

## Workshop 1.4

# Nature of the ligand-binding pocket of estrogen receptor $\alpha$ and $\beta$ : The search for subtype-selective ligands and implications for the prediction of estrogenic activity\*

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*Abstract:* The ligand-binding pockets of estrogen receptor alpha and beta (ER $\alpha$  and ER $\beta$ ) appear to have subpockets of different size and flexibility. To find ligands that will discriminate between the two ER subtypes on the basis of affinity or efficacy, we have prepared compounds of varying size, shape, and structure. We have evaluated the binding affinity of these compounds and their potency and efficacy as transcriptional activators through ER $\alpha$  and ER $\beta$ . In this manner, we have identified a number of ligands that show pronounced ER subtype selectivity. These studies also highlight the eclectic structure–activity relationships of estrogens and the challenges inherent in developing computational methods for the prediction of estrogenic activity.

## INTRODUCTION

The actions of estrogens are mediated by the estrogen receptor (ER), an intracellular protein that functions as a ligand-regulated transcription factor [1]. There are two subtypes of the estrogen receptor, ER $\alpha$  and ER $\beta$ ; these subtypes have different tissue distributions and are thought to regulate different estrogen responses [2]. Therefore, ligands that are selective in activating or inhibiting these two subtypes could have a desirable pattern of pharmacological activity that might be useful in regulating fertility, in treating menopausal symptoms, and in preventing and treating breast cancer [1].

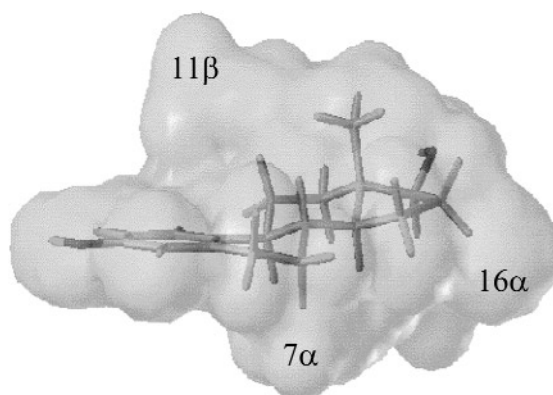
## LIGAND-BINDING POCKET OF THE ESTROGEN RECEPTOR EXHIBITS DYNAMIC AND PLASTIC CHARACTER

Before the structure of ER ligand-binding domains (LBDs) was elucidated by X-ray crystallography, pharmacophore analysis suggested that the hormonal steroid was surrounded by the protein, but that there was not a continuous, intimate contact between the protein and the ligand in the ligand-binding pocket [3]. The fact that strategically placed lipophilic substituents on steroidal estrogens, notably at positions 7 $\alpha$  and 11 $\beta$ , can result in large increases in binding affinity suggested that there were some preformed pockets in the receptor at these points around the ligand (Fig. 1). In addition, the fact that even

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**Fig. 1** Preformed pockets that were predicted to surround estradiol in the ligand-binding pocket of the estrogen receptor, according to pharmacophore analysis based on the affinity of substituted estradiol derivatives. The continuous surface represents the protein surface surrounding the ligand, which is shown as a skeleton representation [3].

