Steroid Hormones, Structure, Function and Disruption

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Steroid Hormone Overview

1. **Derived from Cholesterol**
2. **Our focus:**
   a) Adrenocorticoids, Estrogens, Progestins, Androgens
3. **Nuclear Receptor Mechanism of Action**
   a) Glucocorticoid Receptor (GR)
   b) Mineralocorticoid Receptor (MR)
   c) Estrogen Receptor (ER)
   d) Progesterone Receptor (PR)
   e) Androgen Receptor (AR)
4. **Hormones are Receptor Agonists**
5. **Steroid Drugs are: Agonists or Antagonists**

Steroids

1. **Many hormones and biomolecules are steroids**
2. **Standard nomenclature:**

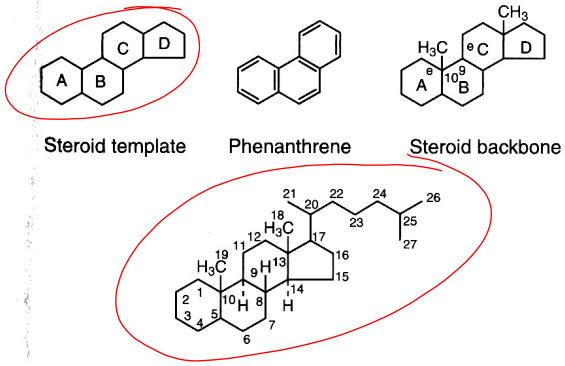


Fig. 28.1. Basic steroid structure and numbering system.

Classes of Steroid Hormones

C20
3 keto
C 19

C19
3 keto
17βOH

Phenolic A-Ring
3OH
17βOH

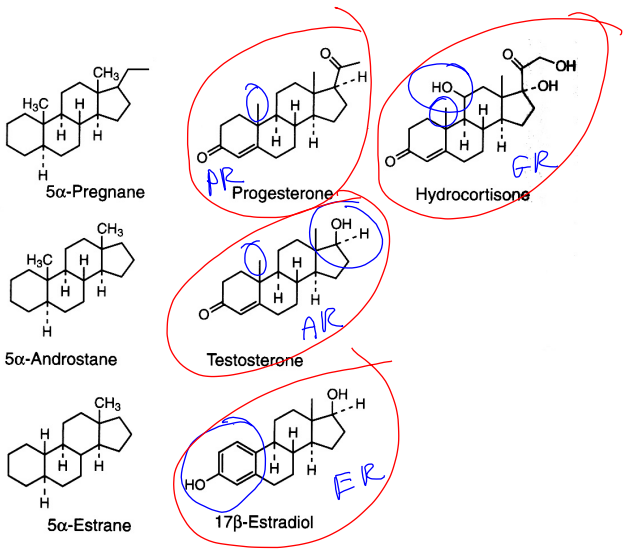
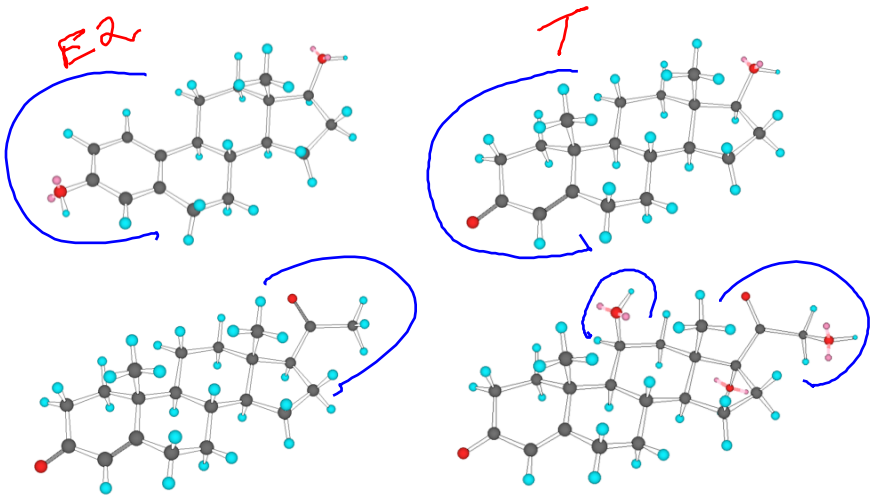
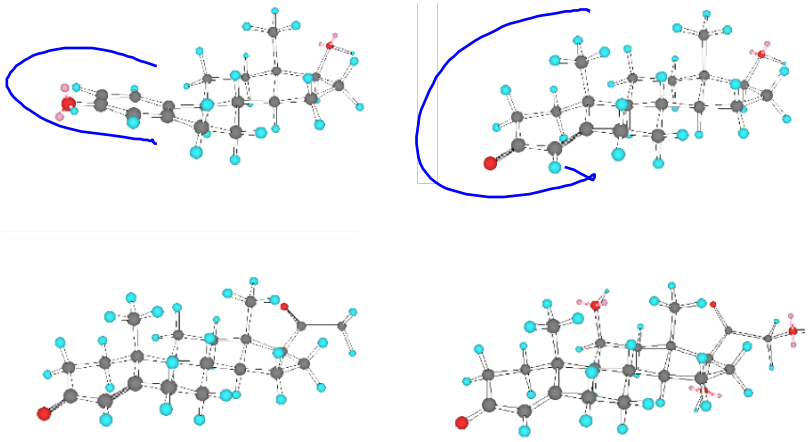


Fig. 28.4. Steroid classes and corresponding natural hormones.

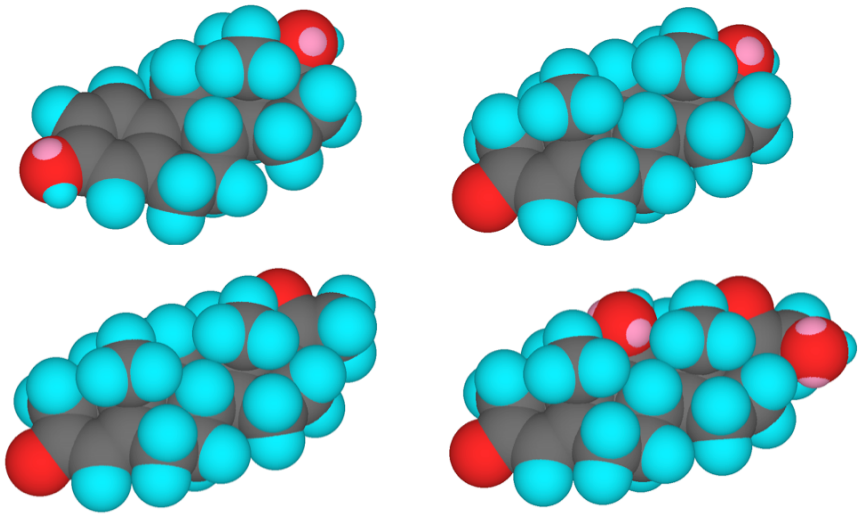
Estradiol and Testosterone
Progesterone and Cortisol



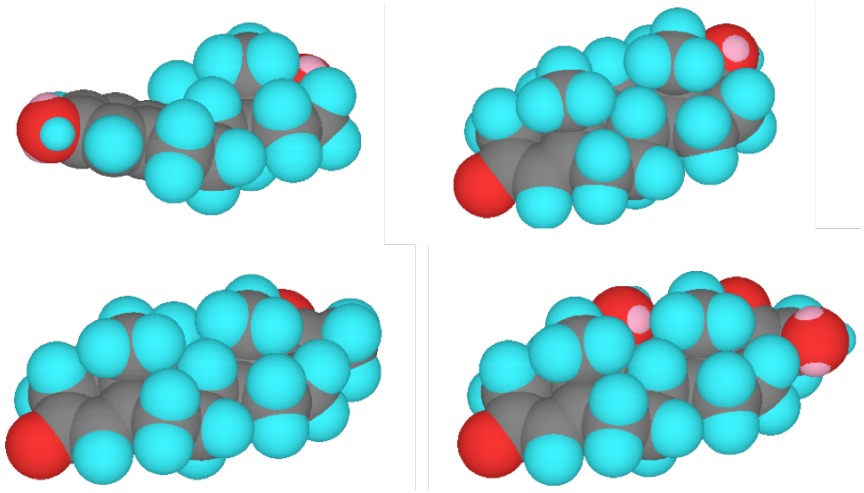
Estradiol and Testosterone
Progesterone and Cortisol



Estradiol and Testosterone
Progesterone and Cortisol



Estradiol and Testosterone
Progesterone and Cortisol



Nuclear Receptors

1. **Ligand activated transcription factors**
   a) Regulate genes in response to hormone
   b) Genes regulated have receptor specific sequence in promoter
   c) Observed effect is from action of gene products
2. **"Super Family" of related nuclear receptors**
   a) Ligands: steroid hormones, vitamin D, retinoic acid, bile acids, fatty acids, eicosanoids, steroid metabolites, xenobiotics, unknown ligands (orphan receptors)
   b) Each type of ligand has unique type of nuclear receptor
   c) Related by sequence and structure of receptor sequence

Nuclear Receptor Action

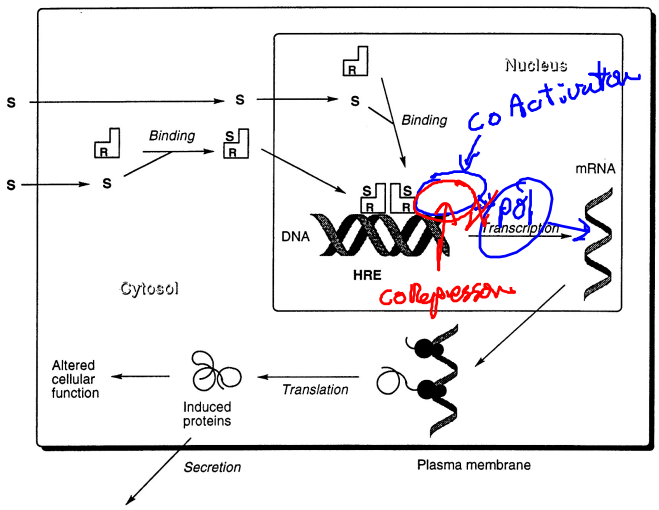
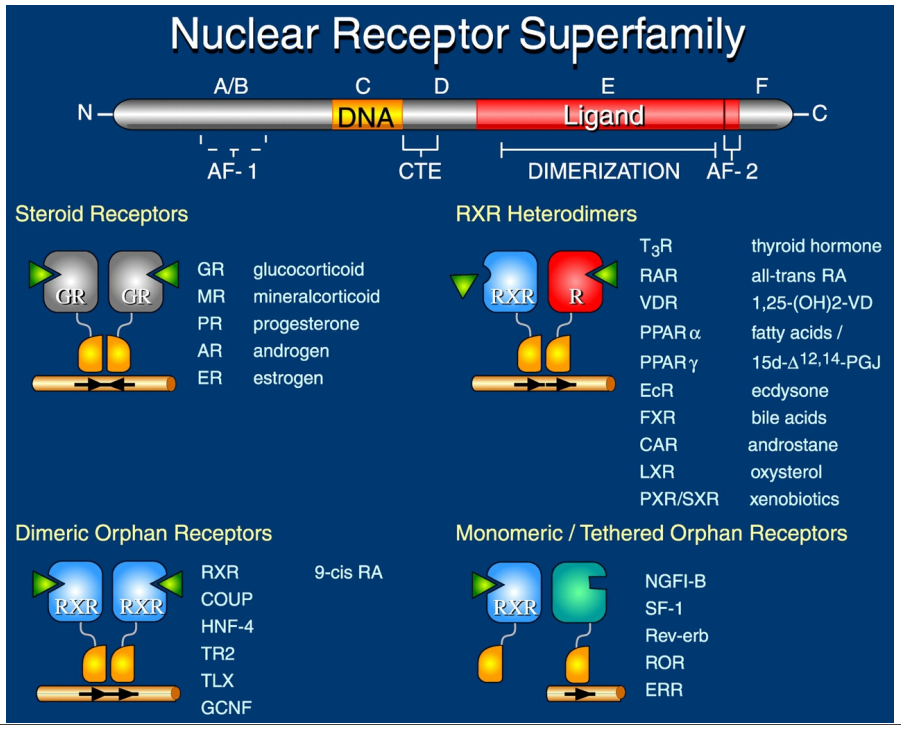


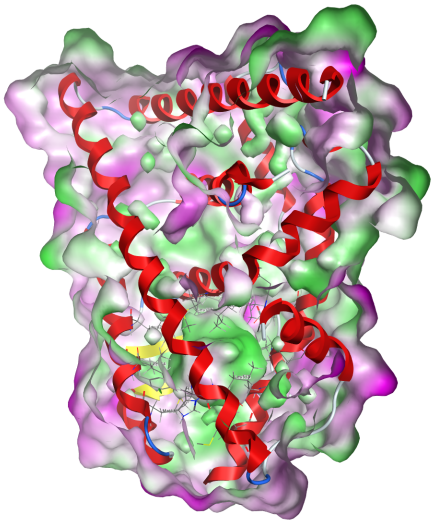
Fig. 28.5. Mechanism of steroid hormone action.



