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

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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)
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, J Med Chem 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.

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 (ER\(\alpha\) and ER\(\beta\))

- 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
ER induces transcription through classical ERE binding directly to DNA or thetering to other transcriptional factors and bind to DNA indirectly!
Estrogen receptor ligands fall into three groups: antagonists, agonists and SERMs
First non-steroidal “hormones”

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.


J.I. Macgregor, 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 (E₂) 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

Brzozowski et al, Nature 1997, 389, 753
Ligand binding – antagonists

Brzozowski et al, Nature 1997, 389, 753
SAR of an antagonist - Raloxofene

Ligand binding – antagonists, agonists and SERMs

Agonist-bound, dimerized ERα with peptide bound at CRD

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

p160s bind AF-2 via an amphipathic helix with the LXXLL motif
Co-regulator binding is ligand – ER complex dependent

AF-2 activation function

H5

H3

H12

Coactivator

LXXLL

Part of NR box binding site is removed and H12 occludes the rest. - No coactivator binding

“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*!

Ways out – E2 and Tam show different agonism mechanisms!

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

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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]
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
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