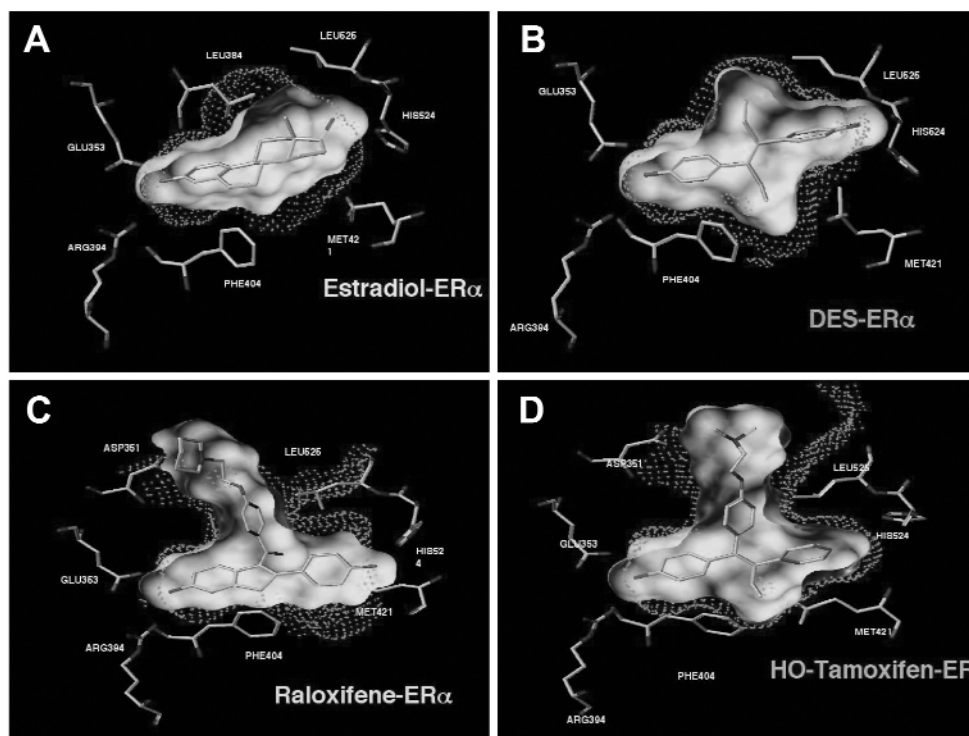
larger substituents were tolerated at these and other sites on the steroid skeleton without major losses of binding affinity further suggested that there were regions of the ligand-binding pocket that were substantially deformable. On the other hand, it was clear that even small substituents at other ligand sites caused dramatic losses of binding affinity. Thus, *the ligand-binding pocket of ER seems to be characterized by regions that had extra space, regions that were readily deformable, and regions that lacked such space or plasticity* [3].

The crystal structures of various ER ligand complexes that have appeared over the past five years have confirmed this general view of the ER ligand-binding pocket and have refined it further to a great degree [4–6]. Overall, all of these structures show the ER LBD to be constituted almost completely of  $\alpha$ -helical elements that are oriented largely in an antiparallel manner and to have a “triple sandwich” structure [7]. There are, however, substantive differences between the ER complexes with ligands of different size and shape.

For example, ER complexes with the agonists estradiol (E2) and diethylstilbestrol (DES) show the ligand engulfed by the LBD, but residing in pockets having substantially different shape (Fig. 2) [5,6]. The ER structure that surrounds E2 showed, as predicted, preformed pockets below the  $7\alpha$  and above the  $11\beta$  positions that are not filled by the ligand. By contrast, in the ER-DES structure, these pockets are filled by the two ethyl groups that extend upward and downward from the ligand. In this structure, however, the portions of the ER that surround the periphery of the ligand in the E2 structure have moved inward to compensate for the lack of bulk in the middle region of the “thinner” DES ligand.

In substantial contrast to the two ER-agonist bound structures, ER complexes with the antiestrogens raloxifene and hydroxytamoxifen have an altered helical topology at the C-terminal region of the LBD [5,6]. Because the large basic side chain of these ligands, which is the signature structural element of their antiestrogenic character, does not fit into a fully closed ligand binding pocket, the C-terminal helix-12 of the LBD becomes repositioned (Fig. 2). By this movement, the ligand-binding pocket is opened up to accommodate the basic side chains, and helix-12 occludes a hydrophobic groove on the surface of the LBD, which in the ER agonist structure functions as a docking site for helical sequences present in certain coactivator proteins.

Thus, the ER-ligand crystal structures highlight the fact that the region of the ER LBD that surrounds the ligand is, in fact, not in uniform, intimate contact with the ligand. Furthermore, it can be reshaped and even reorganized by changes in ligand size and shape. The fact that the ER interacts with its ligands in a dynamic and plastic manner presents special challenges for methods that might be used



**Fig. 2** X-ray crystal structures showing the fit of the ligands, estradiol (A), diethylstilbestrol (DES, B), raloxifene (RAL, C), and hydroxytamoxifen (TOT, D), in the LBD of the ER $\alpha$ . The continuous surface represents the protein surface surrounding the ligands, which are shown as skeleton representations [5,6].

to predict the estrogenic potency of new chemical entities, as well as for the development of various types of selective ligands for this receptor. Nevertheless, methods such as structure-based modeling of ER–ligand interaction and the development of structure/affinity/activity-based pharmacophore analysis provide the best basis for appreciating the “eclectic” nature of structure–activity relationships of estrogens.