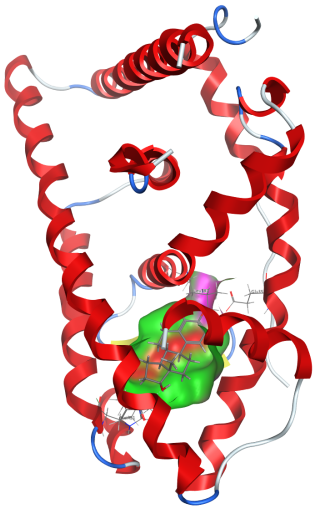
Ligand Binding Domain Structure

1. **X-Ray Crystallography Determinations**
   a) Identify molecular level ligand binding interactions
   b) Design new and more specific drugs
2. **Unique α-helical protein structure**
   a) Found in all Nuclear Receptor Ligand Binding Domains
   b) 12 a-helixes
   c) 2-4 small b-sheets
   d) Ligand binding pocket
3. **Ligand Induces Large Conformational Change**
   a) C-terminus transactivation function (control transcription)
   b) Agonists Activate gene transcription
   c) Antagonists Block gene transcription

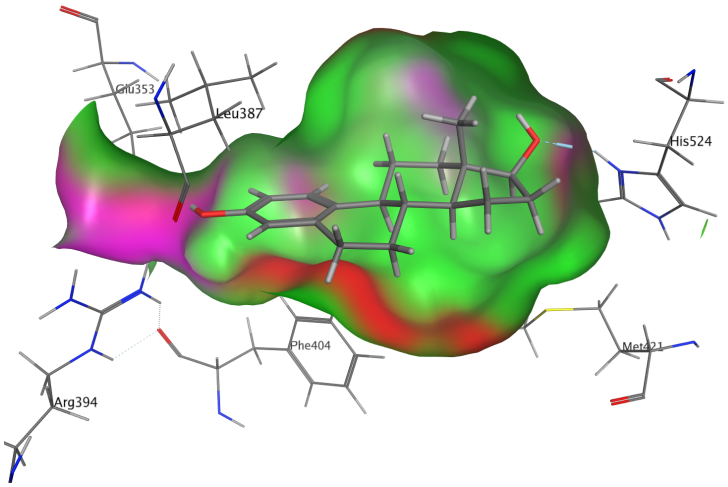
ER Ligand Binding Pocket



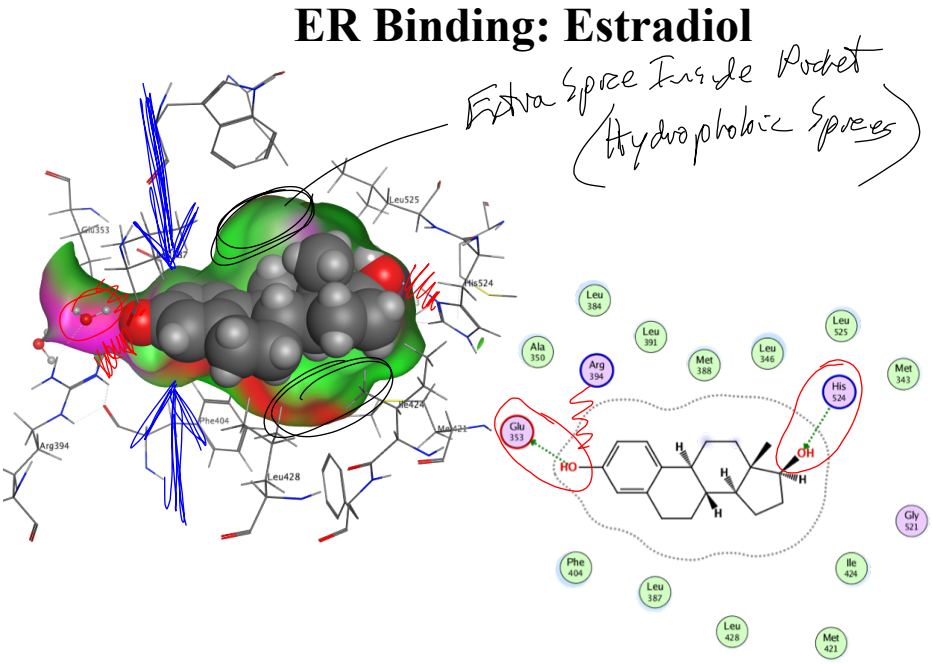
ER Ligand Binding Pocket



ER Ligand Binding: E2



ER Binding: Estradiol

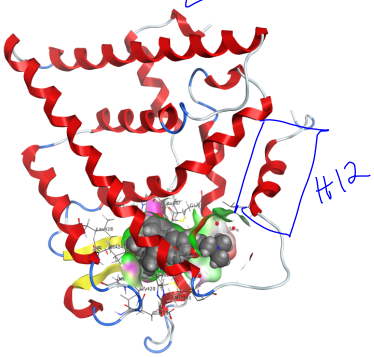


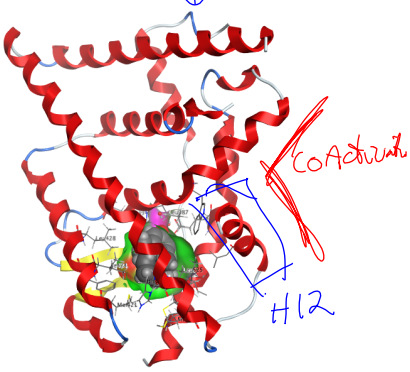
Antagonist and Agonist Structure: ER with Tamoxifen or Estradiol

Lig. Bind Domain

Antagonist

Agonist





Estrogen Metabolism

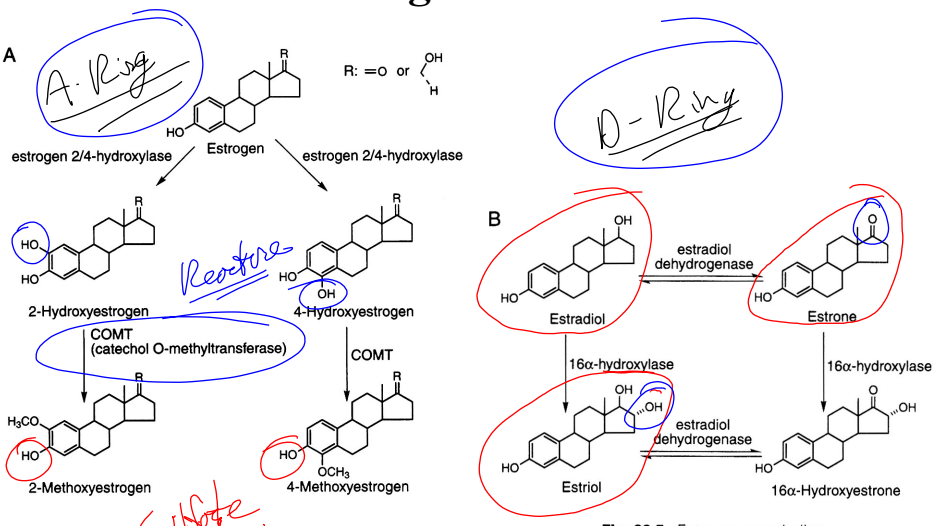


Fig. 29.5. Estrogen metabolism.

Synthetic Steroid Estrogens

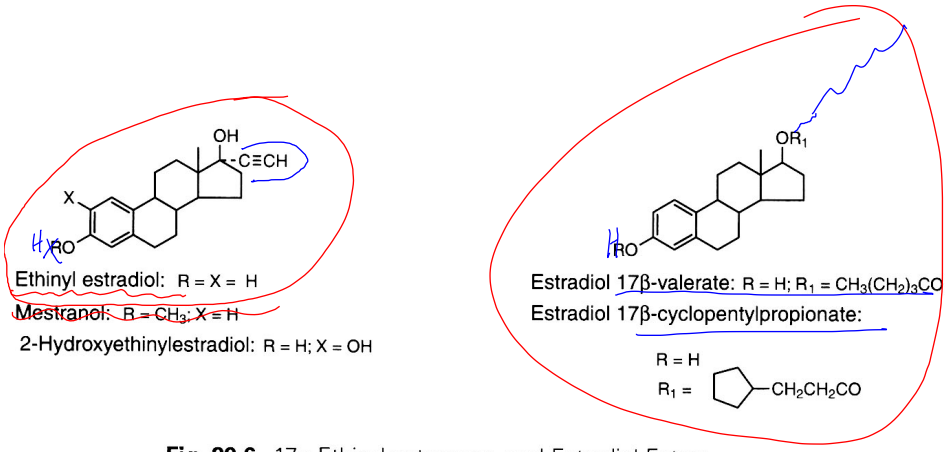


Fig. 29.6. 17α-Ethinyl estrogens, and Estradiol Esters.

(Premarin)

Conjugated & Esterified Estrogens

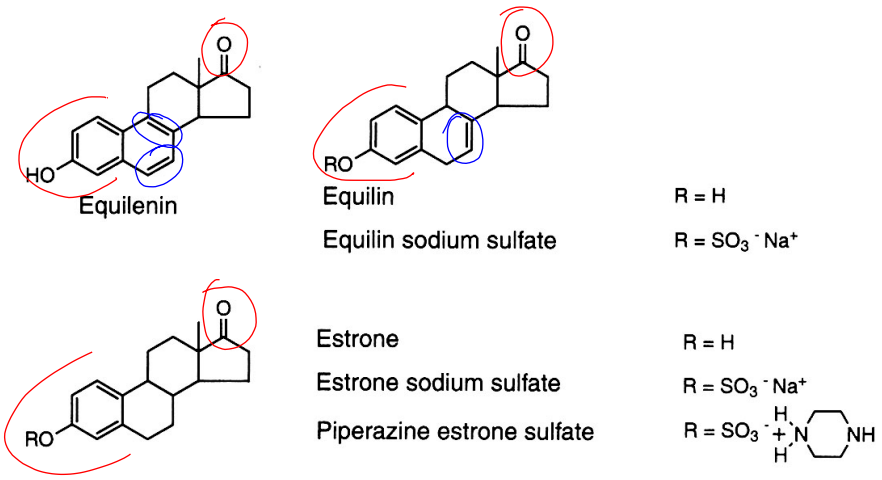


Fig. 29.7. Conjugated and esterified estrogens.

