Design, Synthesis and Biological Screening of Focused Libraries of New Antiestrogens

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Prof. Dr. Peter Wipf's group

Dr. Billy Day's group

Outline

- *Pharmacology side of the story …*

a) History of antiestrogens in breast cancer

b) ER, coregulators and tamoxifen mixed pharmacology

c) Discovery of the lead antiestrogen CK1-183

- *Synthetic side of the story…*

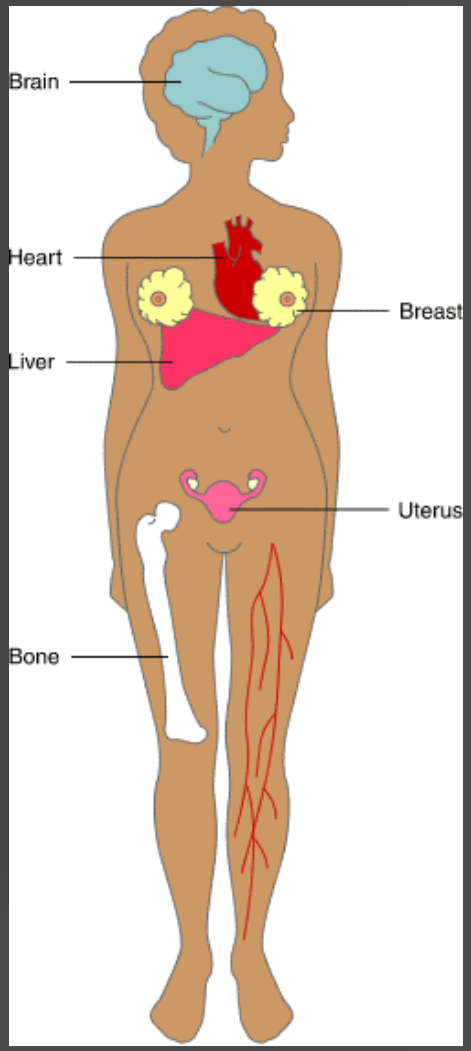
a) Organochlorozirconocene chemistry

b) Focused library synthesis based on the lead compound

c) Using microwave in library synthesis

History behind antiestrogens

- **1896** George Beatson - some premenopausal women with inoperable breast cancer could benefit from removal of their ovaries (**oophorectomy**).
- Stanley Boyd - first “clinical trial overview” of the effect of oophorectomy to treat breast cancer in premenopausal women - 30% (54 women treated) had positive response
- Allen and Doisy in **1923** discovered “**estrogenic principle**” in the follicular fluid of pig ovaries ;
- Doisy crystalized in 1929 first steroid hormone estrone.
- Sir Charles Dodds tested first non-steroidal estrogen diethylstilbestrol (**DES**) in **1930s**.
- Jansen discovered Estrogen Receptor (ERα) in late **1950s.**



Endocrine therapy of breast cancer

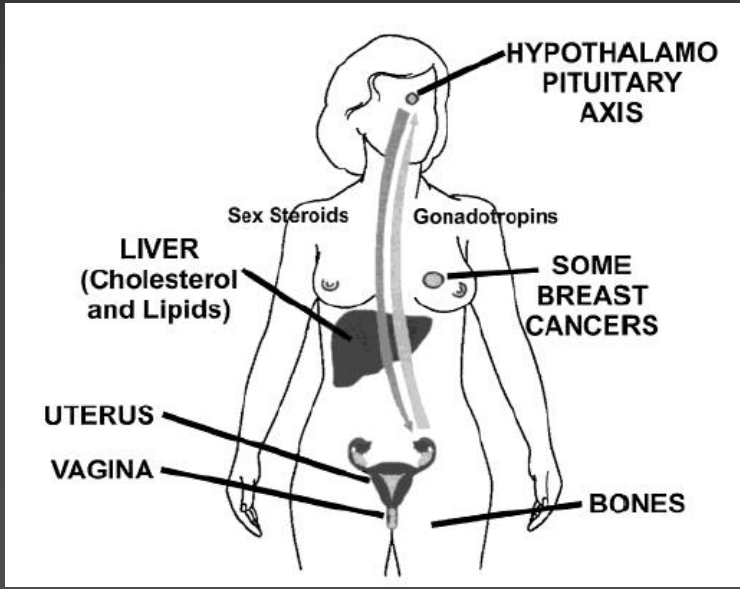
– disruption of estrogen-estrogen receptor (ER) axis by:

1. Inhibition of function of estrogen producing organ (*ablative therapies*) and/or inhibition of estrogen production (*aromatase inhibitors*)
2. Blockade or perturbation of the estrogen-ER interaction (*additive therapies*)

Ref. 2

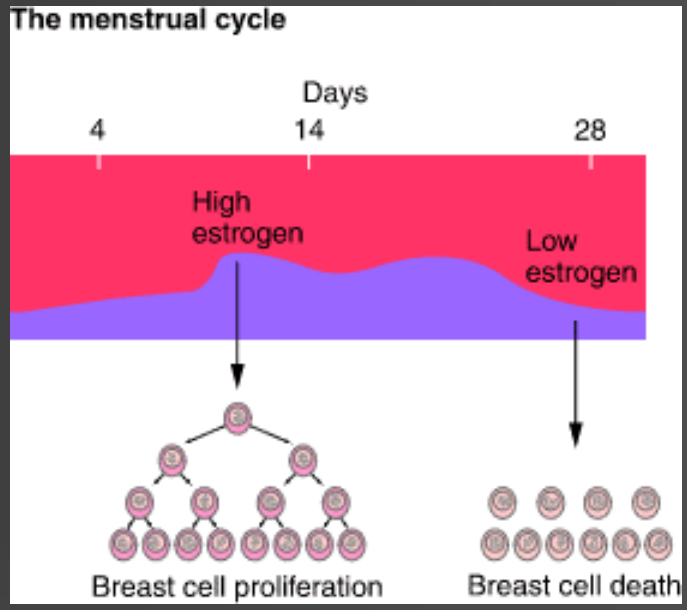
What do estrogens do?

- Estrogen receptors (ERs) in the hypothalamo-pituitary axis regulate the release of gonadotropins by both positive and negative feedback mechanisms.
- The gonadotropins, in turn, control the ovarian synthesis of estrogens and progestins that are essential for maintaining the menstrual cycle and for reproduction.



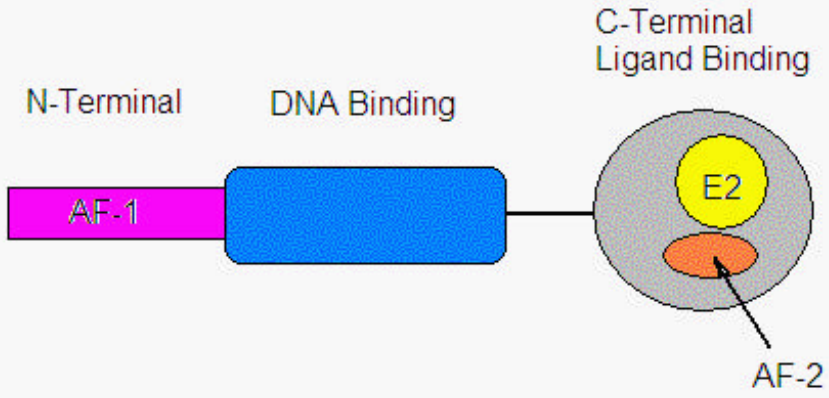
V.C. Jordan, JMedChem 2003

1. Estrogens cause proliferation through the ER in:
   - uterine and vaginal epithelium
   - breast cancer
2. The ERs located in liver and bone cells regulate the circulating levels of cholesterol and lipids and bone density.



