SELECTIVITY OF BINDING TO ESTROGEN RECEPTORS α AND β AS DETERMINED BY FLUORESCENCE POLARIZATION

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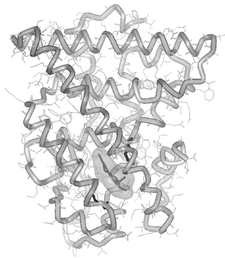
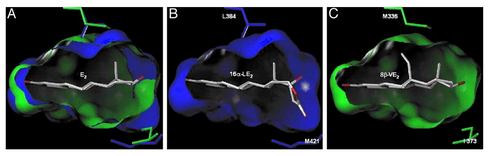
INTRODUCTION

Estrogen's role in cell growth and proliferation has long been appreciated both in the normal development of secondary sexual characteristics and in diseased states in cancers of the breast, ovaries and uterus. We are beginning to appreciate estrogen's expanded role in maintaining such diverse functions as the skin's elasticity, the health of the central nervous system, bone density and cardiac health. Estrogen plays out its roles in varied tissues by binding to two major ligand activated nuclear receptors, estrogen receptor alpha (ER α) and estrogen receptor beta (ER β). Though the ligand binding cavities of the two receptors differ by only two amino acids, the overall degree of homology between ER α and ER β is low. The body uses the receptor selectivity to its advantage by dispersing the receptors in varying ratios to different tissues.



Ligand Binding Domain of ER α and ER β: The residues that differ in the binding pocket between the two receptors are shown in black. These differences are largely responsible for ER ligand selectivity.

Small molecules have been identified which bind each receptor with differing binding affinities. These selective estrogen receptor modulators (SERMs) hold the potential to be pharmacologically effective in treating diseases specific to one type of estrogen receptor while not affecting the other. For example, ER α and ER β are both present in breast tissue, and the ratio of ER β to ER α is being examined as one indicator in determining the likelihood of successful treatment of breast cancer by certain drugs. Here we use changes in the fluorescence polarization to calculate binding affinities for DPN and PPT, two SERMs, to ER α and ER β.



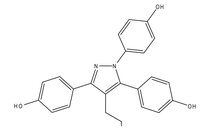
Binding modes of SERMs within the ligand binding pocket of ER α (blue) and ER β (green): The effect of the different shape pocket on ER selectivity is shown.

Various synthetic compounds ubiquitous in the environment have been implicated as xenoestrogens, or chemicals that can bind to the ER thus mimicking estrogen. They have been linked to many health problems including various forms of cancer, sexual and developmental defects, and reproductive abnormalities. Because the ER has a large binding pocket, a diverse range of chemicals can act as xenoestrogens. Though these chemicals are not as potent in binding as natural estrogen, the total xenoestrogen burden due to their ubiquity is cause for concern. This study also examines the binding of bisphenol A and three pyrethroid pesticides, Permethrin, Fenvalerate and Deltamethrin, with both ER α and ER β.

Both images are from Hillisch, A. et al. (2004) Dissecrting physiological roles of estrogen receptor α and β with potent selective ligands from structure-based design. Journal of Molecular Endocrinology 18(7): 1599-1609

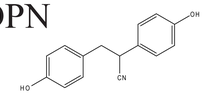
COMPOUNDS TESTED FOR ESTROGENICITY

PPT



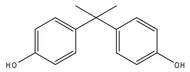
• ER α selective SERM

DPN



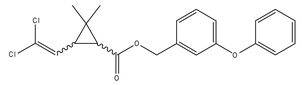
• ER β selective SERM

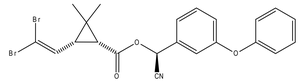
Bisphenol A

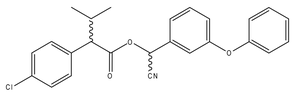


• Found in plastics and epoxy coatings used in food containers

Pyrethroid Pesticides: Permethrin, Deltamethrin, and Fenvalerate

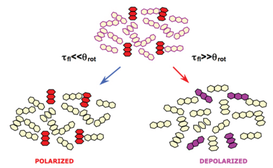




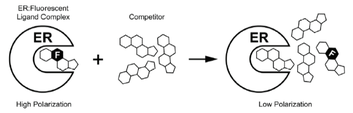


• All three compounds used in agriculture and ranching applications
• Permethrin also found in household products like tick repellent and pet shampoos