Non-steroid Estrogens

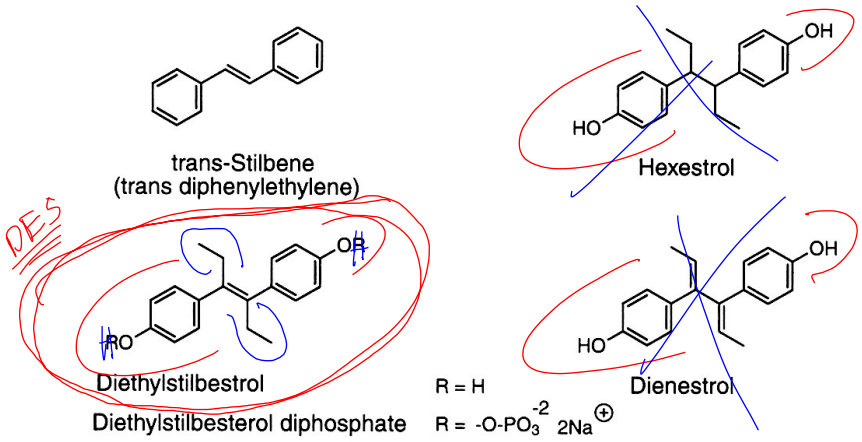
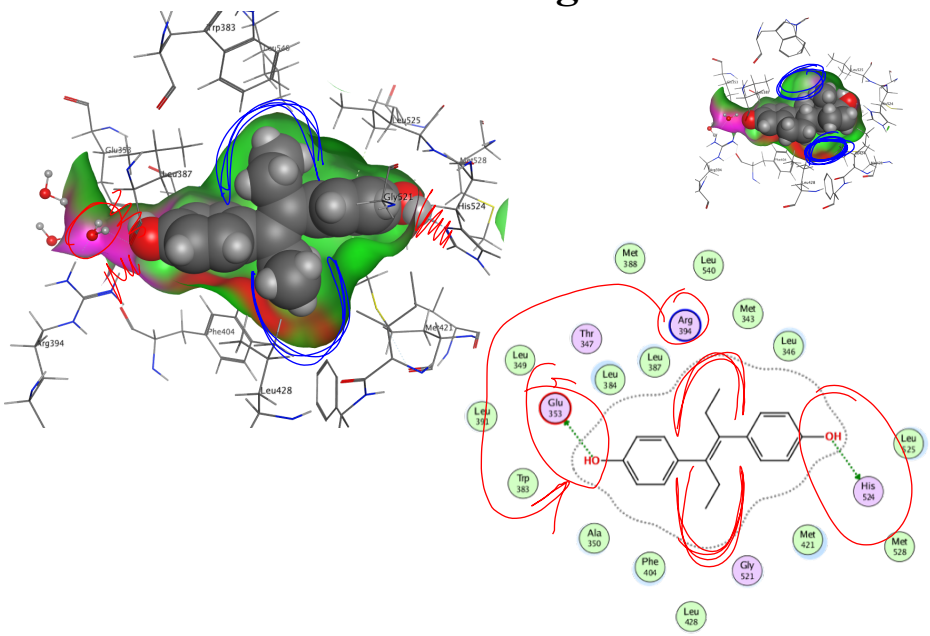
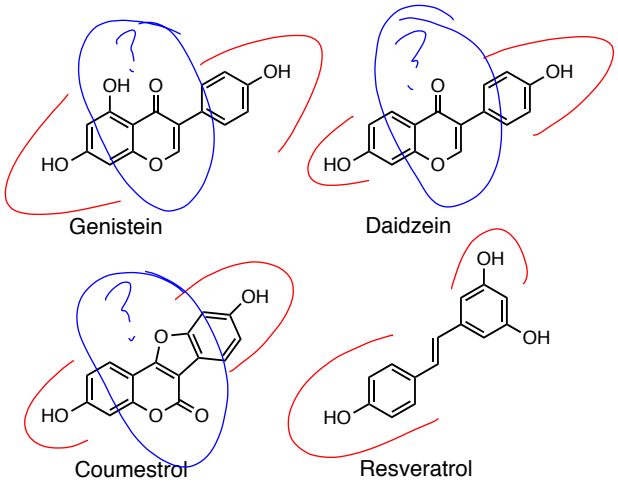


Fig. 29.8. Nonsteroidal estrogens.

ER Binding: DES



Naturally Occurring Estrogens from Plants



Environmental & Xenoestrogens

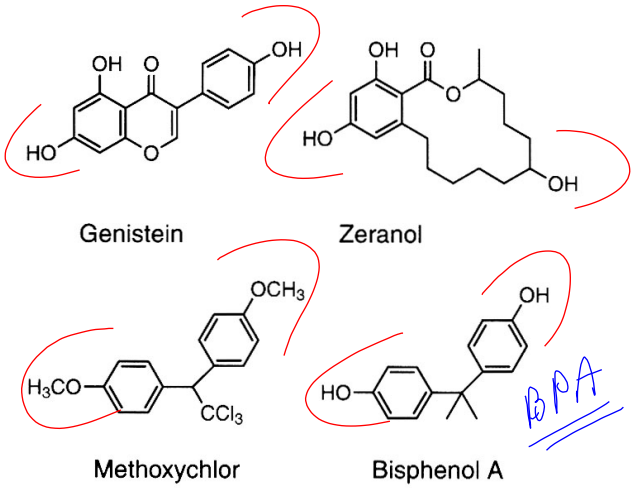


Fig. 29.9. Xenoestrogens.

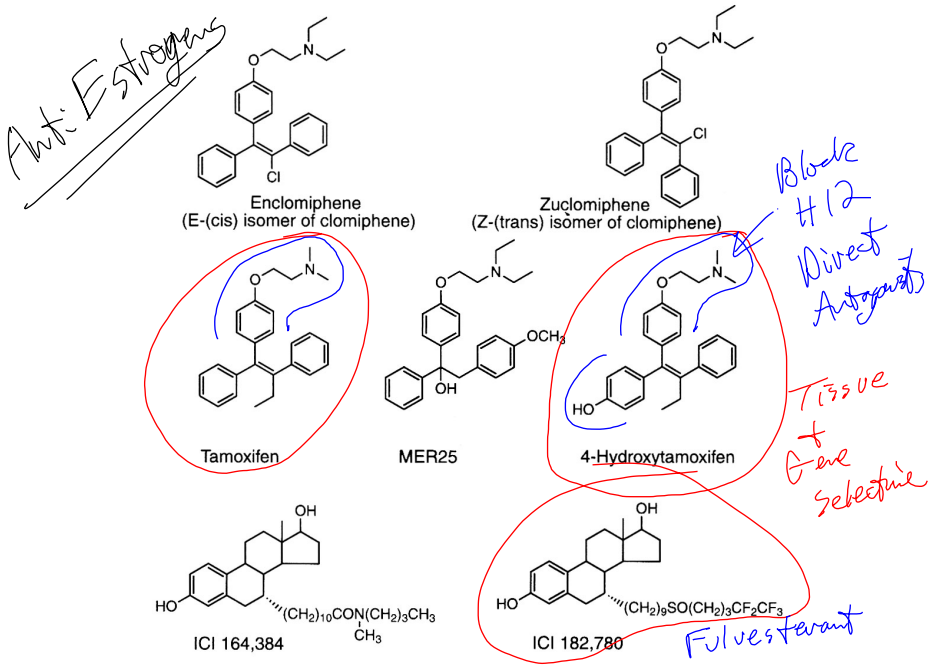
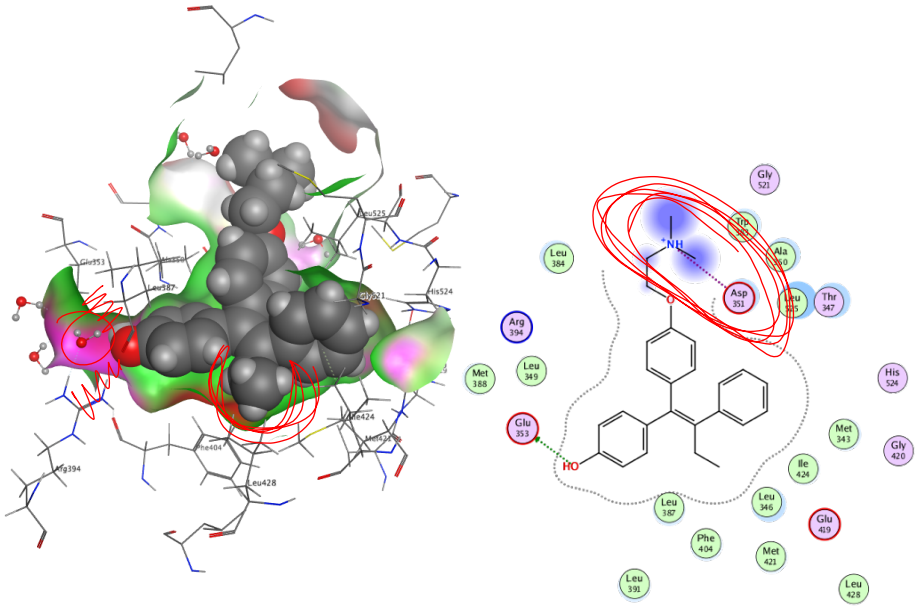


Fig. 29.10. Antiestrogens.

ER Binding: Tamoxifen



Endocrine Disrupting Chemicals

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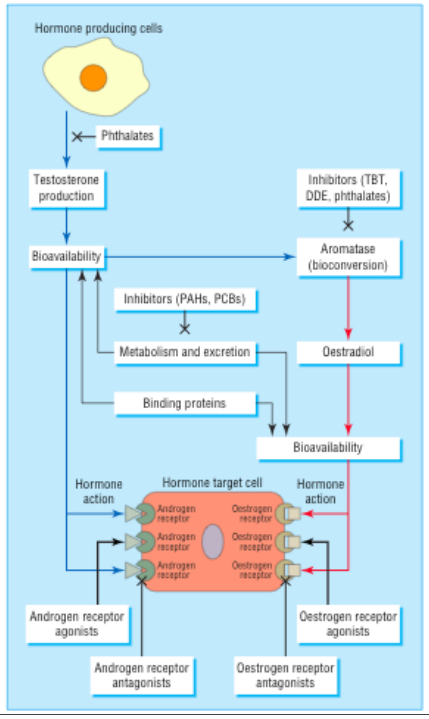
Endocrine Disrupting Chemical (EDC)

An exogenous agent that interferes with the function of natural hormones in the body.

a) Maintenance of Homeostasis
b) Regulation of Development

Interference sites include hormone:

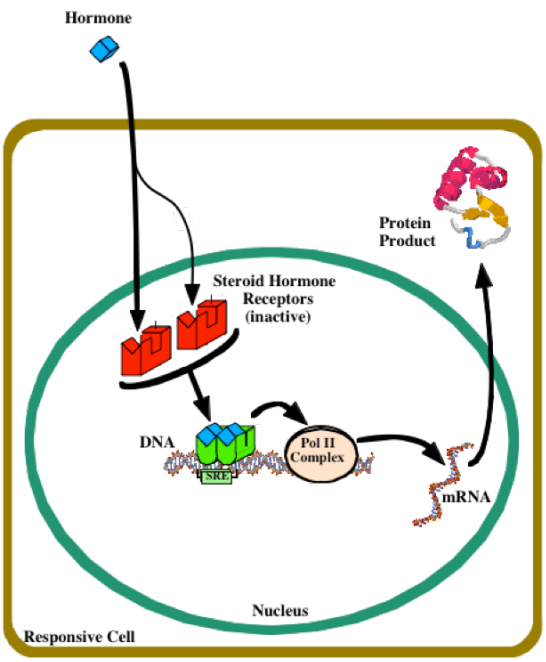
1. Production
2. Release
3. Transport
4. Receptor Mediated Action
5. Receptor Independent Action
6. Metabolism
7. Elimination
8. *Other*



Cellular Pathways of EDC Action

1. Production
2. Metabolism
3. Transport
4. Receptor Agonist
5. Receptor Antagonist

Sharpe 2004



Estrogen Action in Cells Results in:

Gene Regulation

More or Less of Genes Expressed

Steroid Receptors as EDC Targets

1. Multiple Receptor Targets

a) Estrogen Receptor

- Feminization, Promotion-Progression of Hormone Regulated Cancers (Breast, Uterus, Fibroids, etc.)

b) Androgen Receptor

- Male Reproductive Tract Development, Behavior, etc.

c) Progesterone Receptor

- Implantation, Maintenance of Pregnancy, Breast Cancer?, etc.

2. Agonist and/or Antagonist Activity

ER agonists, AR antagonists, PR antagonists

3. What About Other Nuclear Receptors?

TR, GR, VDR, RAR, MR, etc…

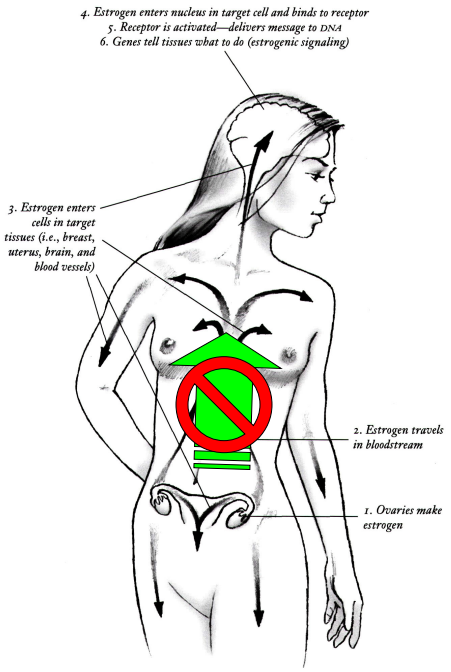


FIGURE 1.4 *How a Hormone Works: Example—Estrogen*

How EDCs Interfere With Hormone Action:

Increase
or
Decrease
Activity

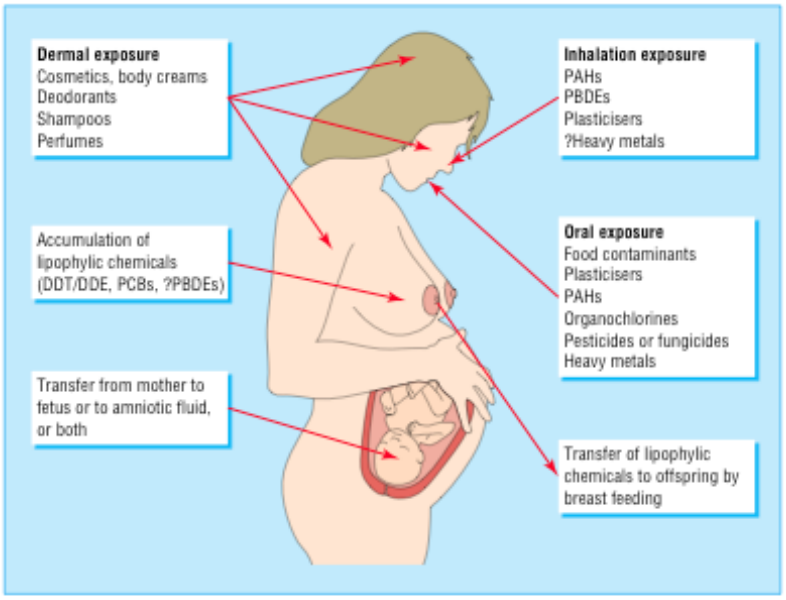
Berkson 2001

Other Ways to Think of EDCs?