- *http://press2.nci.nih.gov/sciencebehind/estrogen/estrogen01.htm*

Structural organization of ERs



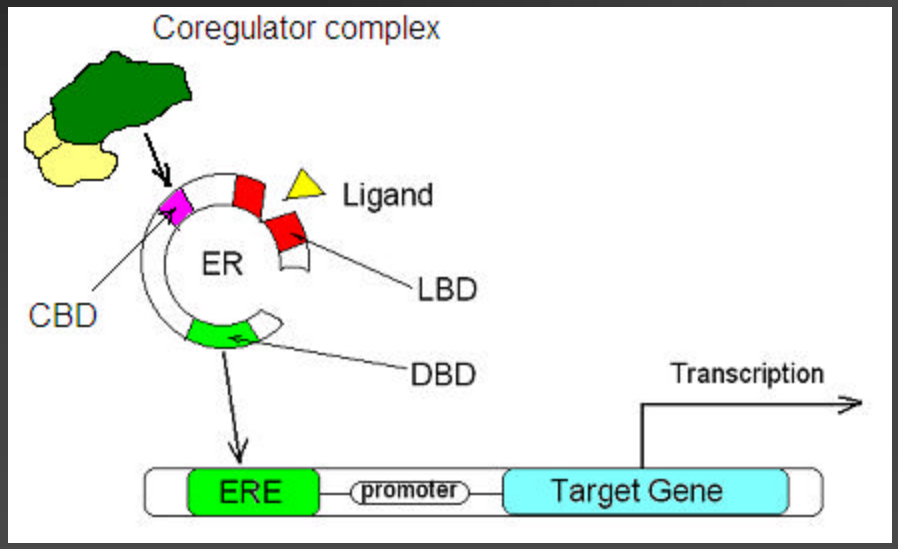
AF-1 = activation function, constitutional and is regulated by MAP kinases

AF-2 = activation function, ligand dependent, a patch on LBD surface

"SERMs: Research and Clinical Applications", A. Manni, M.F. Verderame, 2002

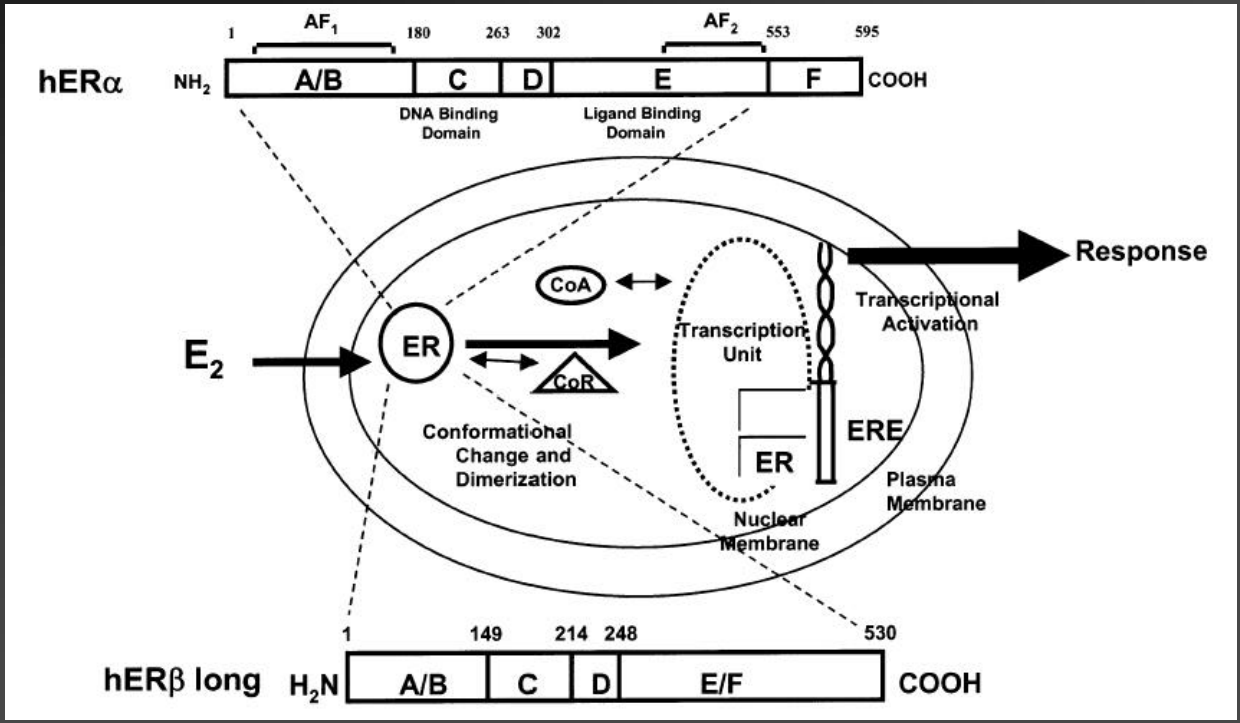
Estrogen Receptors (ERα and ERβ)

- Estrogen receptors are nuclear receptors
- Ligand dependent transcriptional factors regulating different genes



LBD = ligand binding domain, DBD = DNA binding domain, CBD = coregulator binding domain, ERE = estrogen response elements

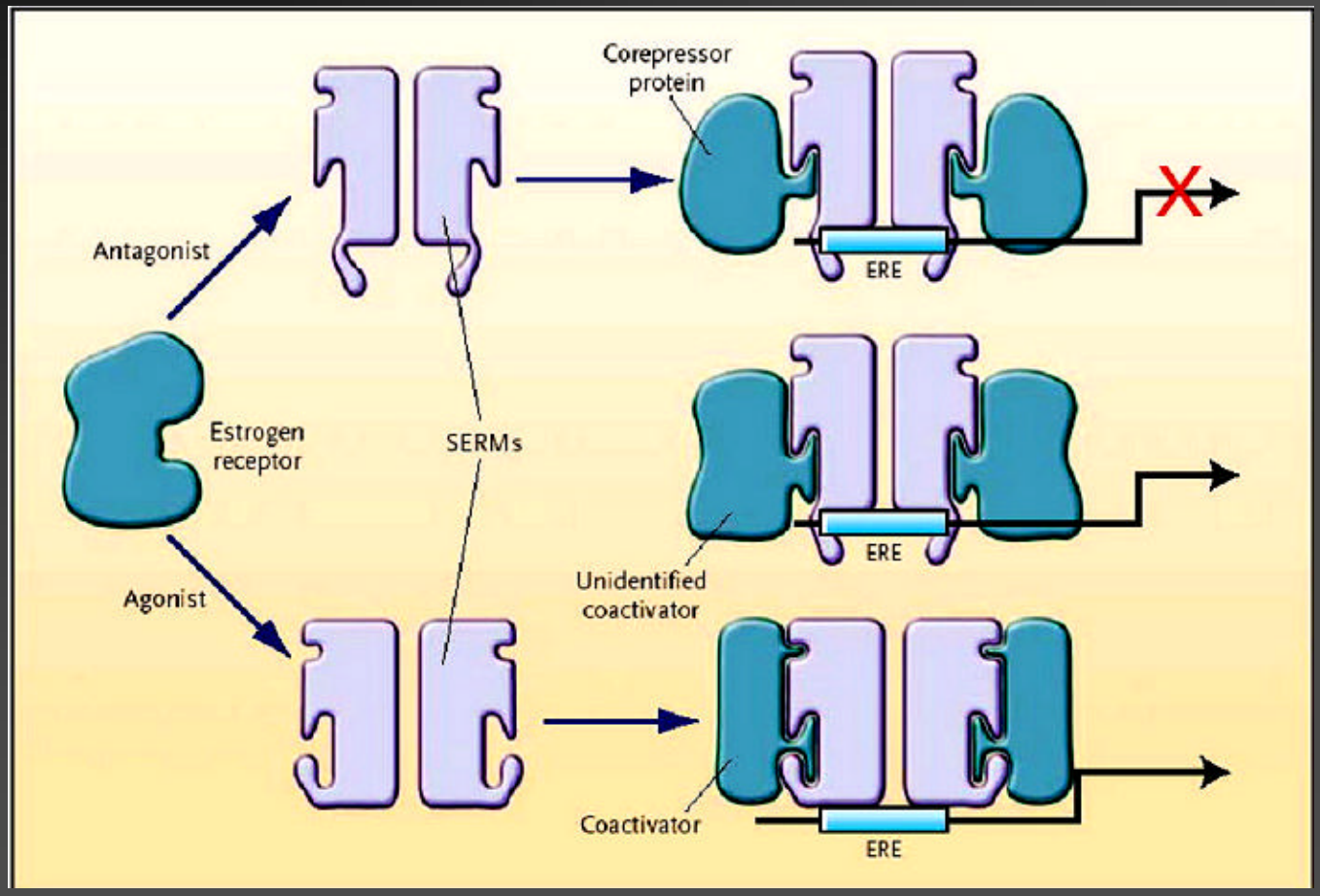
Mechanism of action of estrogen receptors



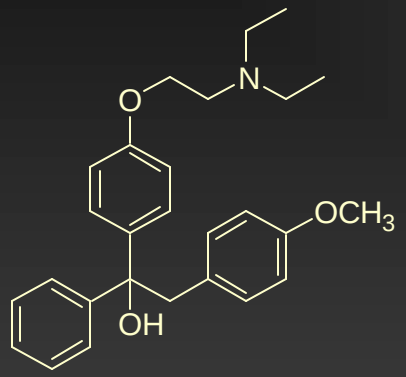
- ER induces transcription through classical ERE binding directly to DNA or thetering to other transcriptional factors and bind to DNA indirectly!

