment.[2,3] ERβ, the more recently discovered ER subtype,[4−6] has proved to be a tantalizing, though challenging target for pharmaceutical development (see more below).[7]

■ BINDING CHARACTERISTICS THAT ARE ECLECTIC RATHER THAN PROMISCUOUS

Among the members of the nuclear hormone receptor superfamily, ER stands out as being responsive to a remarkably diverse set of chemical structures, most of which, but not all, are phenolic in nature (Figure 1).[2,3] Examples are natural endogenous steroidal estrogens, synthetic nonsteroidal estrogens, plant secondary metabolites or phytoestrogens, enteric metabolites of lignans, polychlorinated pesticides, and industrial and research chemicals, such as bisphenol A[8] and an impurity present in the pH indicator dye, phenol red.[9]

Because of its acceptance of such a structurally diverse set of compounds, it has been tempting to label the ER as "promiscuous"; however, closer investigation within any one of these classes reveals that there are striking structure−activity relationships, where a single stereochemical change, such as an epimeric inversion, or the addition or deletion of a small substituent, can shift potency by orders of magnitude. Such specificity within structural diversity is better termed "eclectic", which connotes a distinctive preference, though not one that is easily predicted.[2,3]

■ A LARGE BINDING POCKET WITH CONSIDERABLE FLEXIBILITY

Hints underlying the molecular basis for the acceptance of structurally diverse compounds by the ER came from both modeling and X-ray crystallography.[10−12] Before the crystal structure of the ERα ligand binding domain was known, we analyzed the effect, on ER binding affinity, of the addition of single substituents at most positions throughout the whole steroidal skeleton of the endogenous ligand estradiol.[10] On the basis of that comprehensive analysis, we predicted that the ligand-binding pocket of ERα would be considerably larger than the ligand (350 Å^3 vs 250 Å^3), respectively, with some empty subpockets above the steroid at 11β and below the steroid at the 7α and 16α-17α regions. We also suggested that these pockets could be enlarged even further, at least to some degree, by appropriate substitution (Figure 2).

These predictions proved to be prescient because the crystal structures of the ligand binding domain of ERα showed that the pocket volume of the complexes with estradiol and diethylstilbestrol was in fact nearly 450 Å^3, whereas the ligand volumes were only 250 Å^3 (Figure 3).[11,12] In addition, the structures of ERα complexed with the selective estrogen receptor modulators (SERMs), hydroxytamoxifen and raloxifene, demonstrated that the pocket could be enlarged in the 11β direction to accommodate the bulky and basic side chains of these ligands by reorientation of helix-12. This local remodeling of the protein conveniently removes a leucine residue, allowing passage to the exterior of the protein, and provides access to a surface aspartic acid that forms a salt bridge with the basic amino group of the ligand side chain.[11,12] Somewhat later, a crystal structure of the ERα complex with 17α-(2*E*-trifluoromethylphenylvinyl) estradiol demonstrated that the ligand pocket could also be distorted in the 17α direction to accommodate this substituent group with an overall gain in binding affinity by enlarging the pocket through the uncoiling of two short helices, helix-7 and helix-8.[13] Even larger 17α substituents can be accommodated at this position but with reduced affinity.[14] Other nuclear hormone receptors can also enlarge their ligand binding pockets.[15]

■ WHEN AND HOW DOES THE ESTROGEN RECEPTOR LIGAND BINDING POCKET FORM?

While the larger ligand binding pocket and its flexibility seemed to fit well with the ability of ER to accommodate ligands of diverse structure, we were at the same time perplexed by these observations: How could a pocket with such rather loose protein−ligand contact result in the subnanomolar binding affinity for ligands such as estradiol and diethylstilbestrol?

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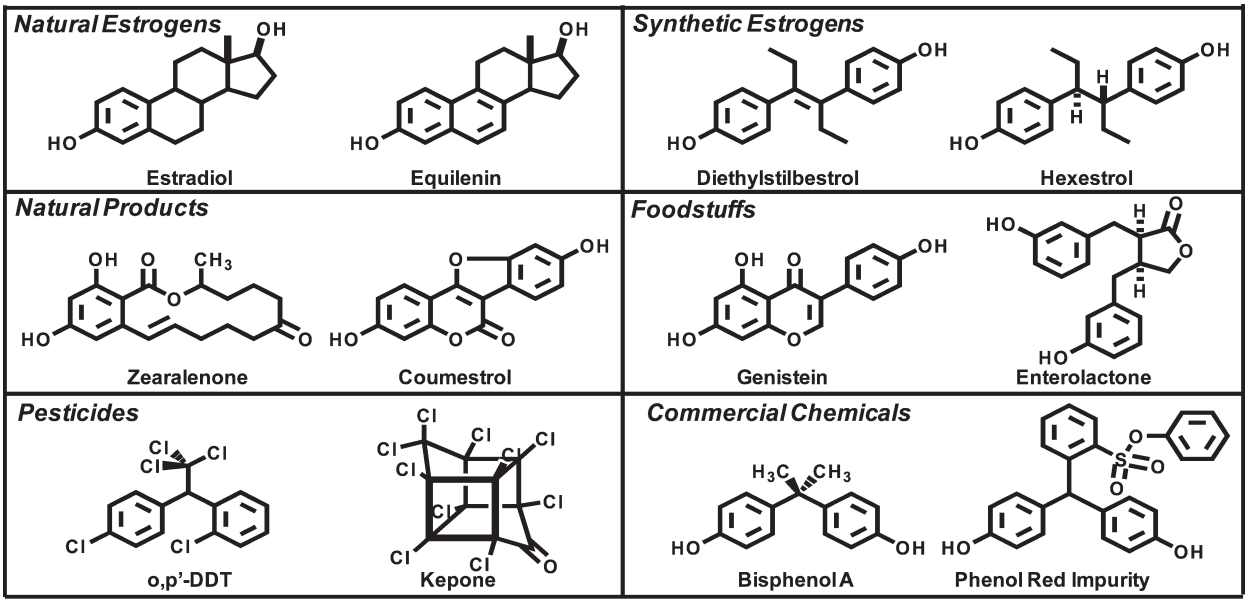

Figure 1. Structural diversity of estrogenic compounds. Molecules from widely different sources and having widely different structures can all exhibit estrogenic activity. Potency, however, covers a very large range, and within each series of compounds, distinct structure−activity relationships are evident such that small changes in structure can lead to large changes in potency.