1. **Hormone Active Environmental Agents**
2. **Environmental Hormones**
3. **"Gender Bender" Chemicals**
4. **Hormone Pollution**

- **Not Like Classic Toxic Chemicals**
  - ✓ Few short term effects
- **Long Term Effect May Be Subtle or Drastic**
  - ✓ Permanent Developmental Changes in Embryo/Fetus
  - ✓ Chronic for Adult: Wrong genes at wrong time…
- **Effect May Not Be Observed for Many Years…**

Routes of Human Exposure to EDCs



Sharpe 2004

Typical Endocrine Disrupting Chemical Research

1. **Hypothesis**
   a) Exogenous chemicals that bind and regulate nuclear receptors can induce altered cell physiology

2. **How?**
   a) Aberant gene regulation through nuclear receptors
   b) Too much, Not enough, Wrong Time, Wrong Gene(s)

3. **Why do we care?**
   a) Promotion and/or progression of carcinogenesis
      ✓ Cell proliferation and survival Pathways
   b) Interference with Development, Differentiation, etc.

How to We Know if a Substance has Endocrine Activity?

1. **Use Bioassays**
   a) *in vivo*: Many animal tests availabe
   b) *in vitro*: Many cell or cell free assays available

2. **What About the Mechanism of Action?**
   a) Need multiple *in vitro* characterizations
   b) Can use *in silico* molecular modeling screens

3. **Tiered Approach**
   a) Start with *in vitro* and *in silico*
   b) Prioritize and move to *in vivo*

Example *in vitro* Hormone Activity Assays

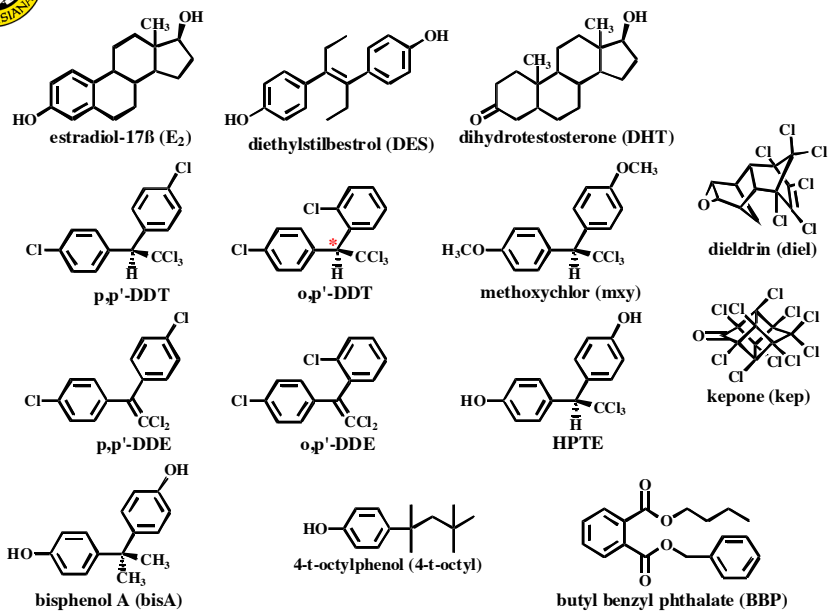
1. **MCF-7 Proliferation**
   a) Estrogen Activity in Mammalian Cells
   b) Agonist & Antagonist

2. **Receptor Specific Reporter Genes (Luciferase)**
   a) ER, AR, PR, GR responsive stably transfected cells
   b) Agonist & Antagonist

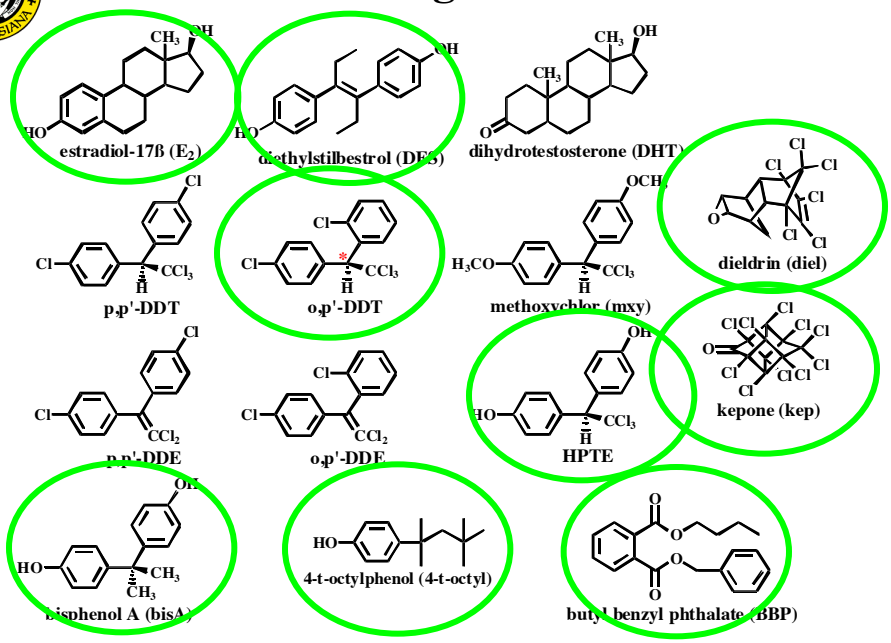
3. **Gene Profiling: PCR Arrays, RNAseq**
   a) Estrogen Activity in Mammalian Cells
   b) Expression of Endogenous Genes
   c) Target Genes by Mechanism, Toxicity, etc
   d) Agonist & Antagonist

4. **Nuclear Receptor Binding**
   a) ER, AR, PR, GR Recombinant FP

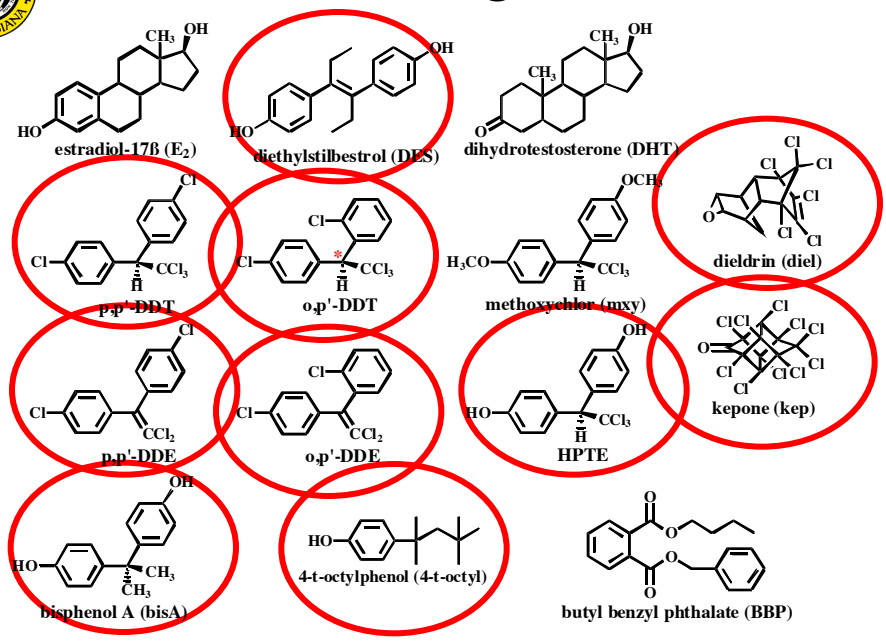
Steroid Hormone and EDC Structure



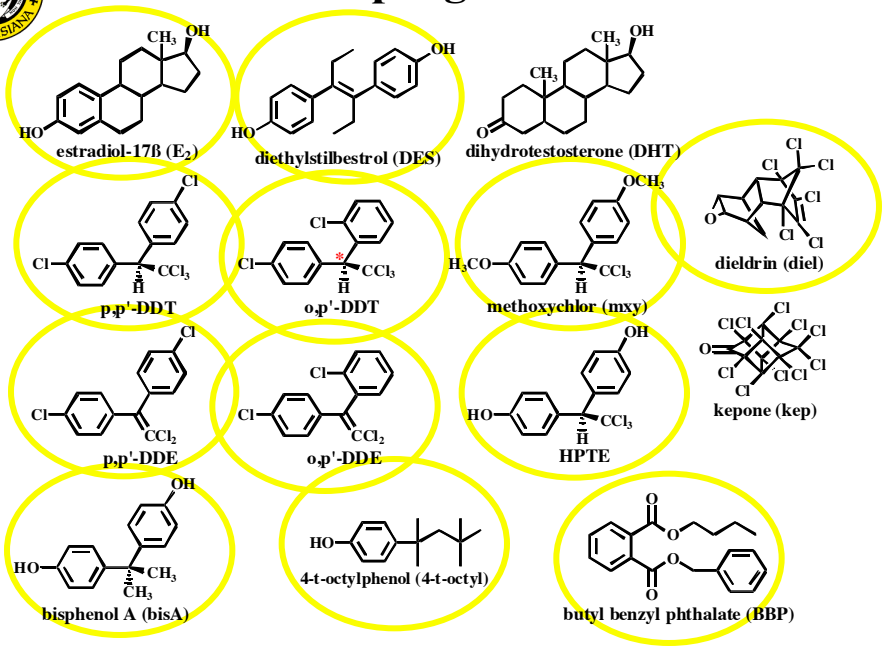
Estrogens



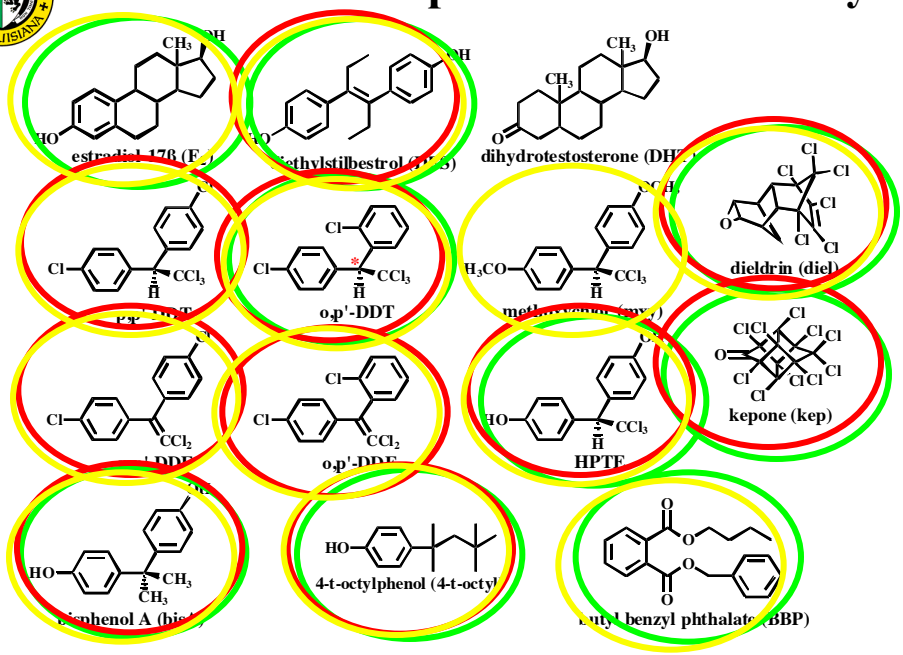
Anti-androgens



Anti-progestins



EDCs with Multiple Hormone Activity



in vitro Hormone Activity of Classic Endocrine Disrupting Chemicals

1. PCR Array Gene Profiling

a) Estrogen Activity in Mammalian Cells
b) Expression of Endogenous Genes
c) Target Genes by Mechanism, Toxicity, etc
d) Agonist & Antagonist

Gene Categories on Breast Cancer-Estrogen PCR Array

Genes Directly Associated with Breast Cancer: CDKN1A ($p21^{Wap1/Cip1}$), CLDN7 (claudin-7), CLU (clusterin), ERBB2 (Her-2), FGF1, FLRT1 (fibronectin), GABRP (GABAa), GNAS, ID2, ITGA6, ITGB4 (ß4 integrin), KLF5 (GC Box BP), KRT19 (Keratin 19), MT3 (metallothionectin-III), MUC1 (mucin), PTGS2 (COX-2), RAC2 (p21Rac2), SCGB1D2 (lipophilin B), SCGB2A1 (mammaglobin 2), SPRR1B (Spr1), THBS1, THBS2, TNFAIP2 (B94).