"Hormone Therapy in Breast and Prostate Cancer"
edited by V. Craig Jordan and Barrington J.A. Furr, 2002

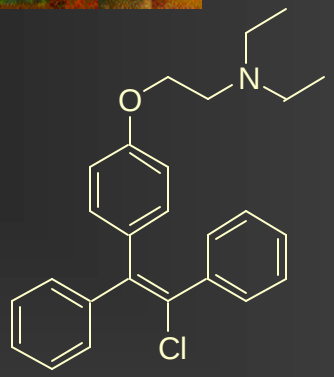
Estrogen receptor ligands fall into three groups: antagonists, agonists and SERMs



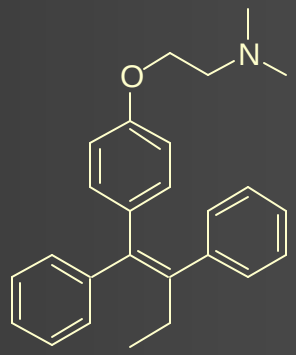
First non-steroidal “hormones”



MER 25



MRL 41
CLOMIFENE



TAMOXIFEN

MER25 - The first nonsteroidal antiestrogen, never developed clinically because of high toxicity and low potency.

Triphenylethylene compounds:

MRL-41, or Clomiphene (developed in 1961)- standard therapy for infertility – induction of ovulation.

Tamoxifen - endocrine treatment of choice for the treatment of breast cancer.

J.I. Maccgregor, V.C. Jordan, Pharm. Rev. 1998, 151-197

What are SERMs?

- The idea of developing selective estrogen receptor modulators (SERMs) arose after discovery that Tamoxifen has mixed effects: it is antiestrogenic in the breast but estrogenic in the uterus and bone.

- PERFECT SERM:

  Estrogens have positive effect on overall health: prevention of osteoporosis and reduced cardiovascular disease. Perfect SERM should combine these effects with selective antiestrogenic action in breast cancer tissues and uterus.

Classes of SERMs

General classes of SERMs base on their chemical and pharmacological properties:

1. High dose estrogens (non steroidal -DES)
2. Triphenylethylene estrogens analogues of tamoxifene (toremifene, droloxifene, iodoxifene, GW5638)
3. Fixed -ring compounds (raloxifene, arzoxifene, EM-800, ERA-923.)
4. Pure antiestrogens (fulvestrant (ICI 182780), SR16234, ZK191703)

The key questions?

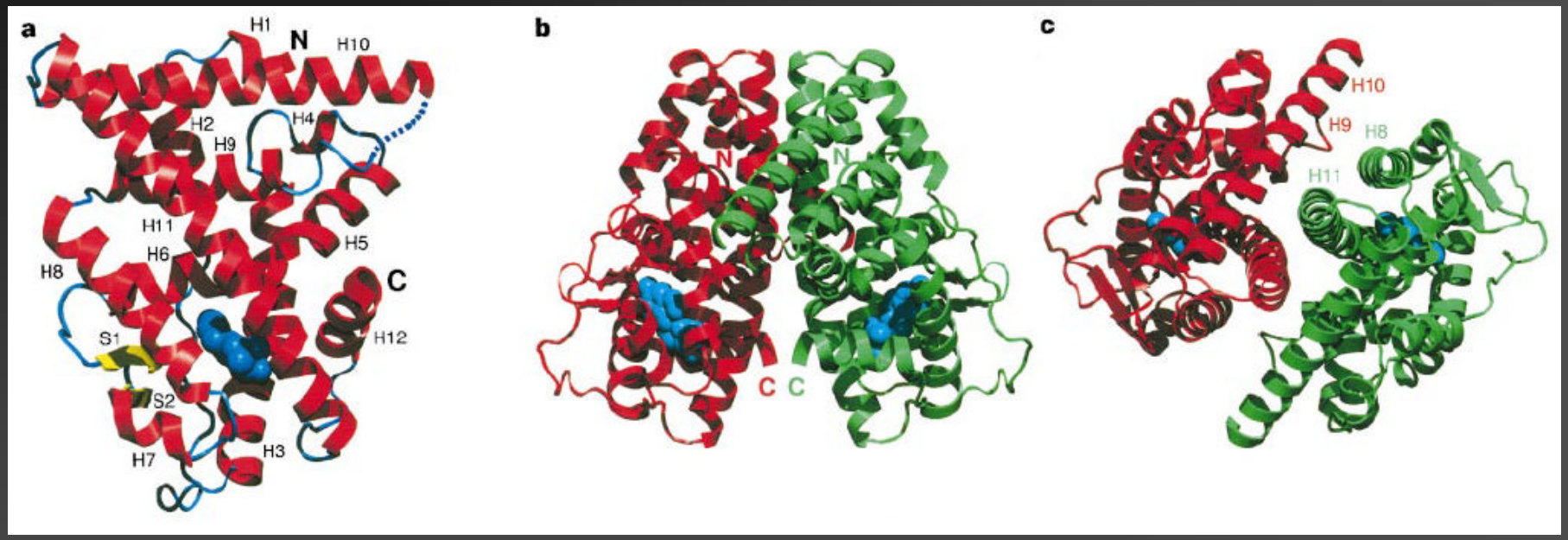
- What is behind tissue selectivity of SERMs?
- Can we design a drug that will be breast cancer tissue selective?

Looking into:

- LBD structure and conformational changes
- Co-regulator binding

Control coregulator binding – control tissue selectivity

ER at the molecular level – looking inside!