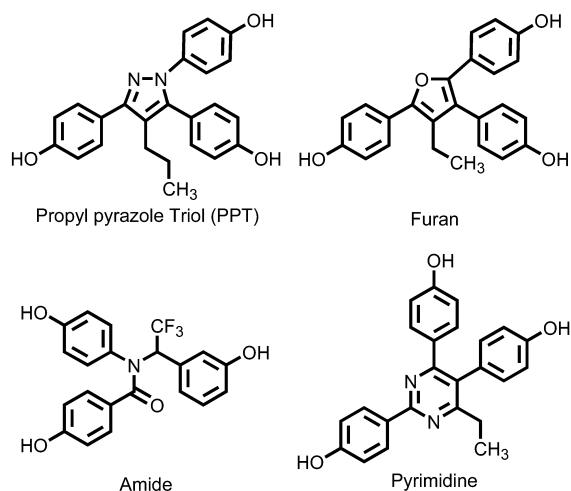
At this point, it is not clear whether other members of the nuclear receptor family have a similar degree of dynamic and plastic character as does ER. The retinoic acid receptors appear to bind their ligands more tightly and show less variation of receptor structure as ligand structures change, but it is not clear how widespread this apparent reduced plasticity is [8–11]. In contrast, other members of the nuclear receptor superfamily, notably the orphan receptors, seem to bind their ligands even more loosely than does ER, with large preformed and unfilled pockets being noted in the crystal structures [12].

### DEVELOPING LIGANDS THAT ARE SELECTIVE FOR THE ER SUBTYPES, ER $\alpha$ AND ER $\beta$

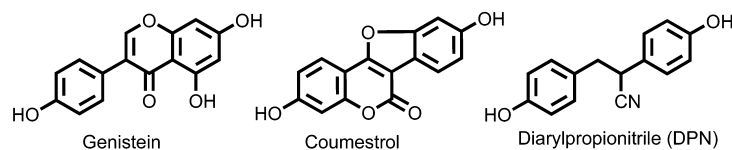
The dynamic, plastic nature of the ER LBD also plays a role in ligand design, especially in the design of ligands that discriminate between the two ER subtypes, ER $\alpha$  and ER $\beta$ . The LBDs of ER $\alpha$  and ER $\beta$  have only 60 % sequence identity. However, the interior of the ligand-binding pockets of the two subtypes is much more similar, with 22 or 24 contact residues being identical [4]. Nevertheless, the two subpockets seem to have somewhat different size and flexibility, and this seems to be a critical distinction that is useful in the development of subtype-selective ligands.

To find ligands that will discriminate between the two ER subtypes on the basis of affinity or efficacy, we have prepared compounds of varying size, shape, and structure. We have evaluated the binding affinity of these compounds and their potency and efficacy as transcriptional activators through ER $\alpha$  and ER $\beta$ . Ligands have been designed in part through a consideration of the structure of the ER LBD and the universe of known ER ligands, and in part through a consideration of their expedited synthesis by combinatorial chemistry means [13]. In this manner, we have identified a number of ligands that show pronounced ER subtype selectivity (Fig. 3).

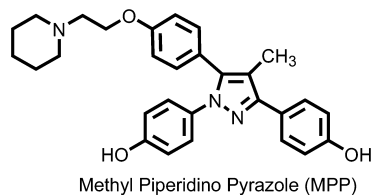
#### ER $\alpha$ -Selective Agonist Ligands



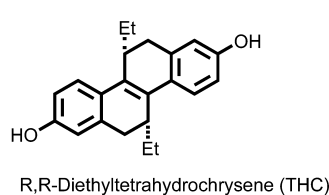
#### ER $\beta$ -Selective Agonist Ligands



#### ER $\alpha$ -Selective Antagonist Ligand



#### ER $\beta$ -Selective Antagonist Ligand



**Fig. 3** Structures of ligands that show selectivity for either the ER $\alpha$  or  $\beta$  subtype. For details, consult references noted in the text [14,15,18,22,25–27].

#### Ligands that are selective for ER $\alpha$

Certain triaryl amides show potency preferences as agonists for ER $\alpha$  that can be as great as 500-fold; they function as agonists on both ER $\alpha$  and ER $\beta$ , but in cell-based assays of gene transcription, they activate ER $\alpha$  at much lower concentrations [14].

Several triaryl-substituted five-membered heterocycles show exceptionally large potency and efficacy preferences for ER $\alpha$  [15–18]. The best of these are triaryl-alkyl-substituted pyrazoles and furans that function as complete ER $\alpha$  agonists, but are almost completely inactive on ER $\beta$ . The very best heterocycle we have found so far is called propyl pyrazole triol (PPT), a compound that is ca. 10 000-fold more potent on ER $\alpha$  than on ER $\beta$  and showed ER $\alpha$ -selective effects in vivo [19], but the molecular basis for its ER $\alpha$  selectivity is not fully understood [15]. Other larger ring heterocycles, such as tetrasubstituted pyrimidines and pyrazines, also retain greater potency and efficacy on ER $\alpha$  than on ER $\beta$  [20]. Ligands in these heterocycle classes can easily be synthesized in a combinatorial fashion [21].