Genes Associated with the Estrogen Receptor Signaling Pathway: AR, C3 (Complement component 3), CCND1, CTSD (cathepsin D), ESR1, ESR2, GATA3, HSPB1 (HSP28), KRT18, KRT19 (Keratin 19), PGR, SERPINA3 (a1-antichmotrypsin), SLC7A5, STC2, TFF1 (pS2).

Genes Associated with Breast Cancer Prognosis: BAD, BAG1, BCL2, CCNA1, CCNA2, CCND1, CCNE1, CDH1 (E-cadherin), CDKN1B (p27Kip1), CDKN2A (p16INK4a), COL6A1, CTNNB1 (b-catenin), CTSB (cathepsin B), EGFR, ERBB2 (Her-2), ESR1, ESR2, FAS (TNFRSF6), FASLG (TNFSF6), FOSL1 (FRA-1), GATA3, GSN (Gelsolin), IGFBP2, IL2RA, IL6, IL6R, IL6ST (glycoprotein 130), ITGA6 (a6 integrin), JUN, KLK5, KRT19, MAP2K7, MKI67 (Ki-67), NGFB (NGF), NGFR, NME1 (NM23A), PGR, PLAU (uPA), PTEN, SERPINB5 (maspin), SERPINE1 (PAI-1), TGFA, THBS1 (thrombospondin-1), TIE1(Tie-1), TOP2A (topoisomerase Iia), TP53, VEGFA.

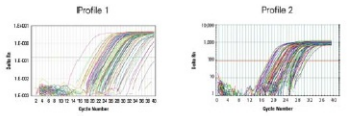
Genes Associated with the Response to Chemotherapy: BCL2BCL2, BCL2L2, CD44, CTSD (cathepsin D), CYP19A1, DLC1, ESR1, ESR2, HMGB1, KIT, NFYB, PAPPA, PGR, RPL27, VEGFA.

How PCR Arrays Work

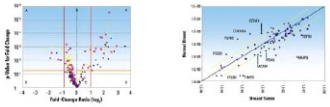
1. Convert Total RNA to cDNA.

2. Add cDNA to RT² qPCR Master Mix & Aliquot Mixture Across PCR Array.

3. Run in Your Real-Time PCR Instrument.

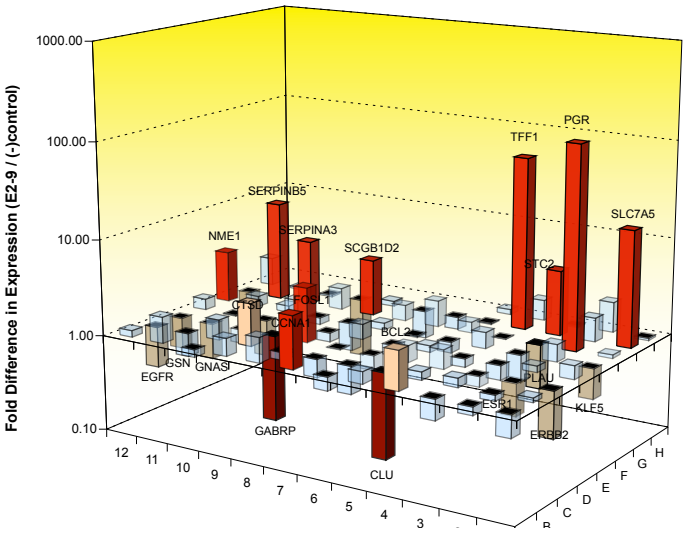


4. Data Analysis.

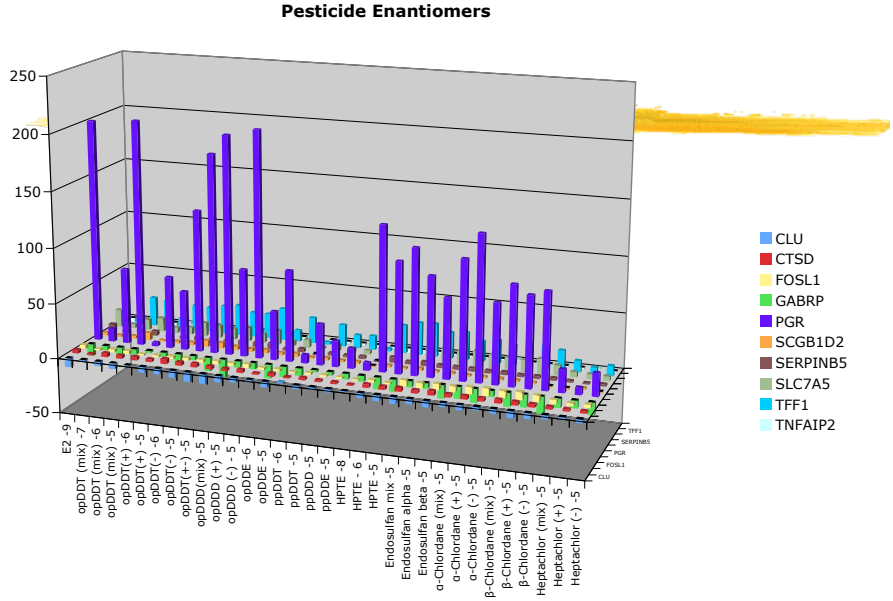


- **cDNA Synthesis (C-03 kit)**
- **Load Plates (Preferably with 8-Channel Pipettors)**
- **Run 40 cycle qPCR Program**
- **Upload and Analyze Data**

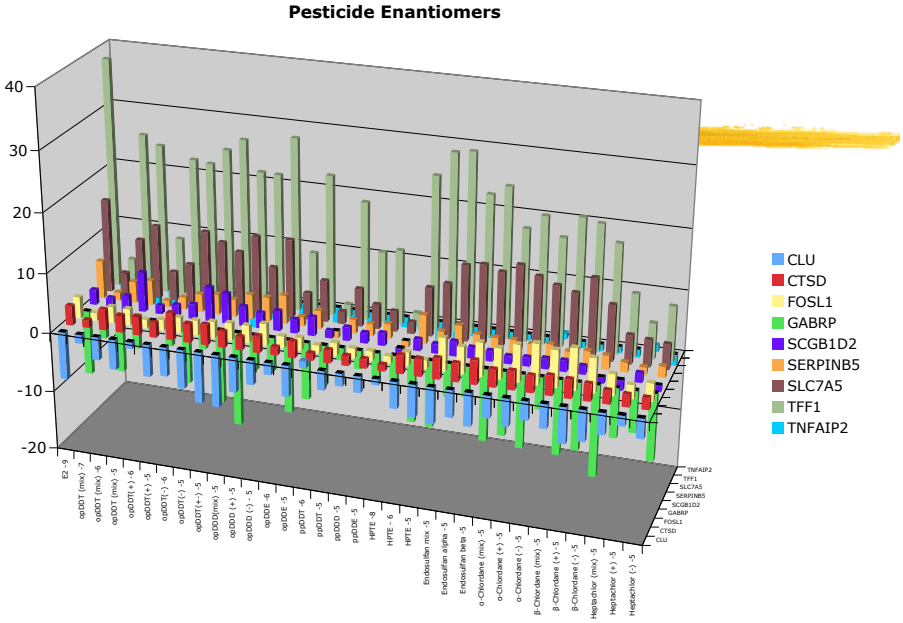
MCF-7 Cells: E_2 10^{-9} M vs Blank



Gene Fingerprints: Big Changes



Gene Fingerprints: Small Changes



Conclusions

1. **EDCs may act through multiple nuclear receptor mechanisms: ER, AR, PR**

2. **Characterizing EDC mechanism of action is more complex than running one bioassay.**

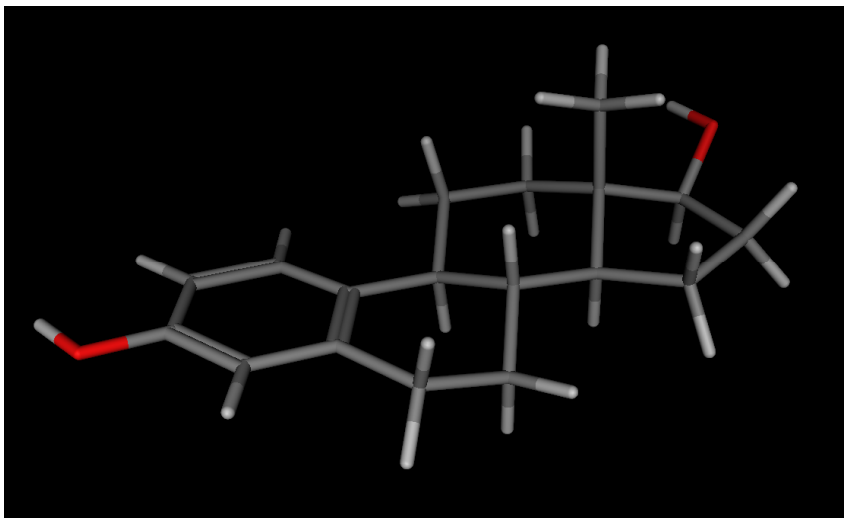
3. **Structure Activity Relationships Exist among EDCs**
   a) **Modeling can explain or predict at least some activity**

How can these compounds bind the estrogen receptor?

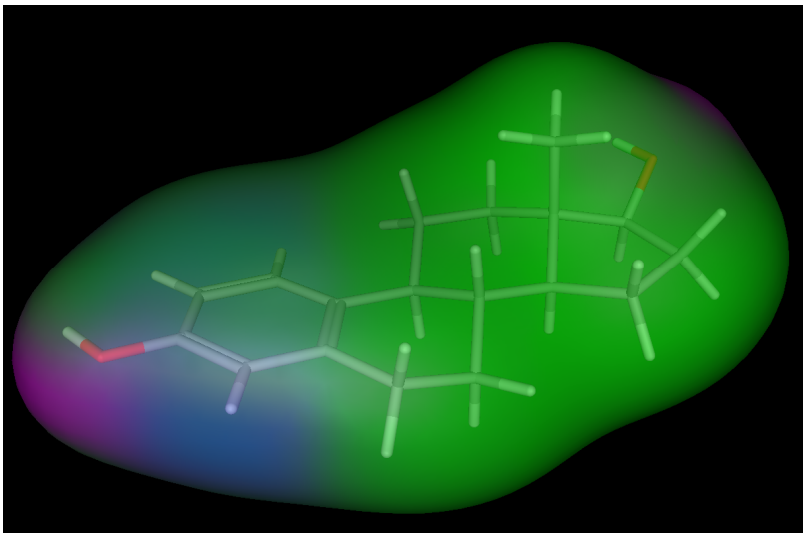
1. **Compare ligands to Estradiol**
   a) Superimpose Ligands (No Receptor Involved)

2. **Use Molecular Modeling: Ligand-Receptor Models**
   a) Obtain crystal structure of Estrogen Receptor Ligand Binding Domain
   b) Make molecular models of EDCs
   c) Use Docking methods to simulate ligand-receptor interactions