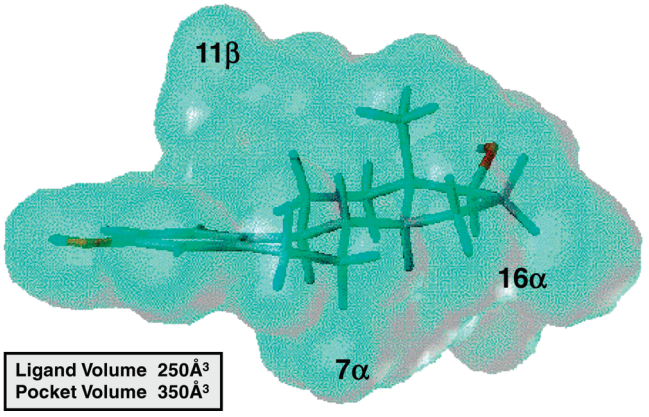

Figure 2. Predicted estrogen receptor ligand binding pocket. Empty space was predicted to surround estradiol when bound by ER, especially in the steroid directions 11β, 7α, and 16α-17α, based on increased binding affinity that results from substitution of lipophilic groups at these positions on the steroid skeleton.[10]

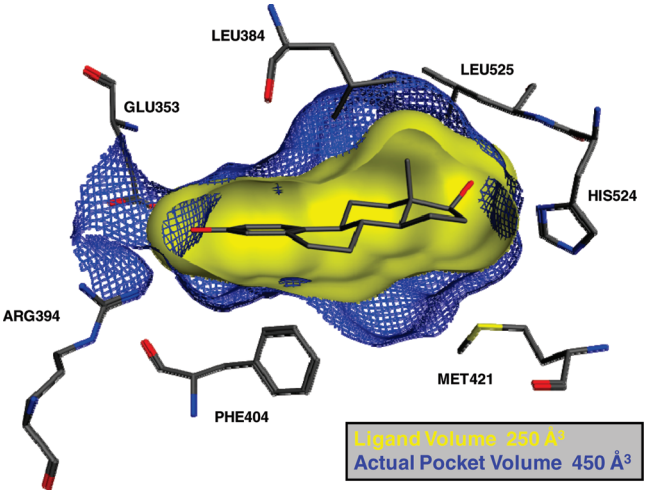

Figure 3. Actual estrogen receptor ligand binding pocket for estradiol. The contour of estradiol is shown as a solid yellow surface, and the contour of the interior of the ligand binding pocket is shown as a green dotted surface.

Through denaturation titration studies using fluorescent probes and intrinsic tryptophan fluorescence, we realized that in the absence of ligand, the lower portion of the ligand binding domain, which accommodates the ligand, is not well folded and could easily be induced to adopt a state that resembled a protein molten globule.[16] Thus, in the absence of ligand, the ligand binding pocket does not exist in a substantial form.

The binding of a ligand involves a coordinated process in which the lower portion of the domain is first able to accommodate the ligand because of its incipient molten globule nature, the initial binding of ligand being almost like a molecule dissolving in a solvent. Then with ligand present, the lower region of the domain completes the folding process, in the process forming a pocket around the ligand and gaining free energy stabilization through new protein−protein contacts and protein−ligand contacts, this final stage being similar to a seed crystal initiating bulk crystallization. Whether this ligand binding process should be called an "induced fit", a "conformational selection", or a "dissolution−crystallization" is more a matter of semantics. The point is this: The pocket that forms around the ligand is as much a function of the size and shape of the ligand as it is of the nature of the folding characteristics of the ligand binding domain. Crystal structures of some nuclear hormone receptor ligand complexes show different degrees of tight packing[17] and even evidence of disorder.[18]

Of note, this view of the ligand binding process corresponds well with the current understanding that ER and other nuclear hormone receptors in their apo state (i.e., without bound ligand)

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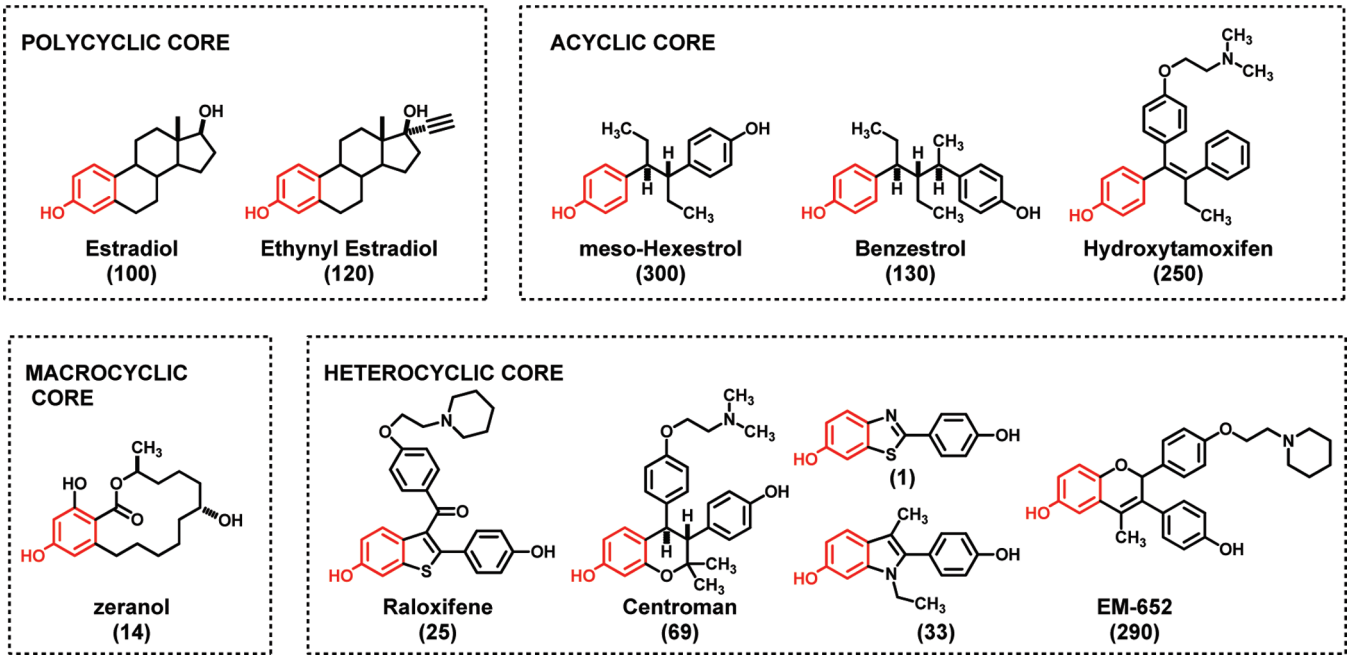