LBD

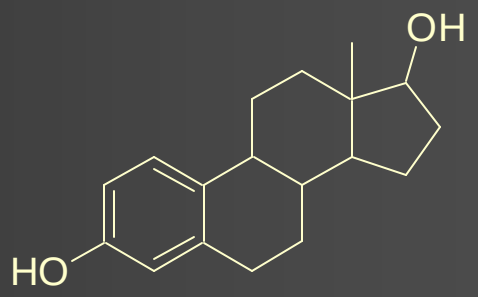
homodimer

Brzozowski et al, Nature 1997, 389, 753

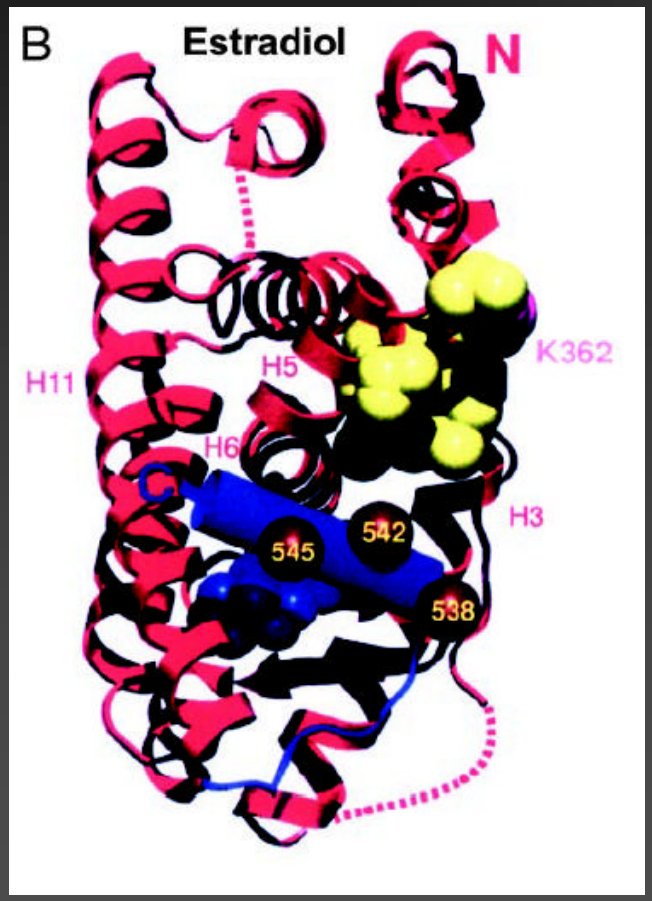
LBD – Helix 12

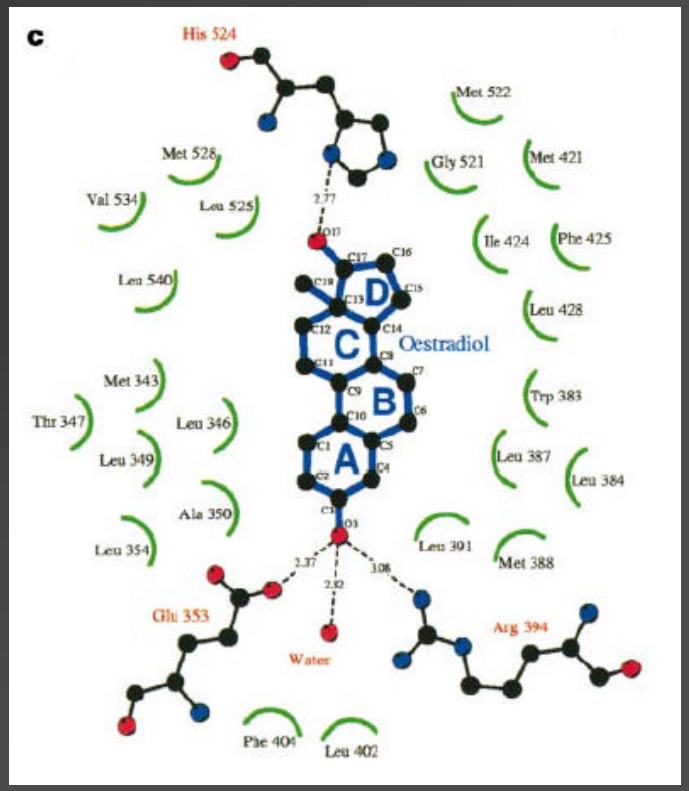
- located in the LBD of the ER
- composition and orientation of helix 12 differs depending on the ligand bound to the ER
- When the ER LBD is complexed with the ER agonists estrogen (E_2) or diethylstilbestrol (DES), helix 12 is positioned over the ligand binding pocket. This proper positioning generates AF2 and forms a surface for the recruitment of coactivators.
- different ligands induce different receptor conformations, and the positioning of helix 12 is the key event that permits discrimination between ER agonists and antagonists by influencing the interaction of the ER with coregulators.