FLOURESCENCE POLARIZATION ASSAY



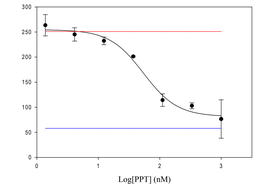
Fluorescence polarization (FP) measures the amount of polarized light emitted by a fluorescent molecule when excited by polarized light. The larger the molecule, the slower is rotates in solution and the more polarized light (the higher polarization) it will have. A small molecule rotates more quickly and will have a lower polarization value. The ability of the compounds shown above to bind to the ER and displace a fluormone (a fluorescent ER ligand) from ER α or ER β was measured using FP. If the compound did not bind to the ER, the fluormone stayed bound to the ER and the polarization of the large complex was high. If the competitor did bind to the ER, it displaced the fluormoe and the polarization of the fluormone alone was low. These assays were conducted in 96 and 384 well plates. A variety of concentrations of the competitors were run with the ER fluormone complex. The polarization was graphed versus the concentration to get a binding curve.



Top image from Terpetschnig, E. Fluorescence Polarization (FP). In Technical Notes. pp.7, ISS, Inc.: Champaign, Illinois
Lower image from Invitrogen (2003). Estrogen Receptor-β Competitor Assay, Green Protocol. 1-4

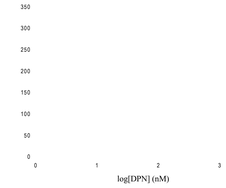
RESULTS AND CONCLUSIONS: ER α

PPT



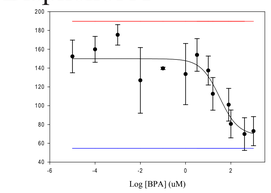
• IC_{50} = 54.2 nM
• K_i = 47.8 nM

DPN



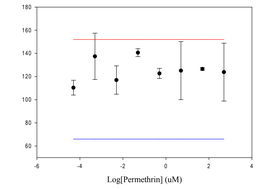
• No binding

Bisphenol A



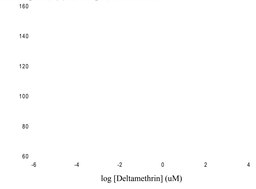
• IC_{50} = 31.7 uM
• K_i = 14.07 uM

Permethrin



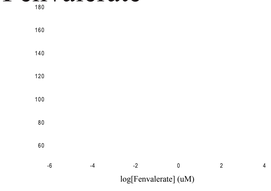
• No binding

Deltamethrin



• No binding

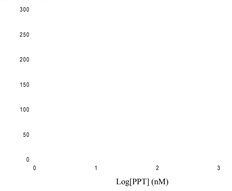
Fenvalerate



• No binding

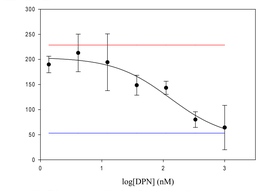
RESULTS AND CONCLUSIONS: ER β

PPT



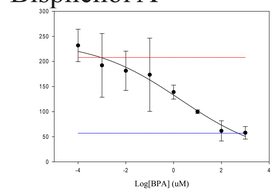
• No binding

DPN



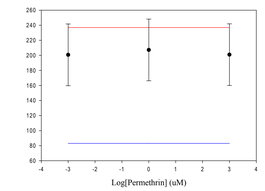
• IC_{50} = 54.2 nM
• K_i = 47.8 nM

Bisphenol A



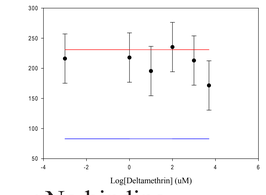
• IC_{50} = 54.2 nM
• K_i = 47.8 nM

Permethrin



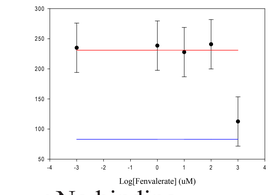
• No binding

Deltamethrin



• No binding

Fenvalerate



• No binding

For all assays, the polarization of the ER fluormone complex is the high polarization standard shown in red, while the polarization of the displaced fluormone is the low standard shown in

ACKNOWLEDGMENTS