Figure 4. Constant phenol but varying cores of high affinity estrogen receptor ligands. Most high affinity ER ligands have a phenol but otherwise can be a variety of core elements, which may be polycyclic, acyclic, macrocyclic, or heterocyclic.

are bound by heat shock chaperone proteins from which they are released only upon ligand binding.[19] In fact, many proteins involved in signal transduction pathways are now thought to be incompletely folded until they interact with their signaling partners; the unfolded state seems more effective in the search for the interacting partner, a process loosely referred to by protein theorists as "fly-casting".[20,21]

■ RELATING LIGAND ON THE INSIDE TO INTERACTIONS ON THE OUTSIDE

At the same time that the crystal structures of the ERα ligand binding domain were appearing,[11,12] cell and molecular biology studies were revealing that the cellular activity of the ER—ligand complexes was being mediated by its interaction with a constellation of coregulator proteins, both coactivators and corepressors.[22−24] These coregulators were recruited to ligand-regulated docking sites on the exterior surface of the ER, and once bound, they acted through physical and enzymatic reactions to change chromatin architecture, loosen nucleosome binding, and activate RNA pol II to transcribe hormone-regulated genes. Thus, it was becoming clear that the molecular pharmacology of ER, as well as that of other members of the nuclear receptor family, involved many cellular partners.

Early on, we embodied these processes in the concept of "tripartite receptor pharmacology", where the activity of a particular ligand was considered to result from the combined effect of the ligand, the receptor, and the aggregate of other cellular factors that were the local mediators of the action of the ligand−receptor complex.[25,26] This multifactorial model (which in reality expands beyond three components)[26] provides a better way to explain the well recognized target tissue selectivity that was known for nonsteroidal ligands, such as hydroxytamoxifen and raloxifene (Figure 4), and that eventually led to their more appropriate designation as SERMs rather than mixed agonist—antagonists.[22,27−30]

What was particularly intriguing to us was the interplay between what was happening on the "inside" of the ER, where the ligand was bound, and on the "outside" of the ER where the coregulators were binding. It was apparent that the size, shape, and functional characteristics of the ligand that induced the binding domain to fold and form a pocket of a particular shape also determined the pattern of surface features of the ER that become rigidified and are thereby made available for differential binding by the constellation of coregulator proteins present in a particular target cell. Thus, it followed that the key to obtaining estrogens having optimized patterns of desirable activity, such as the maintenance of bone and cardiovascular health without stimulating the uterus and breast, might be to create synthetic ER ligands that would challenge the ligand binding domain to induce formation of ligand pockets of new shapes and sizes, thereby engendering novel patterns of interaction with the relevant cellular coregulators.

■ COMMON FEATURES OF ESTROGEN RECEPTOR LIGANDS, OPPORTUNITY SPACE IN THE LIGAND-BINDING POCKET, AND THE PROPER WAY TO EXPLOIT IT THROUGH "GENERIC-CORE" ESTROGEN RECEPTOR LIGANDS

We have noted that compounds of diverse structures can embody estrogenic activity (Figure 1), but the universe of high affinity and high potency estrogens has some restrictions. Typically, but not always, such ligands are phenols. Thereafter, they have a certain size and display an array of substituents; however, these can be borne on a core element or scaffold that can be polycyclic (as in the steroids), acyclic (as in diethylstilbestrol), macrocyclic (as in zearalanol), or heterocyclic (as is typical for many pharmaceuticals) (Figure 4). The diversity and flexibility of the core or scaffold element on which good estrogens could be built seemed to match the fact that the ligand core was in a region of the ER ligand binding domain where there was limited

ligand−protein contacts and even extra space. This caused me to muse that designing an ER ligand was a bit like playing with the original Mr. Potato Head toy (which used a real potato!) but with a twist: Certain molecular features might be essential (nose = phenol; eyes and ears = other substituents), but one could still make a nice face/molecule even if the potato core was replaced by some other fruit or vegetable (Figure 5).

With this general design paradigm (a "generic core" to which essential peripheral groups were attached) I envisioned that the design of ER ligands could be generalized: The phenol was required, but this group would then simply be supplemented with other aliphatic or aromatic substituents that would be appropriately displayed through attachment to a core element. Central to the success of this approach was the presumption that the core element would be functioning as a molecular scaffold that would otherwise be "functionally inert." This seemed reasonable based on crystal structures that show the core of such ligands in a region of the ligand binding pocket with the "extra space", so essentially no contact with the ER protein was expected.

With little interaction with the core, there seemed no reason not to favor cores that could be readily assembled by simple condensation reactions. This would have the bonus that compound libraries could then be prepared in a modular fashion, just by selection of different combinations of the elements needed for their construction.[31] It followed that the size, shape, and topological display of the substituents could be adjusted to generate ligand binding pockets of different size and shape (the inside), the reflection of which on the surface features of the ER (the outside) would then dictate novel patterns of ER interaction with the coregulatory proteins and alter ER function in the sort of nuanced manner that might lead to improved SERM activity.

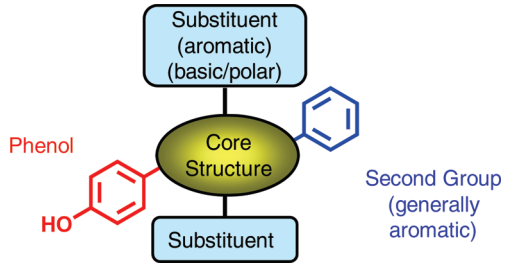

Figure 5. Design for a generic-core estrogen receptor ligand. ER ligands could be assembled in a modular fashion by combining multiple components around a structurally simple and synthetically accessible core that occupies a space in the ligand binding pocket having essentially no contact with the protein.