Ligand binding – agonists



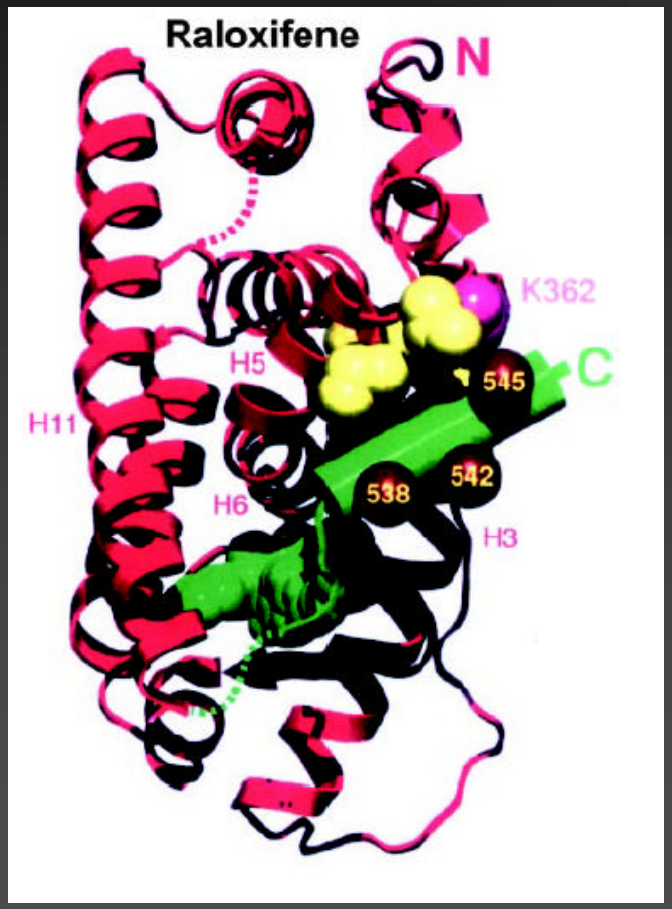
17β-Estradiol

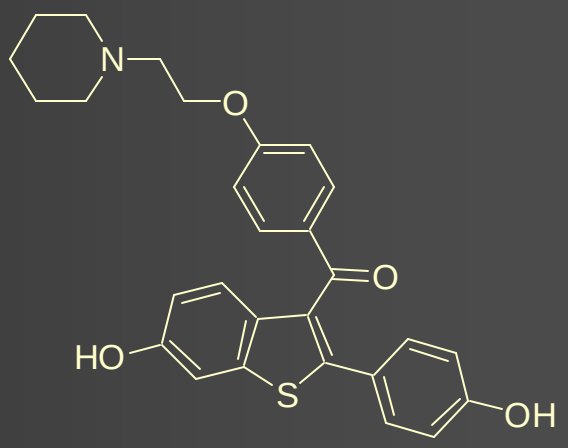




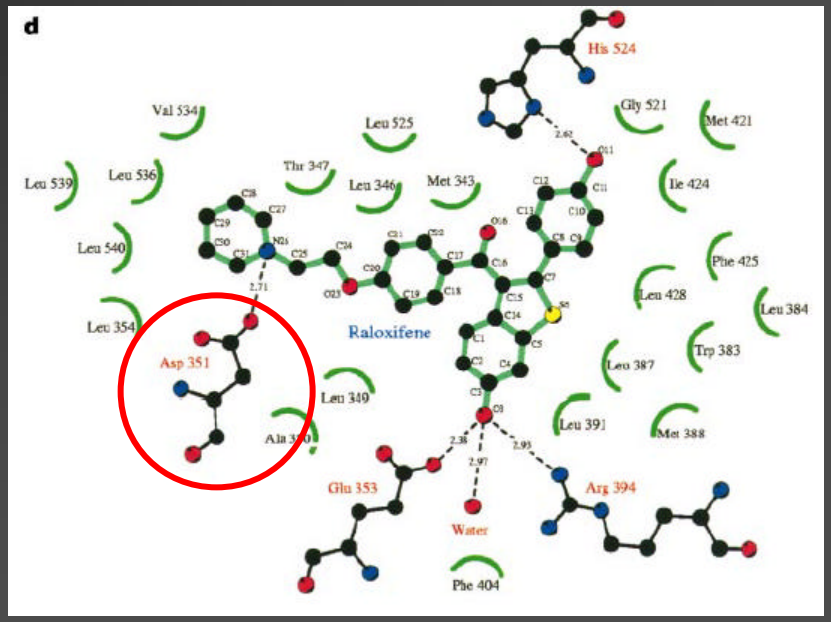
Brzozowski et al, Nature 1997, 389, 753

Ligand binding – antagonists



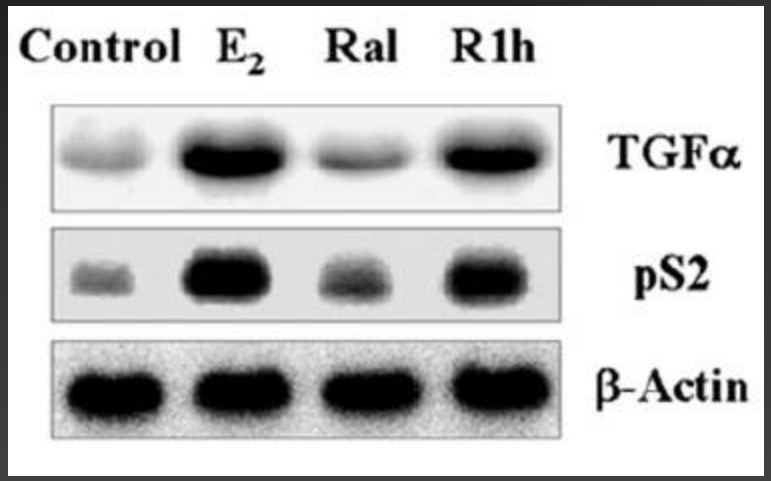


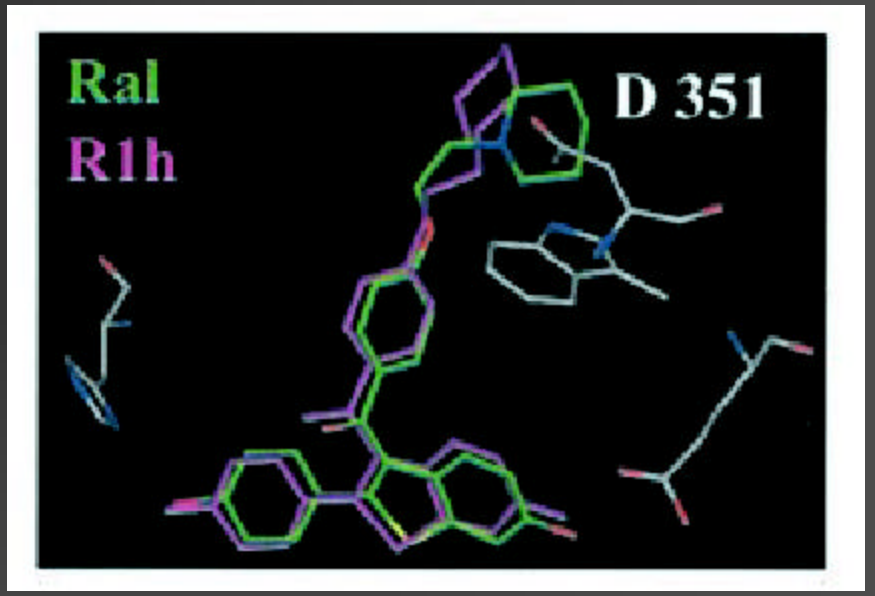
Raloxifene

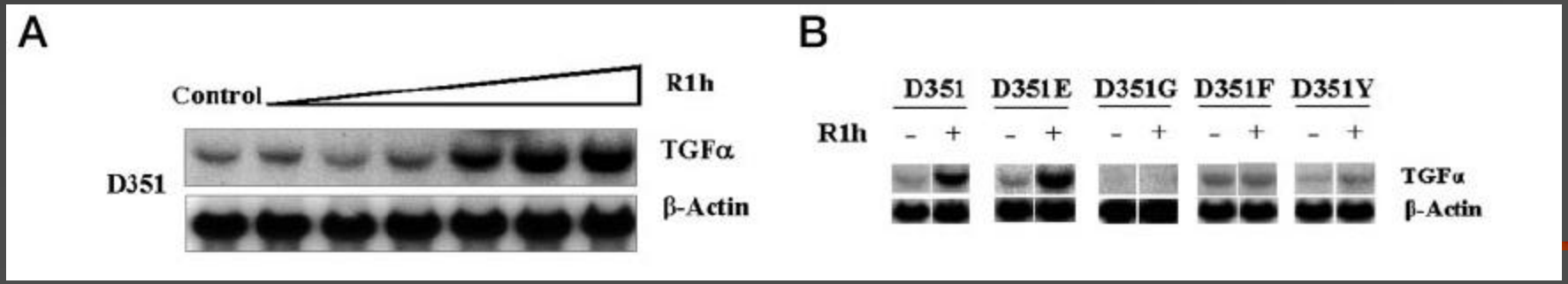


Brzozowski et al, Nature 1997, 389, 753

SAR of an antagonist - Raloxofene

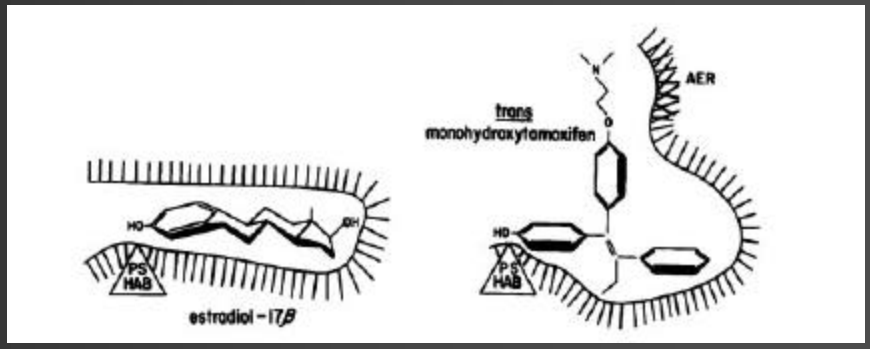