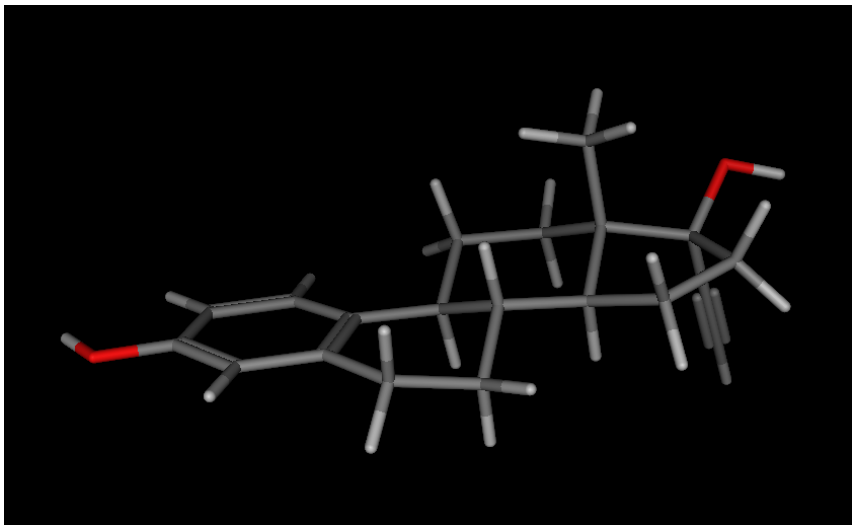
Estradiol



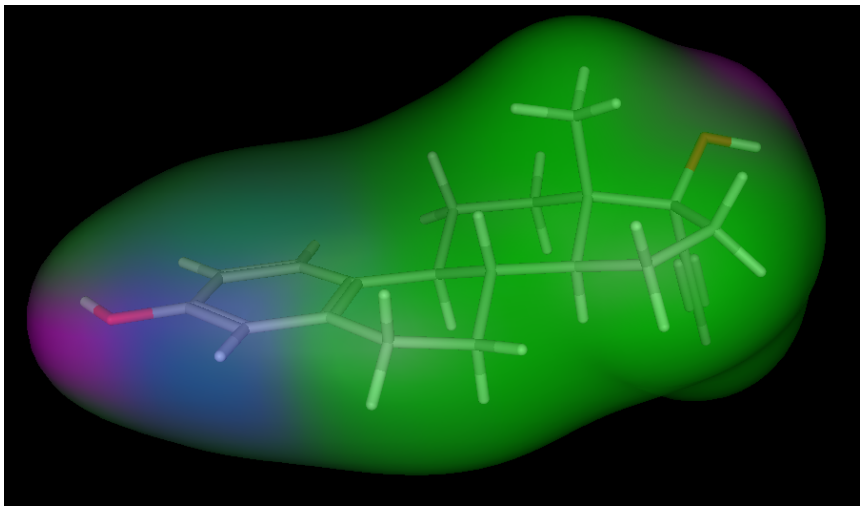
Estradiol



Ethinyl estradiol



Ethinyl estradiol



Non-steroid Estrogens

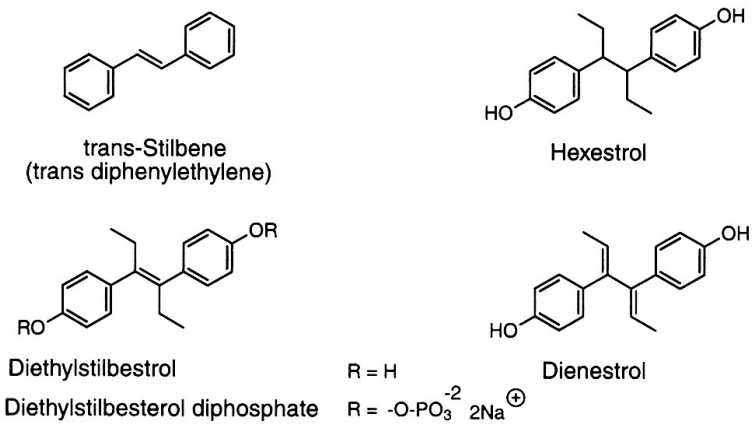
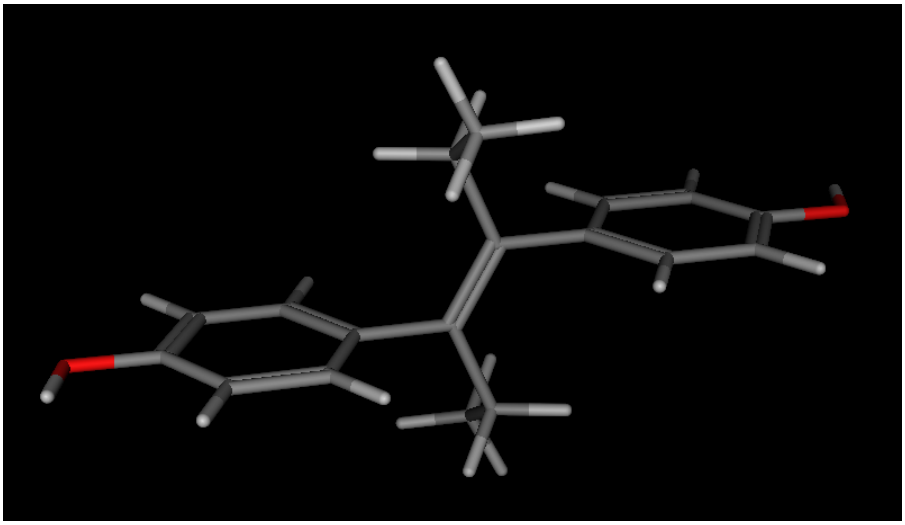
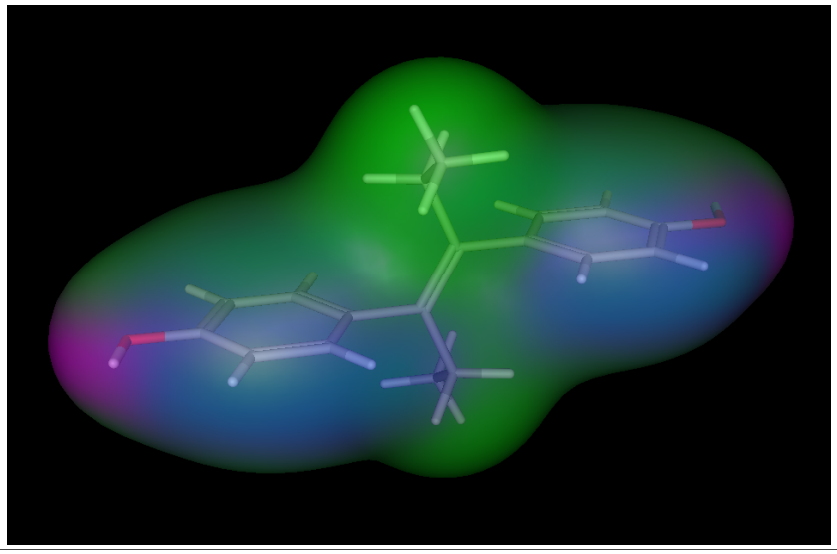


Fig. 29.8. Nonsteroidal estrogens.

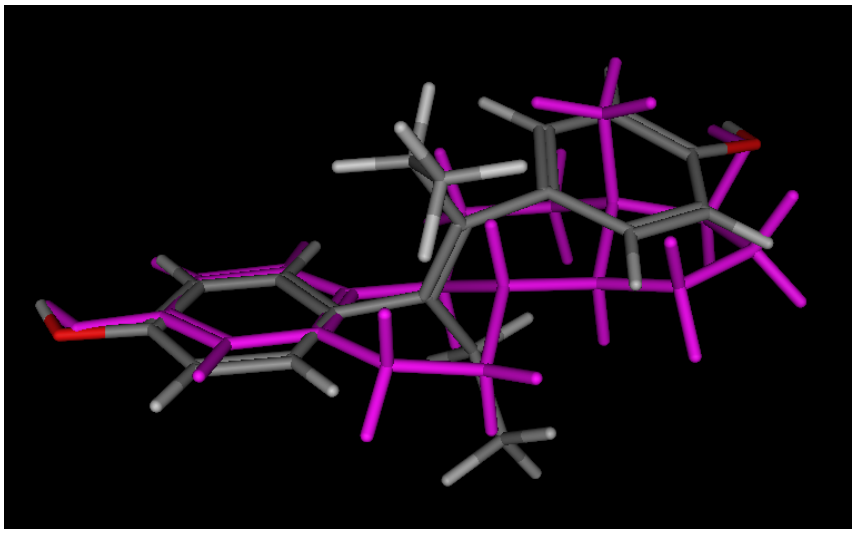
Diethylstilbestrol (DES)



Diethylstilbestrol (DES)



E2 and Diethylstilbestrol (DES)



Environmental & Xenoestrogens

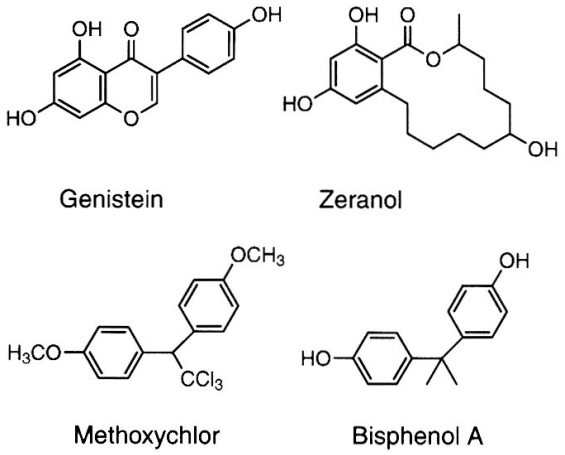
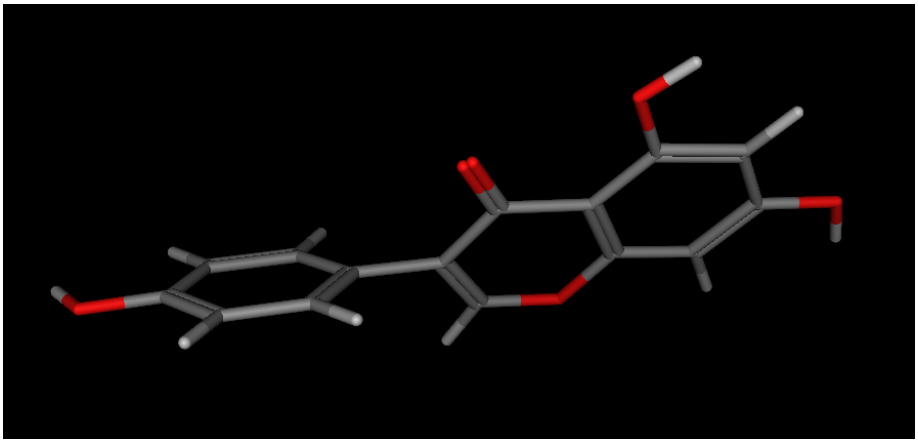
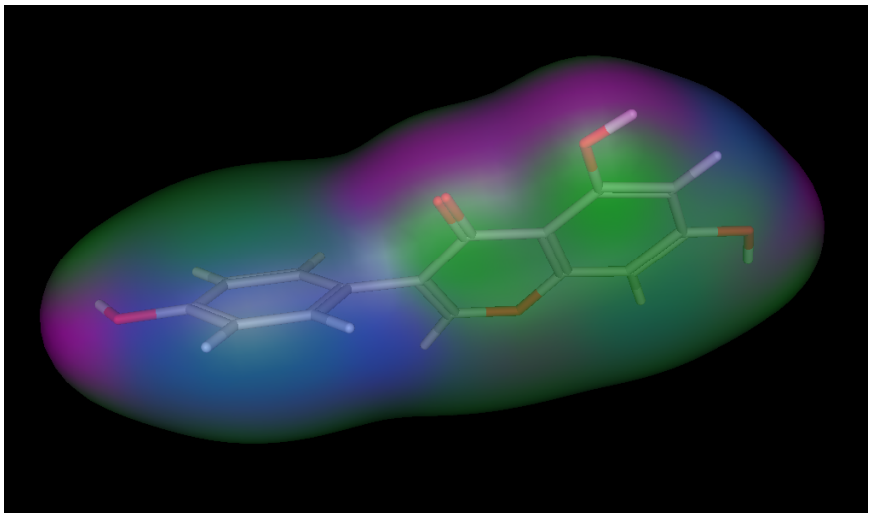


Fig. 29.9. Xenoestrogens.