To guide our investigation of this generalized, generic-core approach to ER ligand design, we extracted from known high affinity ER ligands what appeared to be some important substructural elements beyond the phenol.[31] One of these, a homobibenzyl motif, is illustrated in Figure 6, together with the ligands from which it was extracted (left). Once identified, we sought to incorporate these substructural elements in a simpler context by building them into core elements, such as amides and heterocycles, which could be readily assembled in a modular fashion by condensation reactions (right). In the process, some of the functional and stereochemical complexity of the original ligand would be eliminated, but it was not clear that these were actually needed.

The results of our initial efforts were instructive and ultimately productive. For example, replicating the homobibenzyl substructural motif in a bare-bones 3,5-diarylpyrazole gave a ligand with only 0.01% the affinity of estradiol; even attachment of a third substituent gave only a 2- to 3-fold improvement. Remarkably, with four substituents, the affinity jumped nearly 2000-fold, giving a pyrazole core ligand with an affinity 14% that of estradiol (Figure 7).[31] We encountered similar results replicating the same substructural motifs in amide systems: Only when we built a ligand of sufficient size did binding affinity increase to interesting levels.[32] If the completion of protein folding induced by ligand binding is the source of much of the free energy of binding, then presenting the receptor with a ligand of sufficient size and

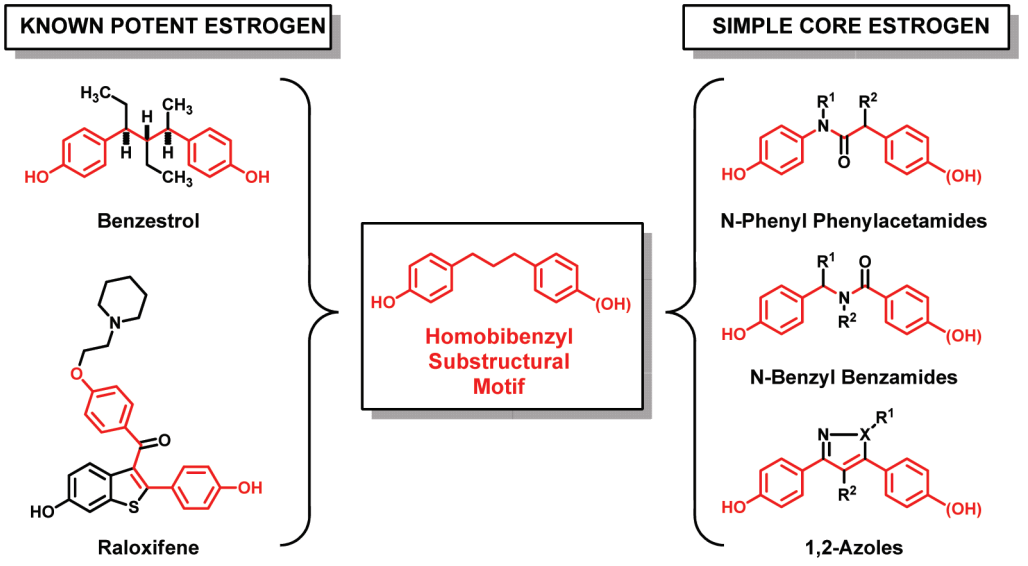

Figure 6. Extraction of a substructural motif to design generic-core estrogen receptor ligands. A homobibenzyl motif, with one or two para hydroxyl groups, found in a number of high affinity ER ligands having core systems with other stereochemical or functional dimensions, might be replicated in ER ligands having simple amide or heterocyclic cores.

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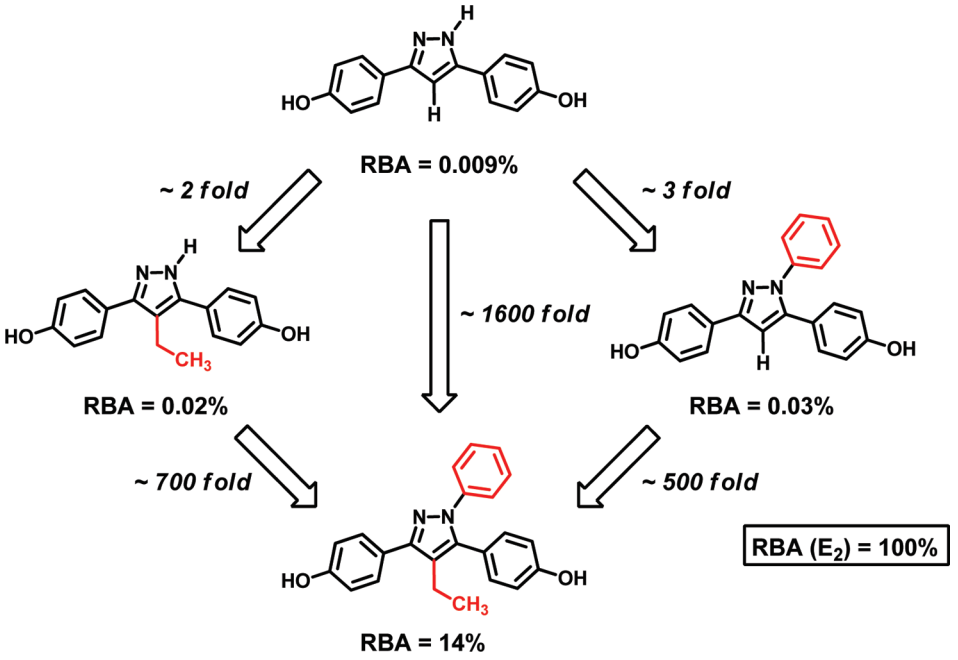

Figure 7. Buildup of a pyrazole to obtain a high affinity estrogen receptor ligand. The basic homobibenzyl system replicated as a 3,5-diarylpyrazole gains high ER binding affinity when it is enlarged with other substituents.