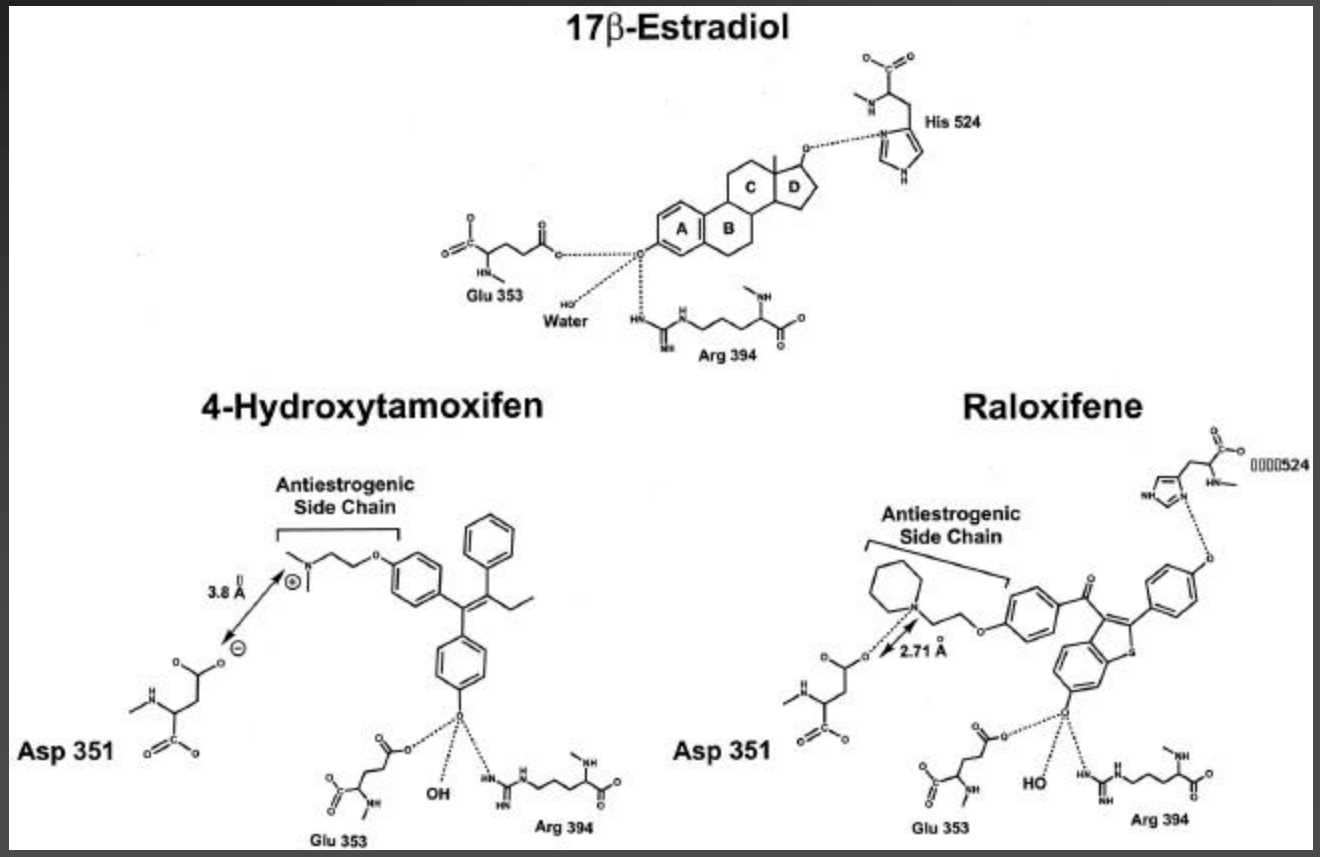




Liu et al, JBiolChem, 2002, 277,9189

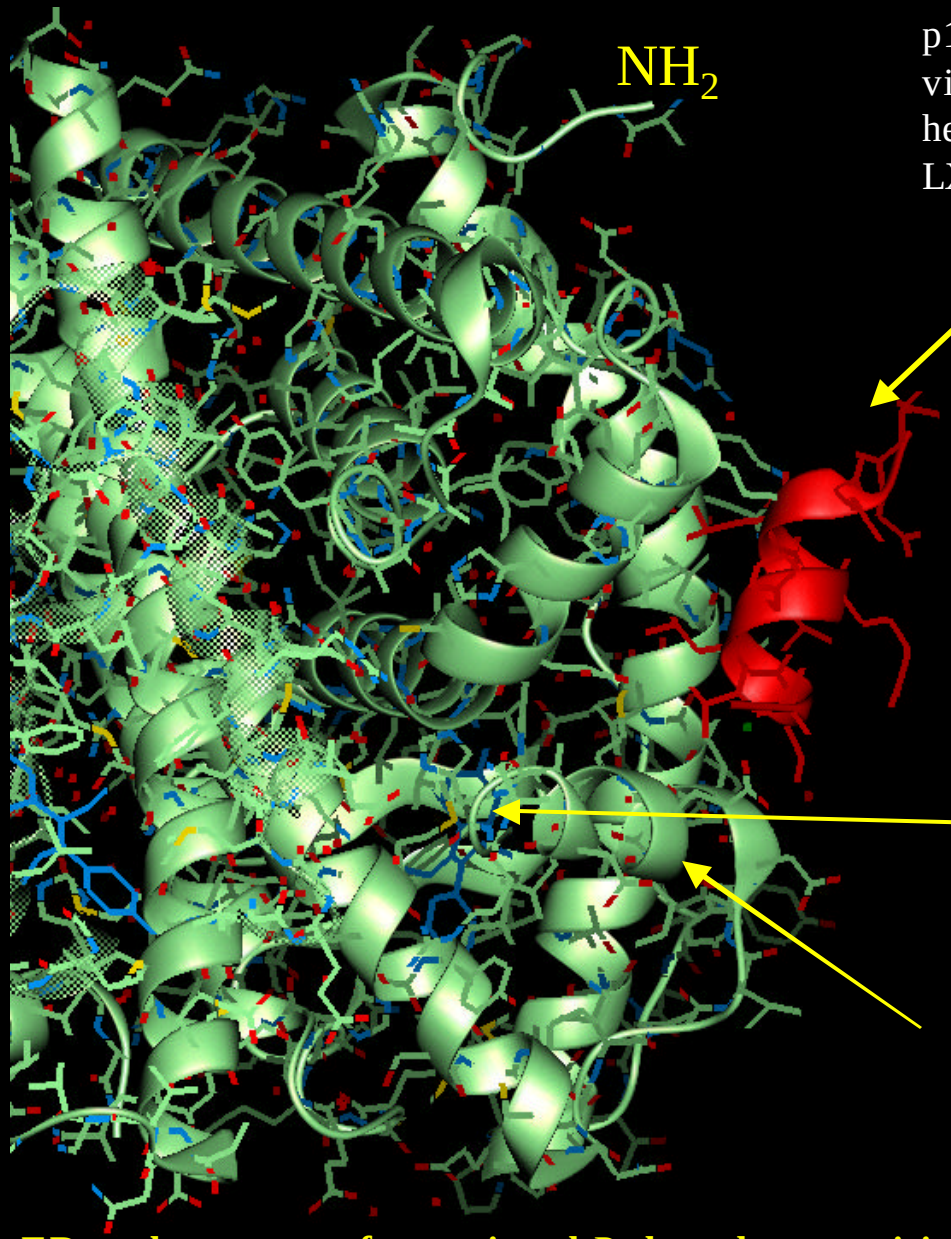
Ligand binding – antagonists, agonists and SERMs





V.C. Jordan, Clin. Can. Res. 2003, 9, 1980-1989
Schafer et al, Can. Res., 2000, 60, 5097-5105

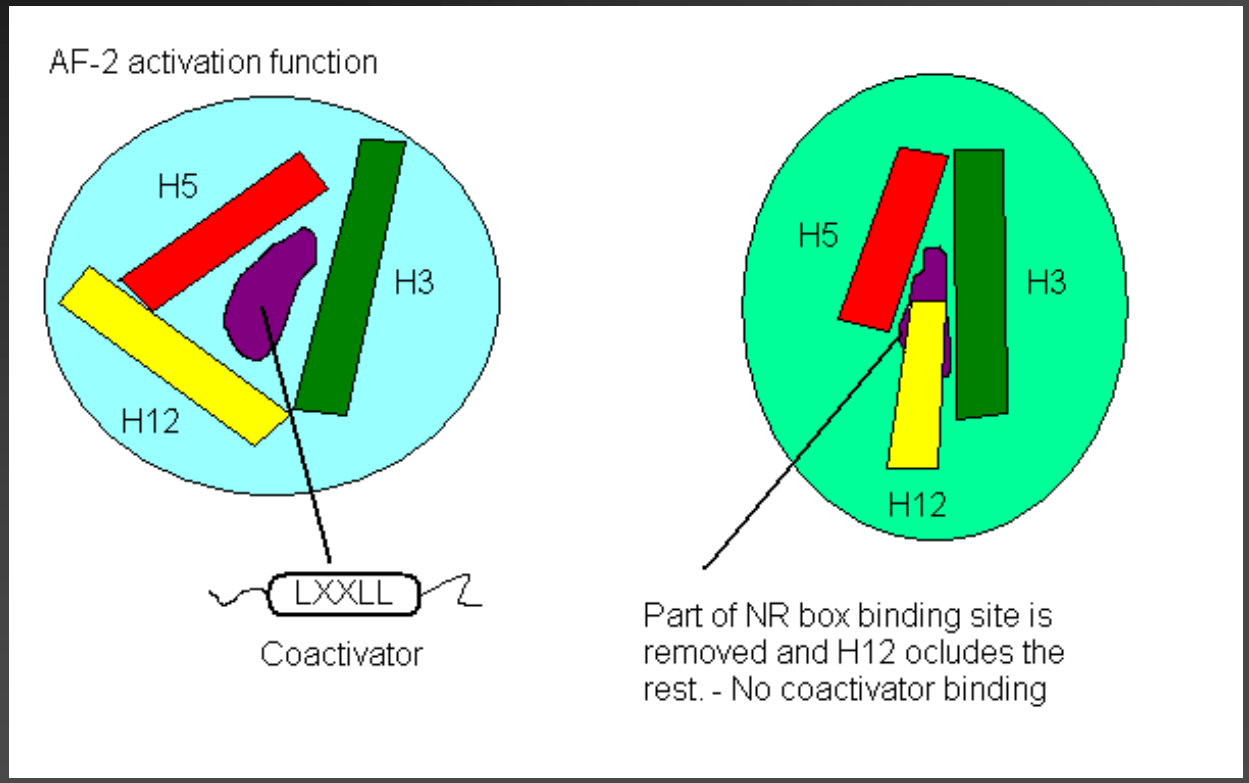
Agonist-bound, dimerized ERα with peptide bound at CRD



When agonist bound, ER undergoes conformational D that alters positions of H3-5 & H12 in the LBD, facilitating formation of a hydrophobic co-activator binding cleft in AF-2