Genistein

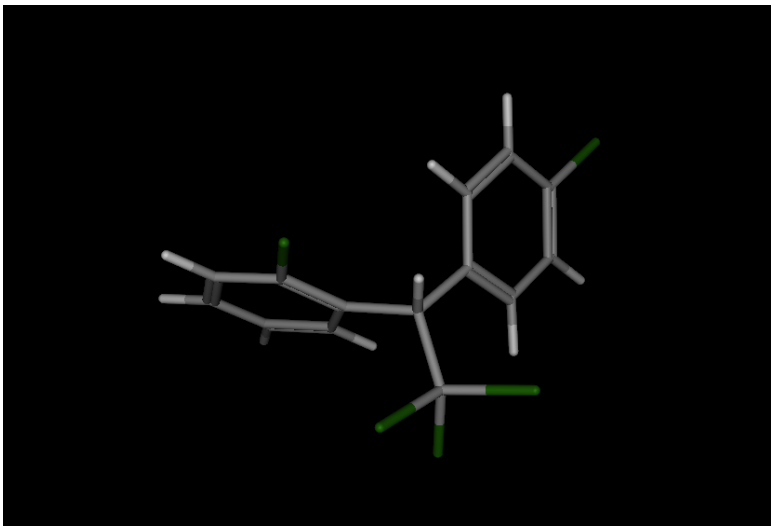


Genistein



E2 and Genistein

opDDT



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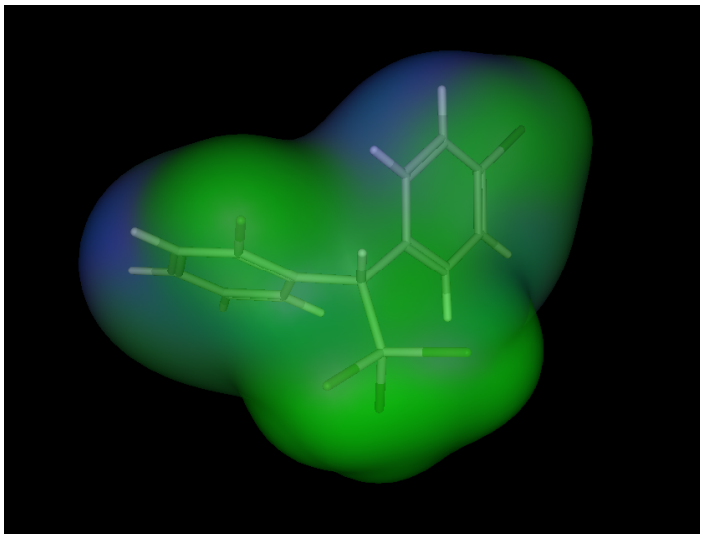
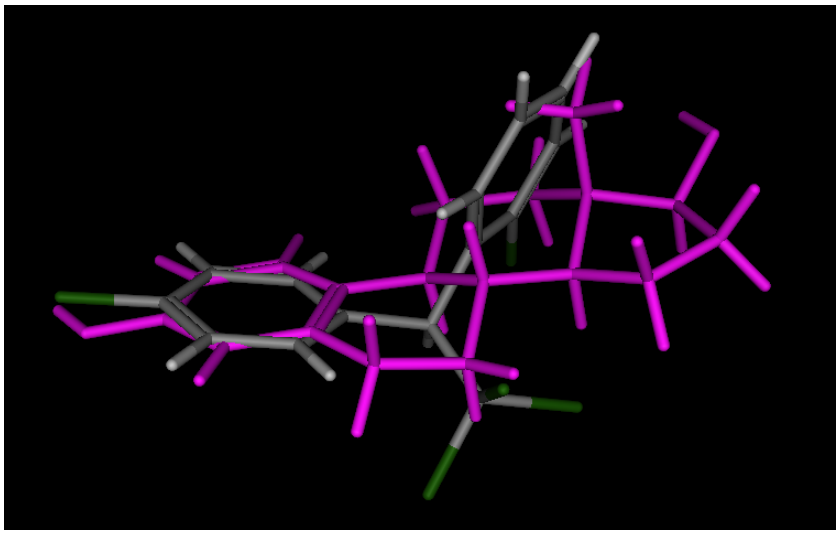
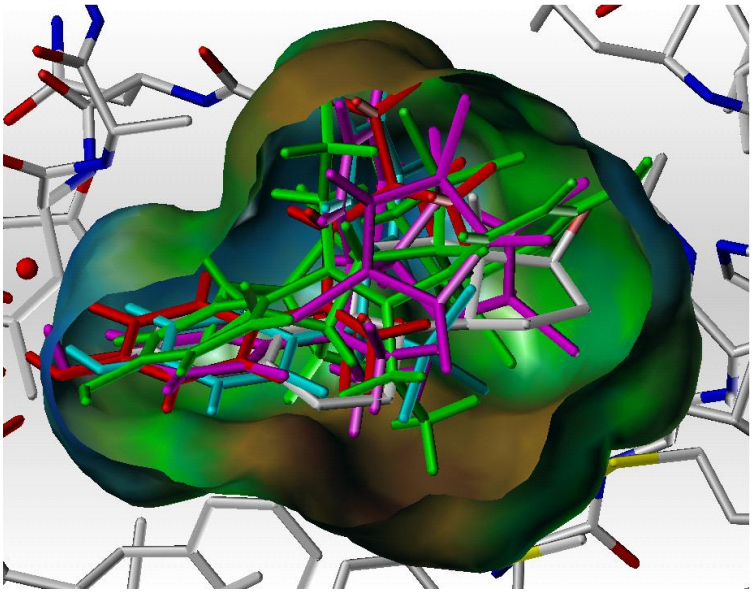
opDDT



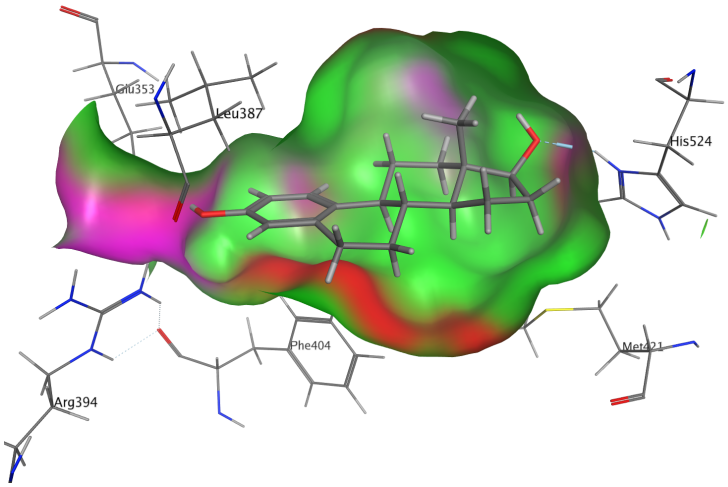
E2 and opDDT



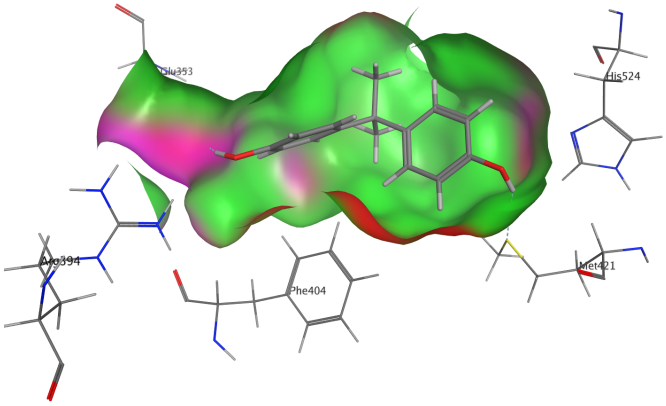
Xenobiotic Binding to Receptors Docked to ERα (1ERE)



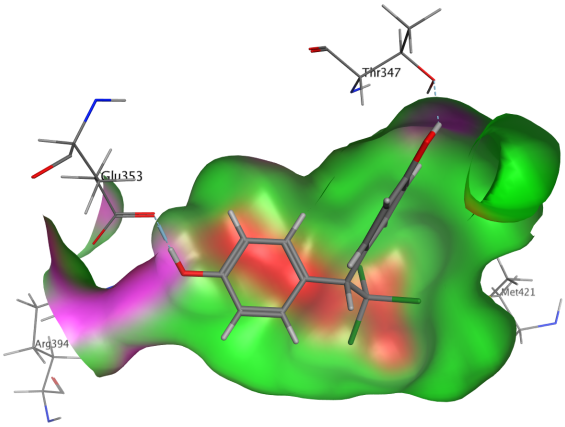
Estradiol Bound to ER



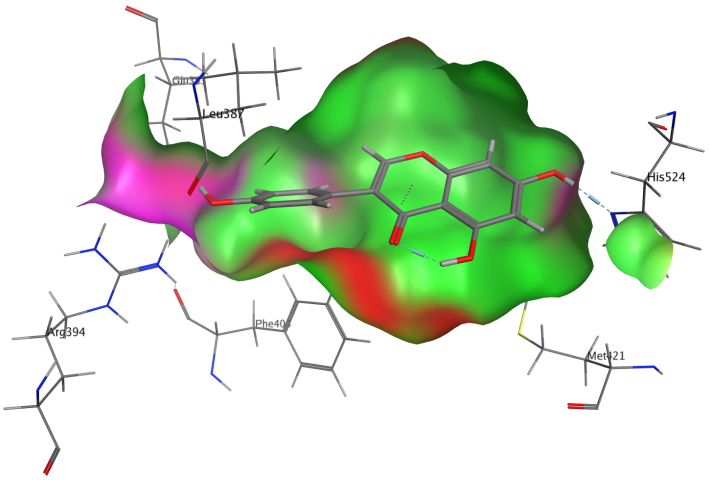
Bisphenol A Bound to ER



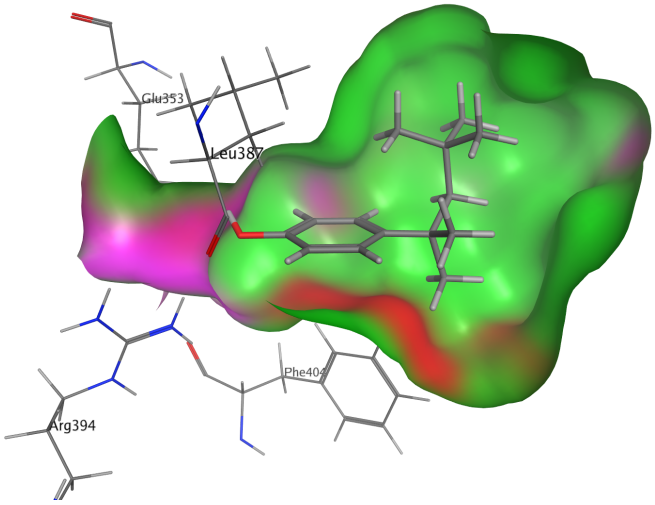
HPTE Bound to ER



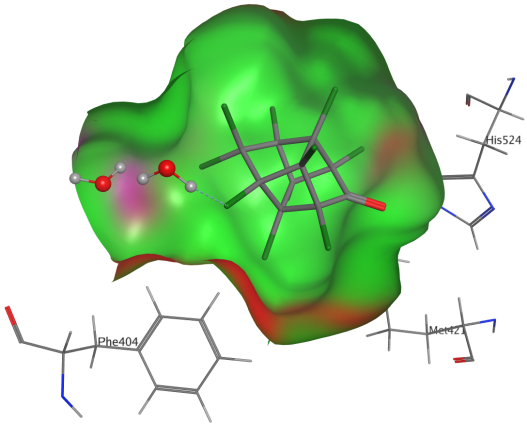
Genistein Bound to ER



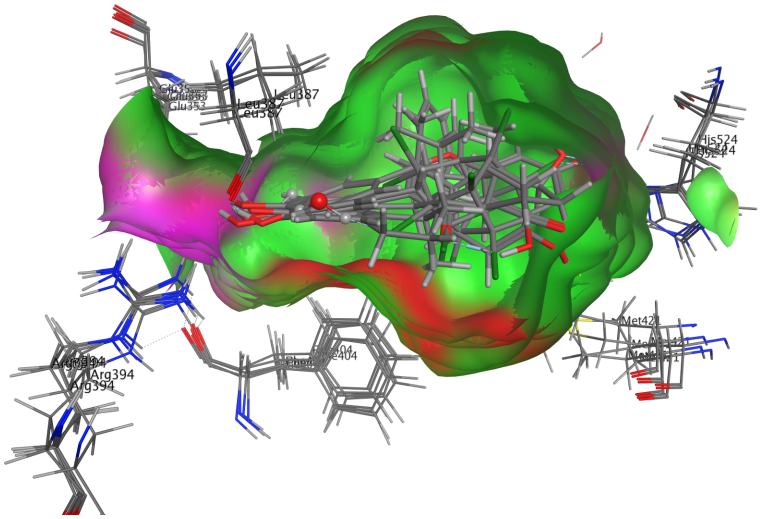
4-P-Octylphenol Bound to ER



Kepone Bound to ER



EDCs Bound to ER



How can these compounds bind the estrogen receptor?

1. **The ECDs can fit into the receptor binding pocket.**

2. **EDCs may use slightly different Binding Modes when bound to ER.**

3. **The Estrogen Receptor Binding Pocket has considerable capacity for a wide range of chemical structures.**

The Estrogen Activity of Bisphenol A Analogues

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Introduction

Bisphenol A

1. Component of some polymers: polycarbonate, lexan, etc. and can leach into water and food.
2. An estrogen *in vitro* and *in vivo*
3. Designed to be an estrogen in 1930s (used in plastics later…)
   a) 1. Cook JW, Dodds EC, Hewett CL, Lawson W (1933) The oestrogenic activity of some condensed-ring compounds in relation to their other biological activities. *Proc Roy Soc B* Vol. 114, pp. 272-286
      2. Cook JW, Dodds EC (1933) Sex Hormones and Cancer-Producing Compounds. *Nature* Vol. 131, pp. 205
4. Contamination is problematic in laboratory studies
5. Shown to be related a number of human health issues: hormone activity, diabetes, cardiovascular disease, development, etc.
6. Bisphenol A is one of a number of Bisphenols used in polymers

Hypothesis and Strategy

Hypothesis

1. General estrogen activity can be characterized by:
   a) ER binding, MCF-7 cell proliferation, MVLN reporter gene assay, PCR Arrays.
2. Some bisphenols may have more hormone activity than others
3. Estrogen activity of bisphenols will relate to structure features allowing interactions with the estrogen receptor.