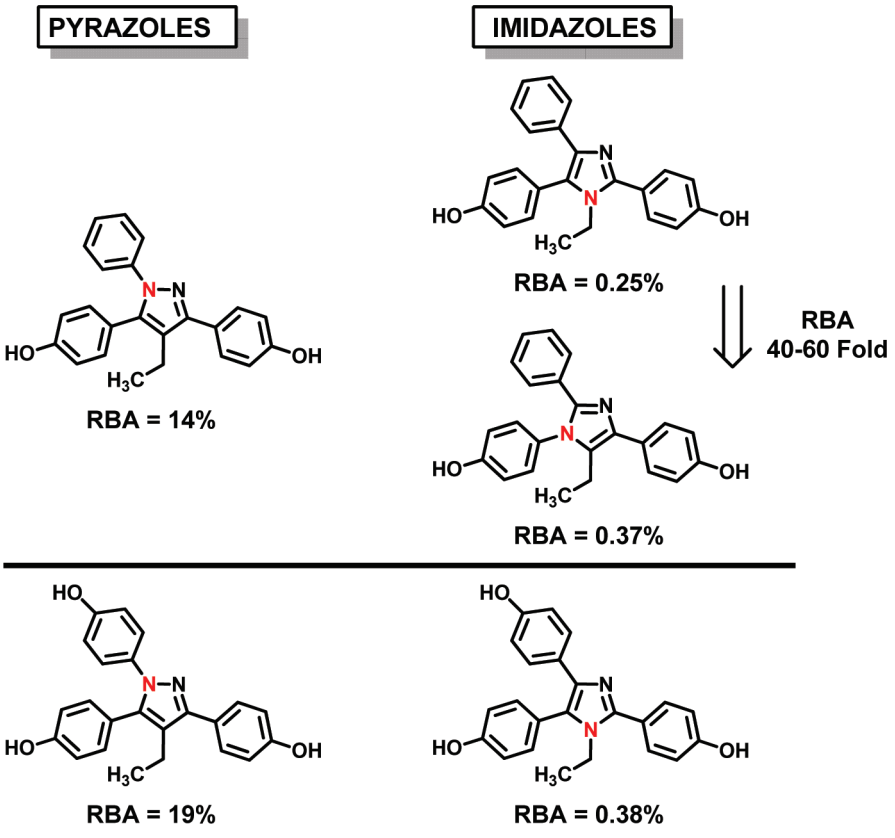

Figure 8. Congruent pyrazoles and imidazoles as estrogen receptor ligands. While the alkyl triarylpyrazoles and imidazoles have otherwise congruent structures, the imidazoles have much lower affinity presumably because the more polar imidazole core suffers a large desolvation penalty upon binding.

shape is required to induce proper folding; this is not unlike finding the right seed crystal to induce the release of energy that occurs upon bulk crystallization.

We were able to use conventional and solid phase synthesis to create libraries of pyrazoles through which we have been able to define structure−affinity relationships in this series.[33] The

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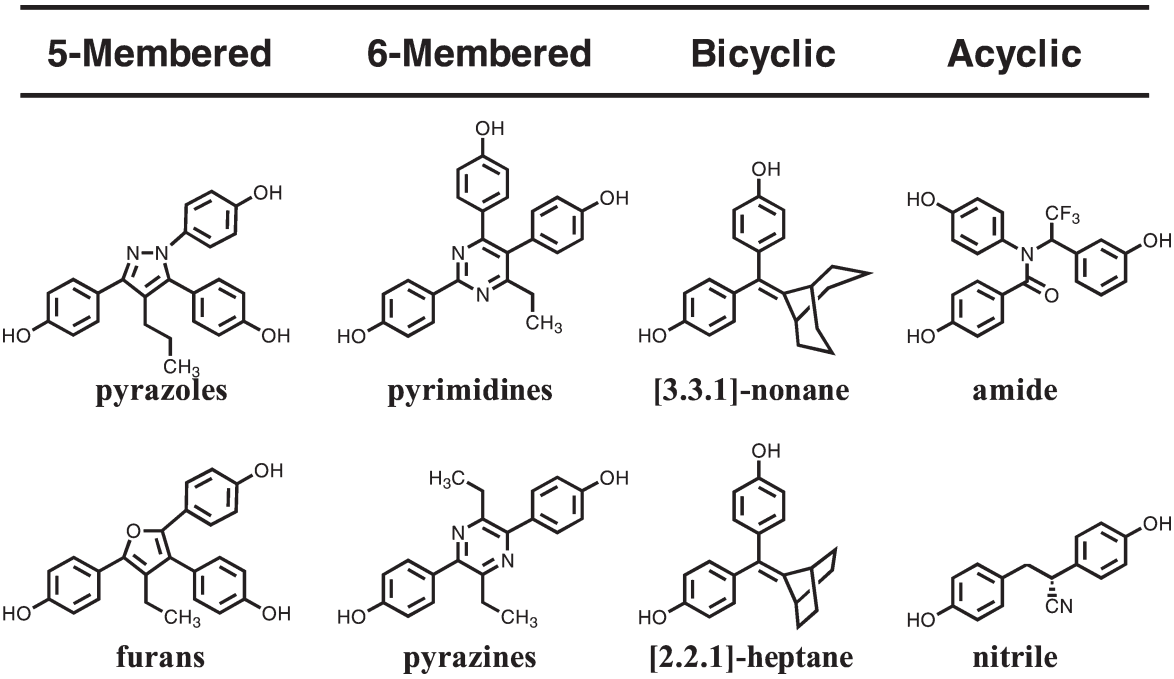


Figure 9. Heterocycle core and related estrogen receptor ligands. Good ER ligands can have a variety of heterocyclic core structures, provided that they are not too polar. Amide core ligands of sufficient sizes are also good ligands. Other cores, such as the off center bicyclo[3.3.1]nonane, can also give high affinity ligands.

optimized pyrazole bore three *p*-hydroxyphenyl substituents and one propyl substituent; it bound about half as well as estradiol to ERα, and we gave it the acronym PPT for propylpyrazoletriol.[34] Others have made additional explorations of pyrazole core ER ligands[35] and related ligands with other heterocyclic cores.[36,37]

We were impressed by the affinity we could obtain with appropriately tetrasubstituted pyrazoles, and this led us to test the central supposition that the core was merely serving a structural role as a central molecular organizer of the substituents. Again, given the lack of core−protein contact, this seemed reasonable. Nevertheless, we were initially taken aback by the fact that the "congruent" imidazoles displaying the same four substituents with the same geometry as did the pyrazole bound to ERα with only 2−4% the affinity of the original pyrazole (Figure 8).[31] This did not seem to square with the lack of core−protein contacts evident in the crystal structures. Upon further reflection, we were reminded that the affinity with which a ligand binds to a protein depends not just on how well the ligand is stabilized in the binding pocket but at the cost incurred by removing the ligand from water. Thus, it made sense that the greater polarity of the imidazole core, which would incur a greater desolvation penalty than the less polar core of the pyrazoles, would systematically lower the binding affinity of the imidazole−core ligands.