B. Day

Co-regulator binding is ligand – ER complex dependent

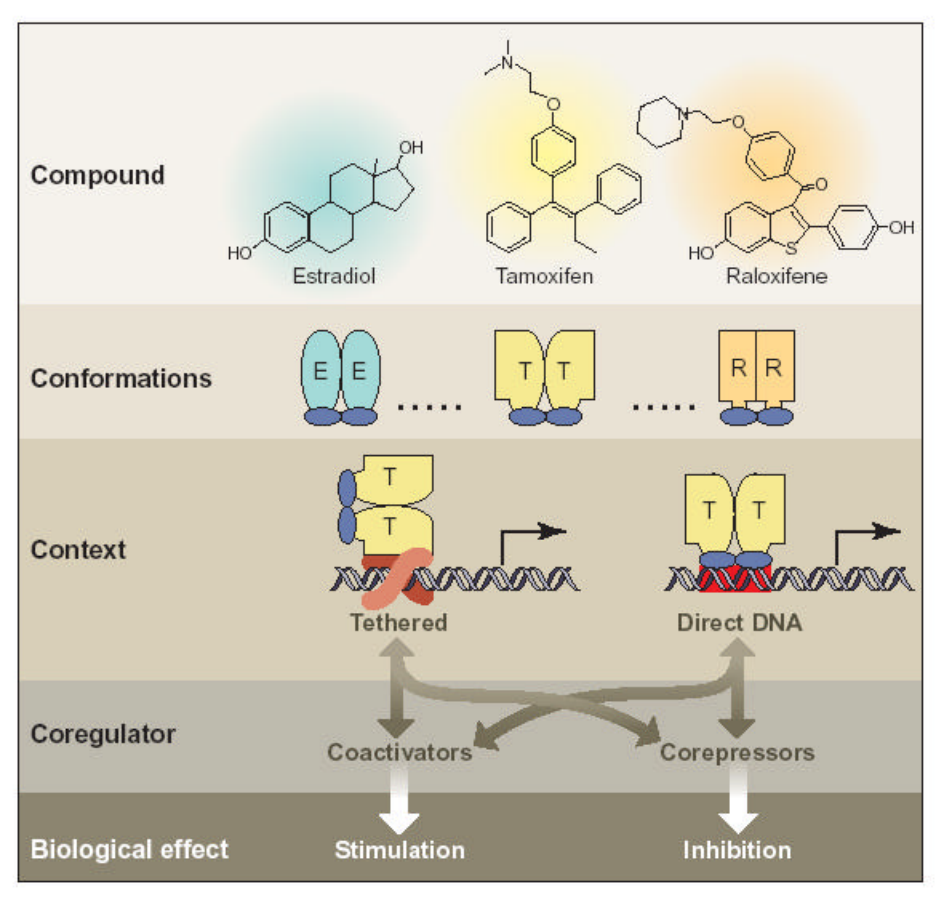


"SERMs: Research and Clinical Applications", A. Manni, M.F. Verderame, 2002

What is behind Tamoxifen and Raloxifene mixed activity?

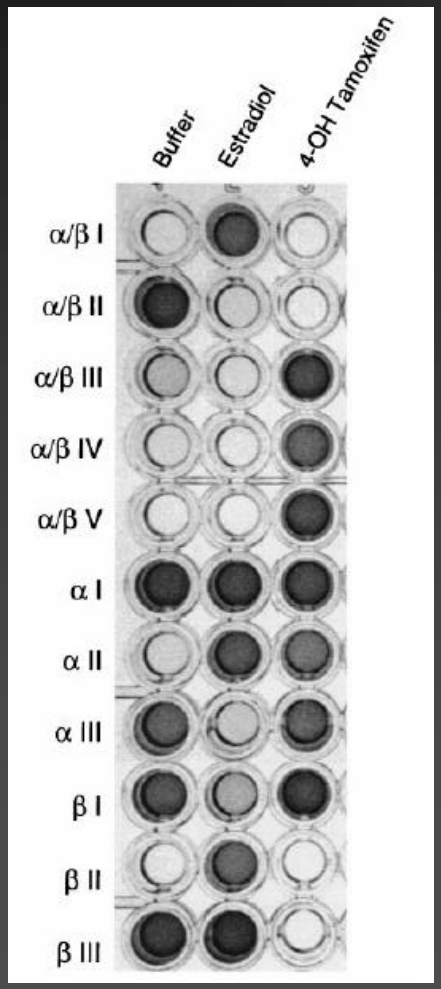
- Antagonists in brain and breast
- Agonists in bone, liver, cardiovascular system
- Mixed agonists/antagonists in uterus

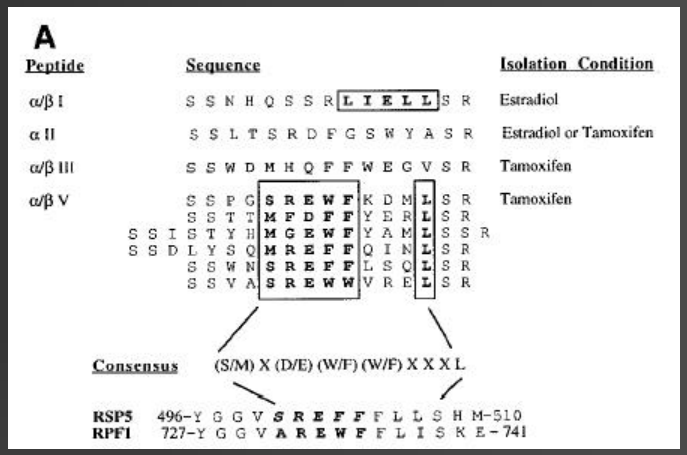
Specific molecular binding partners for ER responsible for breast tissue antagonism of Txf and Ral vs. agonism in uterus are *not known*!

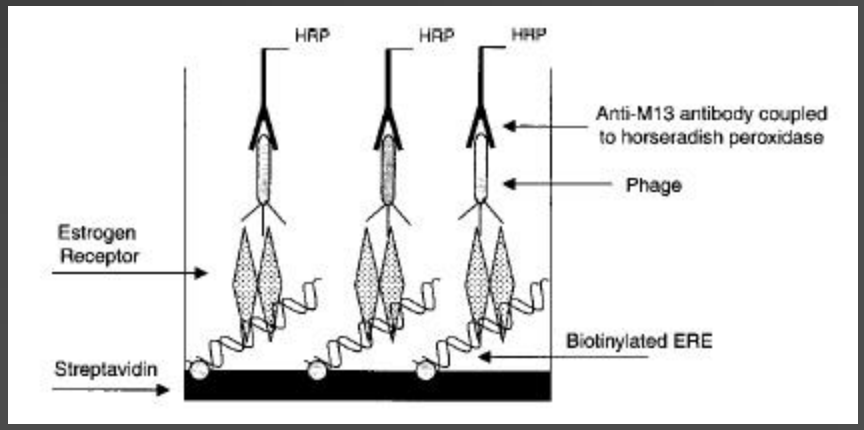


Katzenellenbogen, B. and Katzenellenbogen J., Science 2002, 295, 2380-2381

Ways out – E2 and Tam show different agonism mechanisms!







Norris et al, Science 1999, 285,744-746

PNAS 1999, 96, 3999–4004

How to study new antiestrogens?

- ER transcriptional activation assays – 293, CV1 cells – ER naïve – functional cell based screening
- ER binding assays – in vitro screening
- Antiproliferative assays using ER+ breast cancer cell lines (MCF7)
- Coregulator interaction studies - protein – protein interactions
- Molecular modeling

Stock solutions meet the cell lines…

67 compounds in the discovery library …

⇩

67 stock solutions at 10mM in DMSO …

⇩

Many, many cells …

And…

One target!

Discovery of new antiestrogens

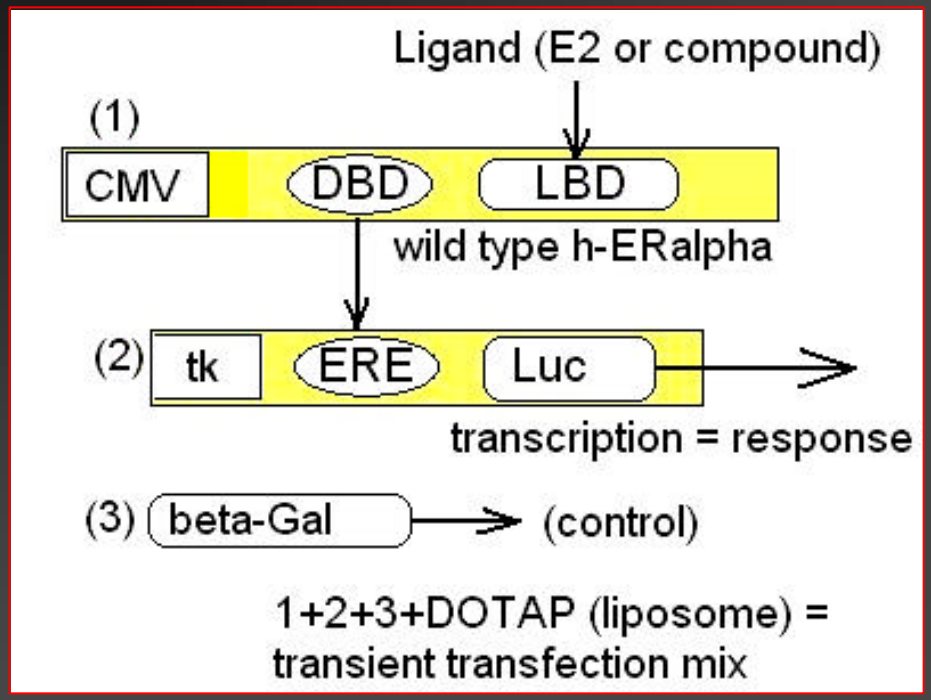
Homoallylic amides
CK3-031
CK2-130A
CK2-124
CK2-057A
CK2-055
CK2-012B
CK2-117A
CK2-053A
CK2-129B
CK2-010B
CK2-010A
CK2-009A