Strategy

1. Obtain commercially available bisphenols (24 including BisA)
2. Evaluate each with ER binding, MCF-7 cell proliferation, MVLN reporter gene assay, PCR Arrays.
3. Examine potential receptor binding interactions using molecular modeling

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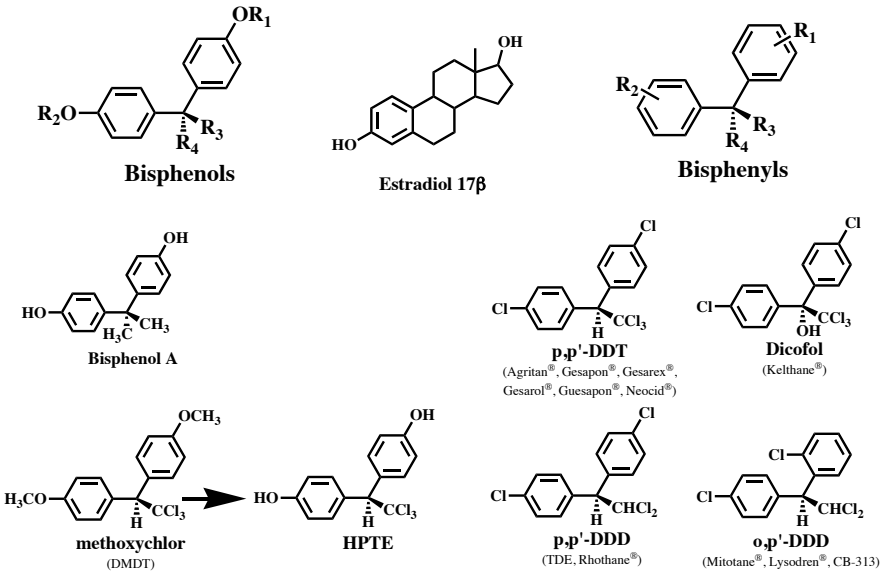
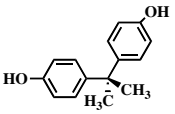
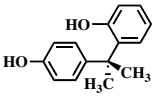
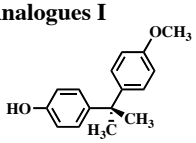
Bisphenols and Estradiol

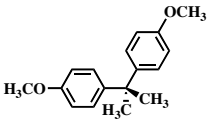


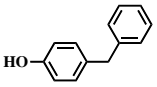
Bisphenol A Analogues I

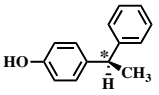

80-05-7 (Bis A)

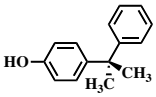

o,p'-Bis A (837-08-1)


Mono Mxy-Bis A


Mxy-Bis A


MH-Bis F (PPP)
620-92-8


MH-MM1 (1988-89-2)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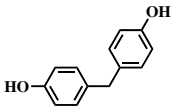
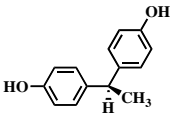
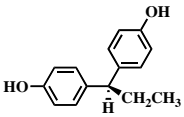
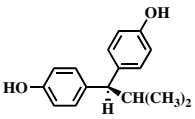
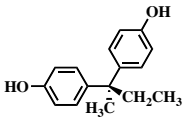
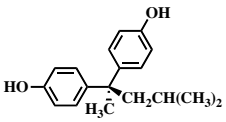

620-92-8 (Bis F)

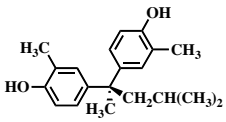

MM1


1576-13-2 (MM2)


1844-00-4

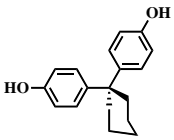

77-40-7 (Bis B, MM3)


6807-17-6 (DMB Bis A)


50628-55-2 (DM DMB Bis A)


MM4

Bisphenol A Analogues II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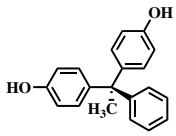
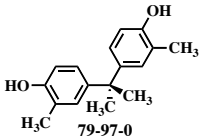
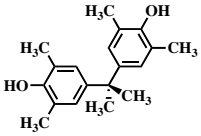

1571-75-1 (P Bis A)


79-97-0
(MM6, DM Bis A)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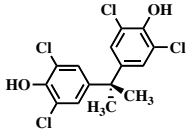
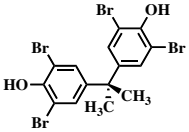

79-95-8 (TC Bis A)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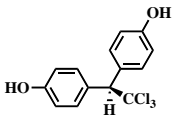
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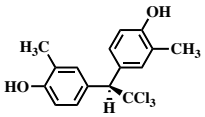
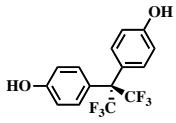

DM HPTE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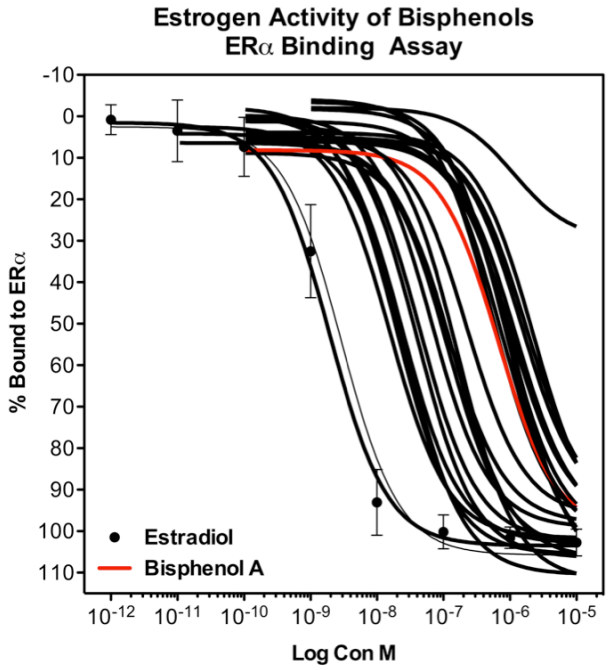
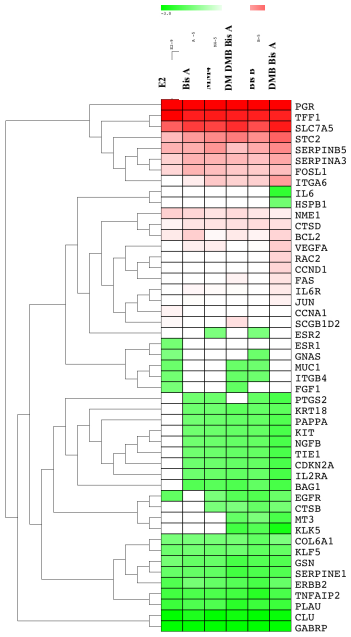
(MM7, HF Bis A)

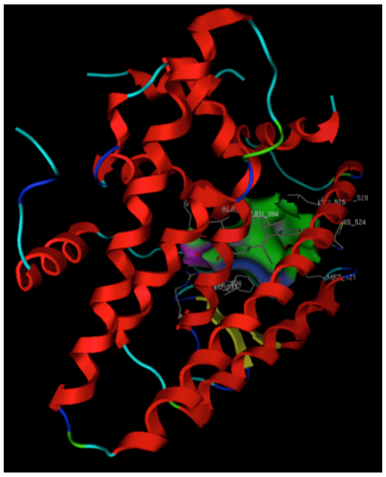


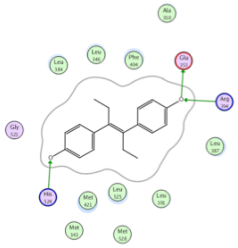
PCR Array of Estrogenic Bis-As

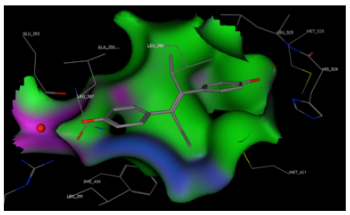


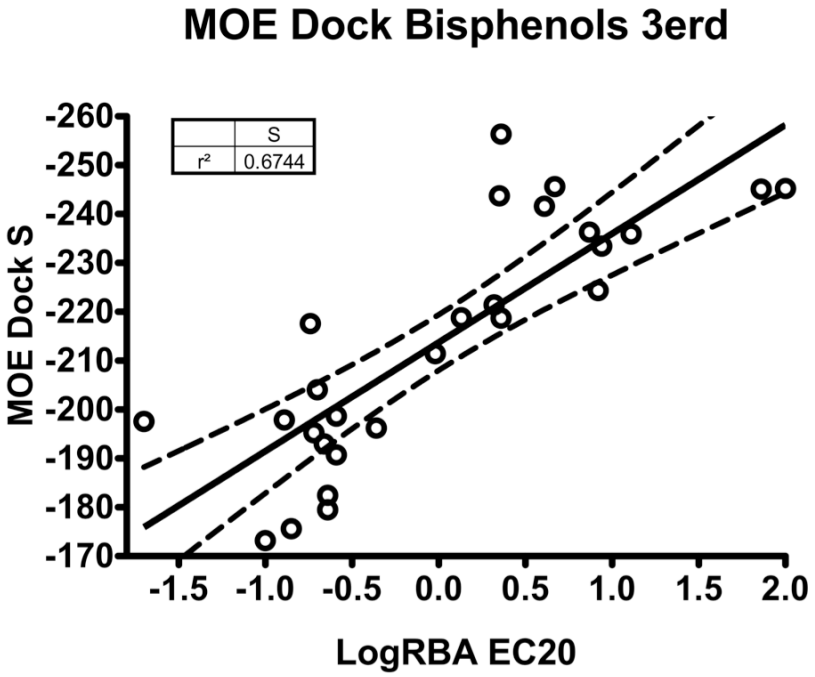
Ligand-Receptor Docking Methods for Bis As

1. ER alpha LBD w/ DES: 3ERD
2. Chemical Computing Group MOE Dock
   - Alpha PMI dock, Alpha HB score, FF opt, Alpha HB score, etc.

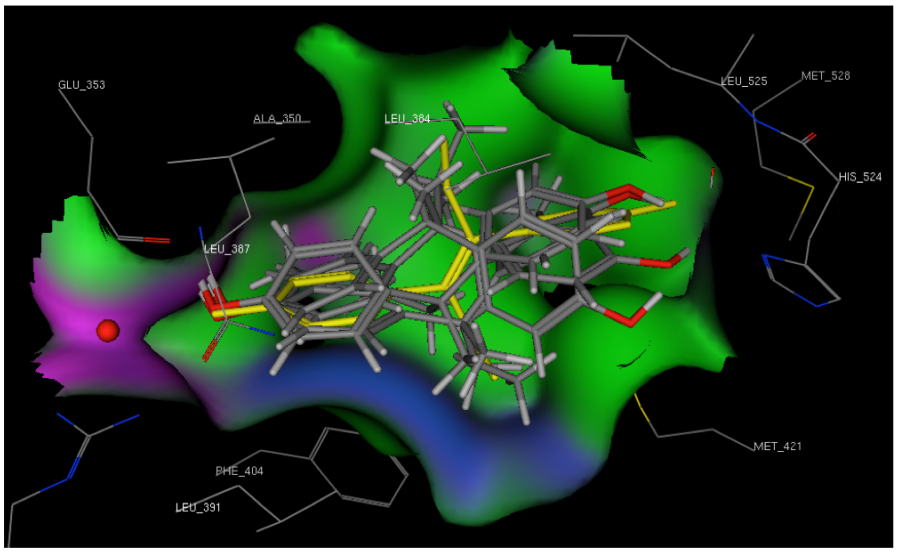








BisAs Docked to 3ERD



Conclusions

1. Some bisphenols are more estrogenic than BisA.
2. Docking can predict ER binding of Bisphenols
3. Bisphenols and E2 Regulate Different Genes in MCF-7 Cells.

Overall Conclusions

1. **Steroid Hormones Bind Receptors**
   a) ER, AR, PR, GR, etc.
   b) Regulate Genes for physiological response.
2. **Small Changes in Steroid Structure (Shape):**
   a) determine Receptor Specificity.
   b) determine Agonist vs Antagonist Activity.
3. **Xenobiotic Chemicals:**
   a) have shapes "similar" to steroid Agonists or Antagonists.
   b) may bind Nuclear Receptors.
   c) may have Physiological Effects: Endocrine Disruption.