With these two simple corollaries added to the ER generic-core ligand design paradigm (the need for a sufficient overall size to induce folding/binding and the need to avoid cores with high polarity) we were able to prepare a number of other heterocycle core ER ligands with high affinity (Figure 9). Ligands with furan cores,[38] which are less polar than pyrazoles, had even higher affinity, up to 3−4 times that of estradiol. Notably as well, in the diazene series, we obtained high affinity ligands with pyrazine and pyrimidine core systems, but not with pyridizine cores presumably because with both nitrogens together, the pyridizine core develops a large dipole moment.[39]

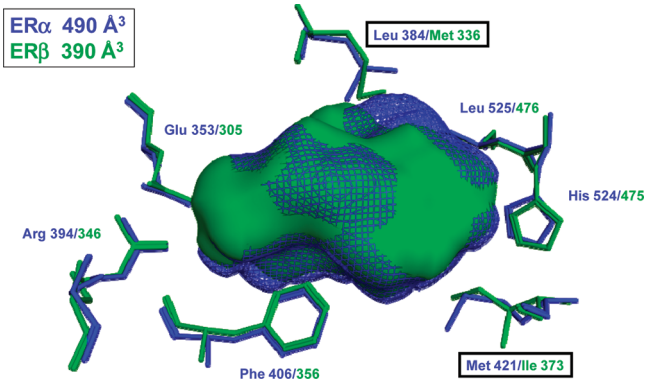


Figure 10. Ligand binding pockets of estrogen receptor α and estrogen receptor *β*. Despite the fact that the ERα and ER*β* ligand binding domains share only 59% amino acid sequence identity, the ligand binding pockets of the two subtypes are nearly the same, with only L384 and M421 in ERα being replaced by M336 and I373 in ER*β*. The volume of the pocket in ER*β*, however, is 100 Å^3 smaller.

THE NEW PLAYER, ER*β*

While much of our work on generalizing the design of ER ligands was done at a time when only one ER was known, the discovery of a second ER in 1995 added another intriguing dimension underlying the diverse and target-selective pharmacology of estrogens.[4−6] The classical ER was dubbed ERα, and the new ER was dubbed ER*β*. Studies quickly showed that the tissue distribution of the two ER subtypes was different. It was found that ERα was predominant in most reproductive tissues,

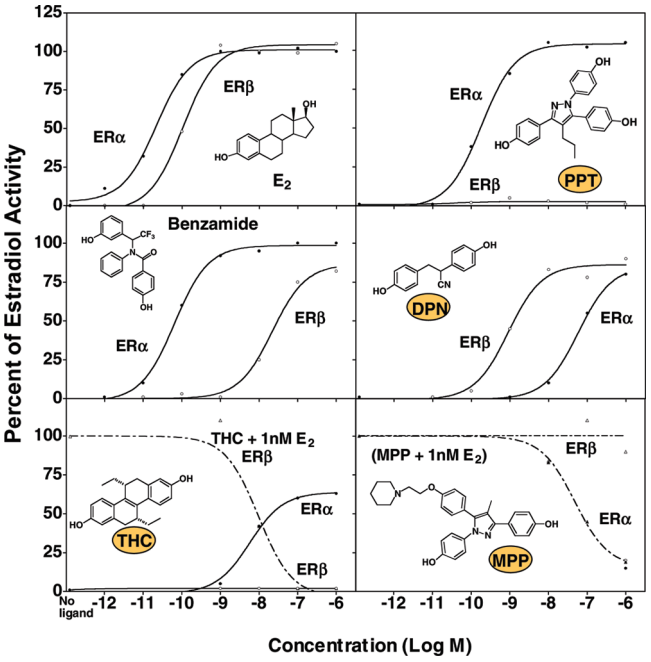


Figure 11. Estrogen receptor subtype-selective ligands. A number of heterocycle core and related ligand were found to have high potency selectivity for activating either ERα (PPT, amide) or ERβ (DPN) or have differential intrinsic activity (THC) or antagonist potency (MPP).

such as uterus and breast, with ERβ sometimes being present along with ERα and other times alone, as in colon, lung, and regions of the brain.[40,41] As a broad generalization, ERα often functioned as a strong driver of proliferation, whereas ERβ was less active and could actually restrain the proliferative drive of ERα.[42,43]

Not surprisingly, great interest developed in creating ER subtype-selective ligands that might differ in either potency or intrinsic activity for activating the two ERs. While the two ERs shared only 59% sequence identity in their ligand binding domains, their ligand binding pockets were lined with nearly identical residues, differing only in the location of a methionine and a leucine or isoleucine residue (Figure 10).[44] Notably, however, the pocket in which ERβ accommodated ligands was generally about 100 Å^3 smaller than in ERα. Early studies also showed that a number of commonly recognized estrogens demonstrated significant ER subtype selectivity.[45]

With our growing collection of ER ligands of novel structure, we quickly found that the rather large ligands, such as PPT and the triarylamide, were excellent activators of ERα, having minimal affinity and activity on ERβ, whereas the more slender ligands, such as the diarylpropionitrile ligand, termed DPN, showed good potency preference for ERβ, observations that were consistent with the size differences in the ligand binding pockets (Figure 11).[46−49] Both PPT and DPN have been used extensively by us and others in wide-ranging studies of the biological effects mediated through ERα and ERβ,[50,51] and they have proved to be effective pharmacological tools that complement studies that rely on ERα and ERβ knockout mice.[52]

Two of our other ligands proved useful as ER subtype-selective compounds. The first, a tetrahydrochrysene, termed THC, is an ERβ antagonist but has substantial agonist activity on ERα (Figure 11).[47] Crystal structures of this ligand with the two ERs confirmed that they adopted the conformations expected for an agonist in ERα and an antagonist in ERβ with the same ligand.[17] THC does not have the bulky, basic side chain typical of SERMs, and a closer look at these structures indicates a new way in which the antagonist conformation can be engendered by a ligand, through distortion of helix-11, a process that was termed "passive antagonism".[17] The second was an analogue of PPT in which, after a comprehensive search, we found the appropriate place to append a basic side chain that engendered SERM activity.[53,54] This compound, which we call MPP (for methylpiperidinopyrazole), preserved the potency preference for ERα characteristic of the parent PPT ligand but behaved as an antagonist (Figure 11).