Substituted tetrahydrochrysenes ligands, in some cases, function as potent agonists on ER $\alpha$ , but are potent antagonists on ER $\beta$  [17,22]. This character is, however, a delicate function of substituent size and stereochemistry, and the best compound is the *R,R*-enantiomer of a diethyl-tetrahydrochrysenes called *R,R*-THC. The difference in efficacy of *R,R*-THC on the two ER subtypes appears to arise from its optimal fit in the ER $\alpha$  ligand-binding pocket and its suboptimal fit in the slightly smaller ER $\beta$  pocket [23,24].

### Ligands that are selective for ER $\beta$

It has proved to be a greater challenge to discover ligands that are highly selective for ER $\beta$ . While certain phytoestrogens such as genistein and coumestrol have higher affinity for ER $\beta$  than ER $\alpha$  [24], in cell-based transcription assays, they do not show much difference in potency. Some simple diarylethane systems do show considerable affinity and potency preference as agonists on ER $\beta$  [25]. The best of these, called diarylpropionitrile (DPN), will activate ER $\beta$  at 100-fold lower concentrations than ER $\alpha$ . The ER $\beta$  selectivity of this ligand seems to result from its preferential interaction with a key methionine residue that is present only in the ER $\beta$  LBD [28]. In general, ER $\beta$  selective ligands seem to be smaller and more polar than ER $\alpha$ -selective ligands.

### ER subtype-selective antagonists

Antagonists based on the highly ER $\alpha$ -selective pyrazoles have been developed that retain a large degree of ER $\alpha$  preference as antagonists [26,27,29,30]. The best of these, a compound called methyl piperidino pyrazole (MPP), can block estrogen action through ER $\alpha$  at a concentration that has insignificant inhibitory effect on ER $\beta$  [27]. So far, no compound has been reported that is more a potent antagonist of ER $\beta$  than of ER $\alpha$ . It should be noted that the THC compound, noted above, is a complete ER $\beta$  antagonist, but has substantial agonist activity on ER $\alpha$  [22].

### GENERAL SCHEME FOR CONSIDERING DIMENSIONS OF ER SUBTYPE SELECTIVITY

Selectivity for the ER subtypes ER $\alpha$  and ER $\beta$  can be based on differences in ligand-binding affinity, ligand potency, or ligand efficacy. In general, one might expect that differences in ligand-binding affinity would be directly reflective of differences in potency; however, this is not universally true, and there can be surprising deviations in both directions. There are compounds that show substantial ER subtype differences in affinity, such as the phytoestrogens, yet do not show major differences in potency; by contrast, certain triaryl amides that have only modest affinity preference for ER $\alpha$  show large potency differences in favor of ER $\alpha$  [14]. These disjunctions between affinity selectivity and potency selectivity probably have several origins, but most likely reflect preferential interactions the ER subtype ligand complexes have for the various coregulatory proteins that mediate their effects on transcription [31].

Ligands that show subtype differences in efficacy or degree of agonist vs. antagonist character between the two ER subtypes seem, at least in all reported cases, to show higher levels of agonism on ER $\alpha$  than on ER $\beta$ . The structural basis for this trend is not yet clear [23], but it might indicate that the most

difficult pharmacological class of ER subtype-selective ligands to develop will be those that are more agonistic on ER $\beta$  than on ER $\alpha$ .

## CONCLUSIONS AND IMPLICATIONS FOR MODELING OF ESTROGEN ACTIVITY

Our studies on the development of ER subtype-selective ligands, as well as the crystallographic determination of the structure of ER ligand complexes, illustrate the complexity of the structural response of the ER to ligands of different shape and size. In the process, these results also highlight the eclectic structure–activity relationships of estrogens. In the face of clear evidence for the dynamic and plastic character of the estrogen receptor, it can be anticipated that the most successful approaches to structure-based modeling of ER ligands or of structure–activity based pharmacophore modeling will be those that incorporate, in some appropriate fashion, consideration of the dynamic and plastic elements involved in ER–ligand interaction. It is hoped that such considerations will enable these models to be raised to a higher degree of reliability and utility for both pharmaceutical development and regulatory consideration.

## ACKNOWLEDGMENTS

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