Cyclopropyl Amides
CK2-299
CK2-299
CK2-171
CK2-244
CK2-209B
CK2-209A
CK2-213
CK3-117
CK2-189B
CK2-048
CK1-183
CK3-083
CK2-223
CK1-181
CK3-089
CK3-016B
CK1-139
CK2-059
CK2-060
CK2-130B

Allylic Amides	
CK2-270	CRS4-097
CK3-079	CRS4-098
CK2-184	CRS4-099
CK2-183	CRS4-100
CK1-175	CRS4-101
CK3-082	CRS4-102
CK1-173	CRS4-103
CK3-086	CRS4-104
CK1-137	CRS4-105
CK2-176	CRS4-106
CRS4-087	CRS4-107
CRS4-088	CRS4-108
CRS4-089	CRS4-109
CRS4-090	CRS4-110
CRS4-091	CRS4-111
CRS4-092	
CRS4-093	
CRS4-094	
CRS4-095	
CRS4-096	

C. Kendall and C. Stephenson Discovery library

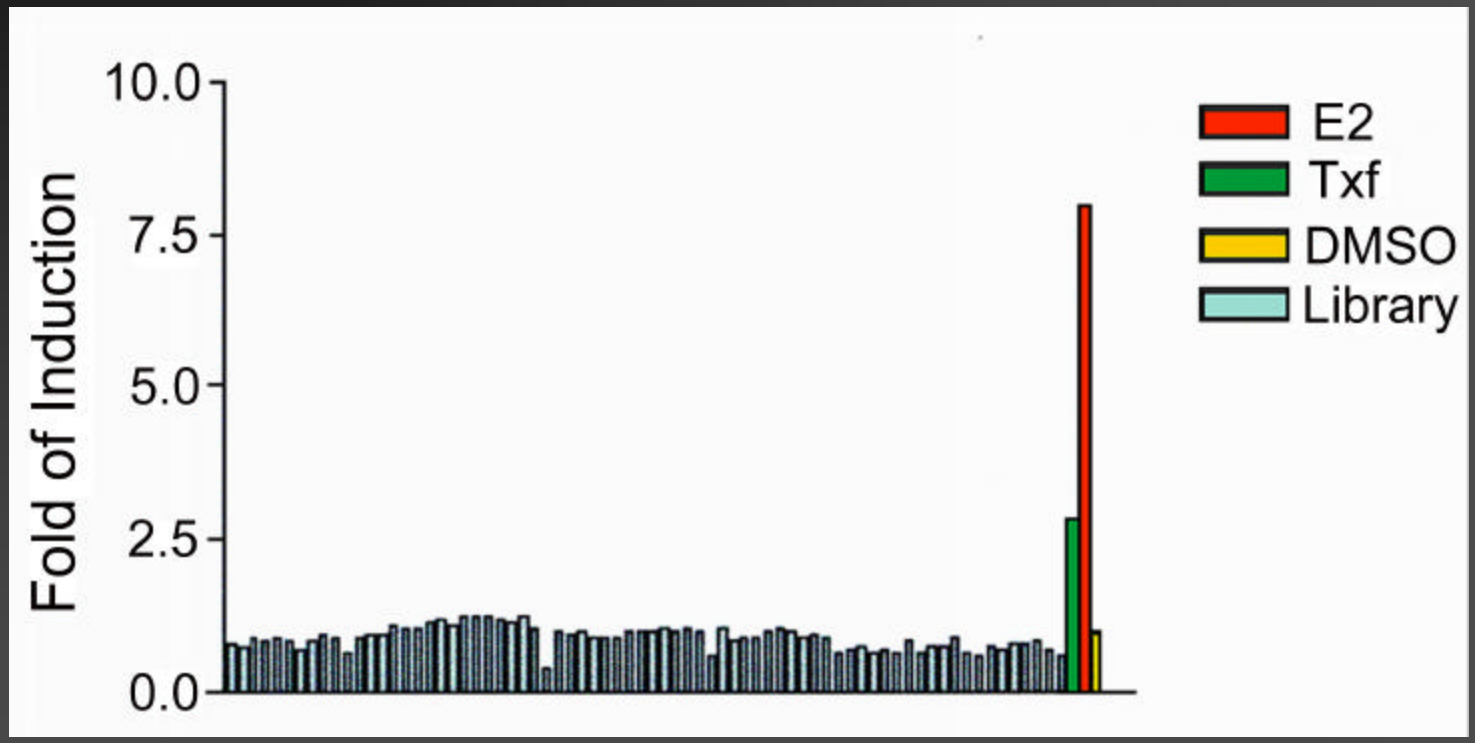
ERα Transcriptional Assay



Fold induction: ligand or E2 / DMSO (DMSO set to 1 as a base line).

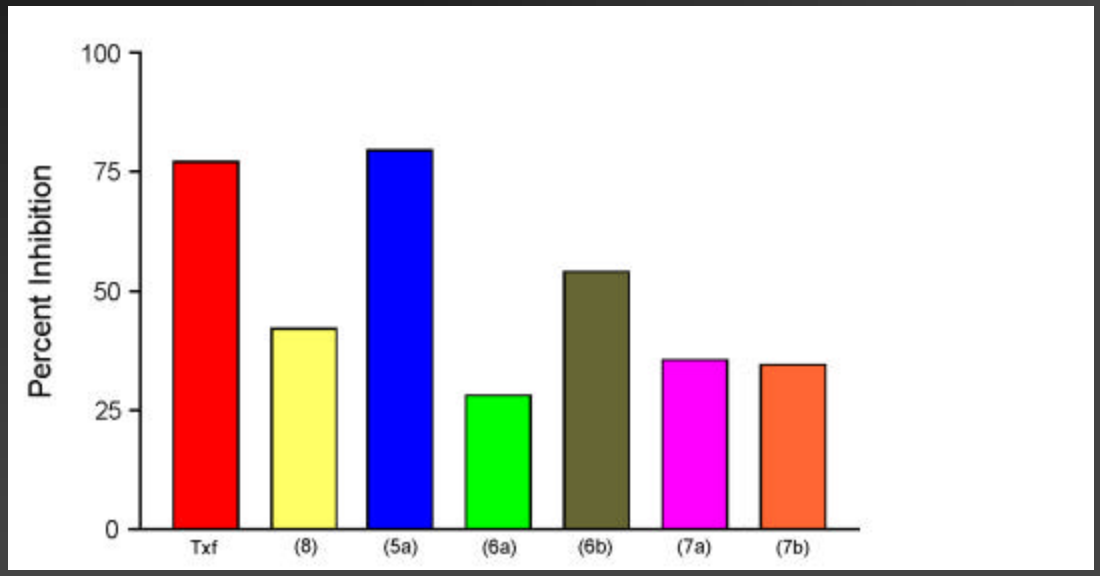
% Inhibition: 100 – [(ligand +E2 count) / (E2 count.) x 100]

Discovery Library Screen – no estrogenic compounds



Initially, library compounds were screened for estrogenic activity in the transcripional based assay.

Discovery of New Antiestrogens



Library compounds tested for antiestrogenic activity in the transcriptional activation assay revealed new antiestrogens.

Compound 5a (CK1-183) is the lead compound for our new focused library.

Future directions:

1. QSAR based on first generation focused library
2. Using feedback from QSAR to design new library of antiestrogens with high potential for hits
3. Implementation of microwave methodologies to library synthesis protocols

Thank you to:

Prof. Dr. Peter Wipf

Wipf group - Chris, Corey – discovery library

Bojan – alkyne precursors

Dr. Billy Day

Day group - Raghavan Balachandran, Brianne Raccor,Ying Lu – antiproliferative assays

Dr. Wen Xie

Xie group - Dr.Ying Mu – functional ERa assay

NIH