We have continued our efforts to generate novel ERβ-selective ligands, as have other academic and industrial laboratories.[7] In many cases, these ligands preserve the slender and slightly polar core exemplified by DPN and also apparent from earlier studies of the phytoestrogen genistein.[45,55] Nevertheless, slender ligands with fully nonpolar cores can also have high ERβ selectivity,[49,56,57] as can some ligands that are not so slender but have specific projections that are only accommodated by ERβ.[58] High selectivity for both ERα and ERβ can also be obtained on steroid-core ligands by appropriate substitution at the 16α,17α positions (for ERα) and the 8β position (for ERβ), these sites corresponding to size differences in regions of the ligand binding pocket where the methionine and the leucine/isoleucine residues are differentially disposed.[59,60]

■ DEVELOPING PHARMACEUTICALS THAT TARGET ERβ

The spectrum of ligands selective for ERβ that have been described and the variety of processes that might be regulated by them (prostatic hypertrophy or prostate and breast cancer, fertility, anxiety, depression, vascular protection, etc.)[7] offer opportunities for the development of useful therapeutics based on ERβ as a target. It is clear that this is challenging, however, because nuclear receptors typically have effects in multiple tissues, with a mixture of beneficial and detrimental actions. In this regard, the fact that estrogen action through ERβ appears not to stimulate the breast and reproductive tract is encouraging. A particularly intriguing aspect of ERβ action is the following: While a set of ERβ ligands may have a similar profile in terms of ERβ affinity and intrinsic activity and ERβ/ERα selectivity in binding and cell-based reporter gene activity assays, their biological effects in complex contexts and in vivo can be very different.

This was exemplified in a recent study in which we showed that an ERβ-selective cloroindazole we prepared had potent neuroprotective effects in the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis, where it was highly protective and could even reverse established disease.[61] These effects were clearly mediated through ERβ because the compound had no activity in ERβ knockout mice.[61] Extensive studies of the cellular activity of this chloroindazole in microglia and astrocytes showed that it had a unique pattern of cytokine regulation that was not found with ERB-041 and only minimally with DPN, both of which are nominally similar ERβ-selective agonists.[61] Thus, only one of these three ligands had this desired neuroprotective activity. Undoubtedly, there are (or will be) other examples of this compound-specific ERβ activity that may lead to useful therapeutic agents.

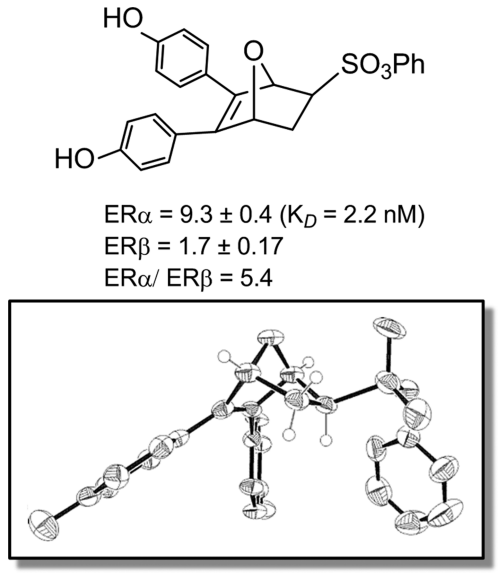


Figure 12. Oxabicyclo[2.2.1]heptene sulfonate (OBHS) estrogen receptor ligand. This high affinity ligand has a three-dimensional core structure, illustrated nicely in the ORTEP rendering of the X-ray crystal structure.

This unexpected pattern of selectivity suggests that at the molecular level, ERβ may be capable of adopting a wider array of conformational states than ERα in response to the binding of ligands that are only subtly different in structure, thereby offering more diversity and selectivity in interactions with coregulator proteins and more nuanced patterns of cellular responses. There are hints of this in some recent in vitro and cell-based studies with ERβ.[62−64] Work with the androgen receptor on aspects of coregulator recruitment preference with ligands of different structure is more advanced,[65,66] and these studies suggest that more sophisticated, conformational probing screens (e.g., phage display studies[67−70]) and more phenotypic assays will be needed to adequately discriminate among otherwise similar ligands in the search for effective and selective therapeutic agents that operate through ERβ.

EXPLORING THE THIRD DIMENSION AND PROBING ELEMENTAL DIVERSITY

We continued to be intrigued by the empty, opportunity space that was apparent in the crystal structures of most ER-ligand complexes, and this prompted us to explore the generation of ligands with additional novel features, such as more pronounced three-dimensional character and elemental diversity. In other work, we had prepared ER ligands that had a cyclopentadienyl core onto which was affixed a tricarbonylrhenium unit. This organometallic species was an analogue of a technetium-99m compound of interest as an imaging agent for ER in breast cancer using single photon emission computed tomographic (SPECT) imaging.[71] Despite the marked extension of the core element by the appended organometallic unit, the best compound in this series bound to ERα with an affinity 25% that of estradiol.[71] Clearly, there is space near the core that can be exploited by appending steric bulk normal to the plane of the ligand. Others have prepared high affinity ER ligands having different bulky cores, such as carboranes.[72]

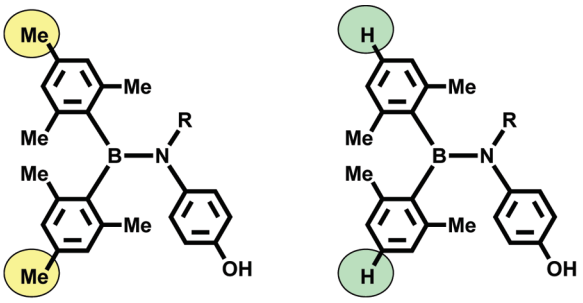
To explore this third dimension in a more systemic fashion, we prepared a series of bicyclic core ligands, first exploring the bicyclo[3.3.1]nonane system.[73] This bridged system proved difficult to be accommodated at the center of the ligand binding pocket; however, when it was moved a bit further from the phenol, very high affinity ligands were obtained.[74] In fact, large bridged and fused bicyclic and higher polycyclic systems, including adamantane, could be accommodated when appended to the end of a 1,1-bis(4-hydroxyphenyl)methylidine system.[74]

With a smaller 7-oxa-bicyclo[2.2.1]heptene core system, however, we were able to get high affinity ER ligands having inherently three-dimensional central core elements. These systems could be prepared by a facile Diels−Alder cycloaddition of 3,4-diarylfurans with a variety of dienophiles. Here, a *cis*-1,2-bis(4-hydroxyphenyl)ethene unit, contributed by the furan diene, appeared necessary for high affinity, with actual affinity being dependent on the precise nature of the groups contributed by the dienophiles. The best compound in the series that we have thus far explored has an exo-disposed phenyl sulfonate group, which we termed OBHS (for oxabicycloheptene sulfonate). The affinity of OBHS is ∼10% that of estradiol, and it profiles as an antagonist on ERα and ERβ (Figure 12).[75]

Like the THC compound described earlier,[17,47] OBHS does not resemble a classical SERM, and despite our initial speculations that the extended phenyl sulfonate was mimicking the bulky and basic side chain of the SERM raloxifene,[75] crystal structures of other members of this series suggest that the antagonist character is more likely the consequence of a passive antagonism mechanism, involving distortion of helix-11.[76] An intriguing characteristic of OBHS is that it has the antiproliferative activity of an ER antagonist, yet it preserves the anti-inflammatory activity of an ER agonist. Compounds having this pattern of pharmacological activity have been the subject of a considerable search in other laboratories,[77] and such a compound might prove uniquely effective in the treatment of estrogen-dependent breast cancer in which an inflammatory component is contributing to the virulence of the disease.

Physical chemists have noted that a boron−nitrogen single bond (C−N) is isoelectronic and isolobal with a carbon−carbon double bond (C═C). In fact, taken together the nucleon count for a ^{10}B−^{14}N bond and a ^{12}C−^{12}C double bond is the same, 12 protons and 12 neutrons, as is the sp^2 hybridization state of both of the atoms in the bond; consequently an effective B−N π-bond can form.[78,79] The major difference is that in the C═C double bond, each atom contributes one p-oribtal electron to the π-bond, whereas in the B−N π-bond, both electrons are contributed by the nitrogen lone pair and are accommodated by the empty p-orbital on the boron in forming the π-bond.

Carbon−carbon double bonds are found in the core of a number of nonsteroidal ER ligands, such as those of the cyclofenil and triarylethylene class, and we wondered whether it might be possible to replace these carbons with a B−N bond. It proved relatively easy to make such molecules from diarylboranes and amines, but simple systems in which the empty p-orbital on boron was not sterically shielded proved to undergo hydrolysis to the borinic acid.[80] Flanking the ortho position of both phenyl groups with methyl substituents, however, gave very stable B−N products that could withstand vigorous hydrolysis conditions. With the help of a key crystal structure obtained on one of the analogues, we were able to optimize the binding of these B−N compounds, with the best compound demonstrating an affinity for ERα ∼20% that of estradiol (Figure 13).[80]



R	RBA (E_2 = 100)			
	ERα	ERβ	ERα	ERβ
H	0.013±0.001	0.18±0.01	--	---
CH_3	1.3±0.2	3.1± 0.8	23±0	16±1
CH_2CH_3	1.3±0.0	1.1±0.0	---	---
CH_2CF_3	2.2±0.1	1.3±0.4	10±0	5.8±2
C_5H_{11}	0.16±0.04	0.12±0.01	---	---

Figure 13. Estrogen receptor ligands with boron−nitrogen core elements. ER ligands with a B−N bond core in place of a C=C bond have high affinity, and they are stable when the boron is protected by ortho-substituted arenes. Removal of the two *p*-methyl groups increases binding affinity by many fold.

These compounds are both remarkably stable and easy to prepare, despite their extreme steric congestion, a fact driven home by our inability to prepare the C=C analogues of these systems, despite our investigation of several methods well suited to the preparation of tetrasubstituted ethylenes.[80] The facility with which these compounds can be synthesized suggests that B−N for C=C substitution could be widely used for the preparation of analogues of drug candidates, a process that might be loosely described by the oxymoronic term "elemental isomerism".[80]

LOOKING FORWARD

The estrogen receptors continue to prove intriguing in a number of respects. They are established targets for fertility regulation (enhancement and contraception), for menopausal hormone replacement, and for breast cancer prevention and therapy, and they offer tantalizing opportunities for novel prostate cancer and behavioral therapies and for selective cardiovascular and neuroprotection. Novel estrogen pharmaceutical agents might be able to exploit the different biologies of ERα and ERβ, given careful appreciation for the different spectrum of activities that can be displayed by nominally similar ERβ ligands.[61] Also, though not described here, certain polymeric conjugates of estrogens also appear capable of activating only the extranuclear pathway of estrogen action,[81−83] thereby affording the long-sought-after cardiovascular protection without stimulation of the breast and uterus.[84] Furthermore, estrogen receptors can function as targets for imaging breast cancer by PET using estrogens labeled with the short-lived ($t_{1/2}$ = 110 min) positron-emitting radioisotope fluorine-18,[1,85,86] and ER can be used in hormone challenge tests to evaluate the functional status of this receptor in breast tumors.[87−89] Such imaging can be used to select patients most likely to benefit from endocrine therapies.[87−89]

All of these applications rely on the development of well optimized ER ligands in which the nature of the core of the ligand can be the core issue in ligand design.

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ABBREVIATIONS USED

DPN, diarylpropionitrile (an ERβ-selective ligand); EAE, experimental autoimmune encephalomyelitis; ER, estrogen receptor; MPP, methylpiperidinopyrazole (an ERβ-selective antagonist); OBHS, oxabicycloheptene sulfonate; PET, positron emission tomography; PPT, propylpyrazoletriol (an ERα-selective ligand); SERM, selective estrogen receptor modulator; SPECT, single

photon emission computed tomography; THC, tetrahydrochrysene (an ERα agonist, ERβ antagonist)

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