Current Literature Presentation

Date: March 27th, 2004

Design, Synthesis, and in Vitro Biological Evaluation of Small Molecule Inhibitors of Estrogen Receptor Coactivator Binding

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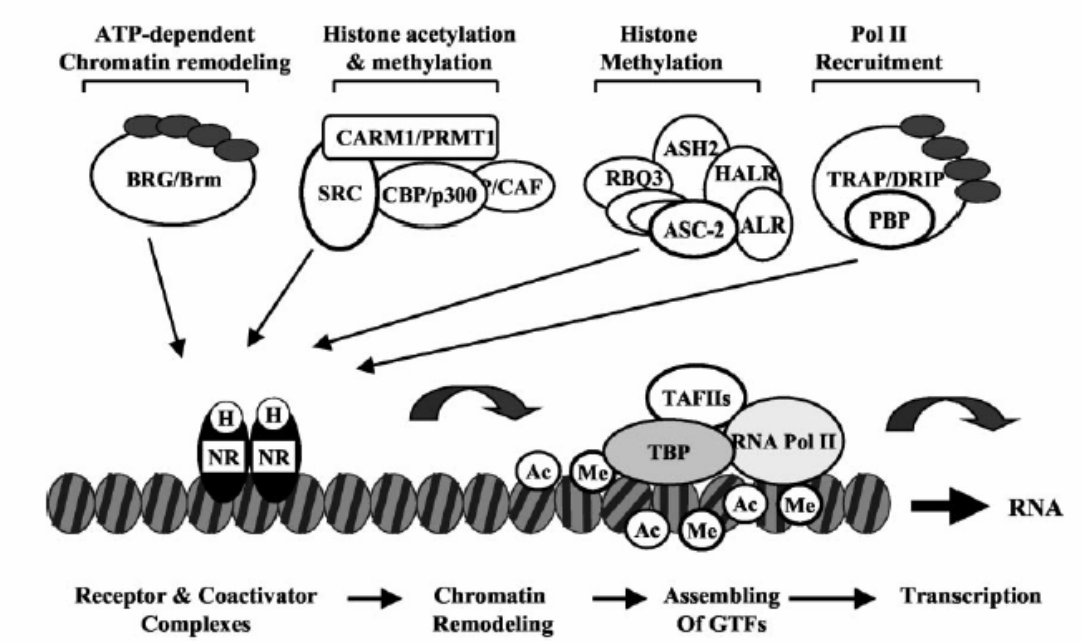
J. Med. Chem. **2004,** *47*, 600-611

Presented by: Jelena Janjic

Nuclear Receptors

- Transcriptional factors
  - Ligand-dependent activation of gene transcription by nuclear receptors is dependent on the recruitment of coactivators, via a region containing three helical domains sharing an LXXLL core consensus sequence



Genes Dev. 1998, 12(21):3357-68

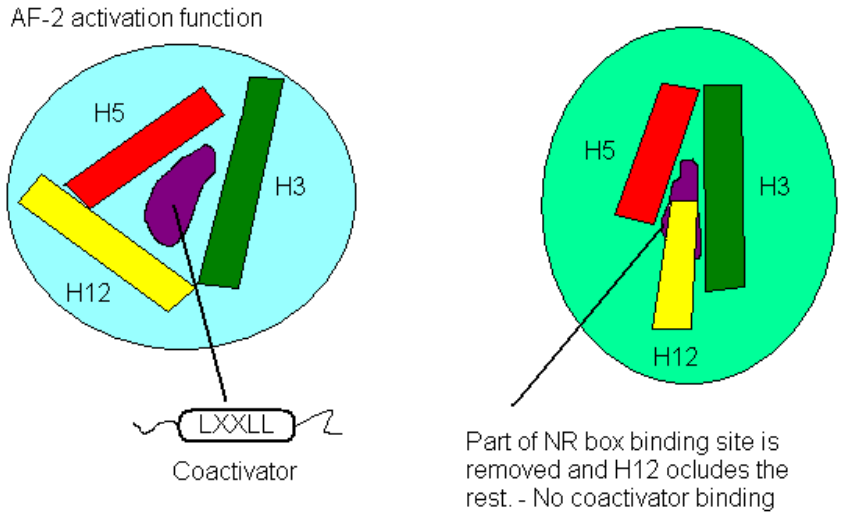
Genes Dev. 1998, 12(21):3343-56

Molecular Endocrinology 2003, 17(9):1681–1692

Co-regulator binding is ligand – ER complex dependent

Coregulators

1. Coactivators: AIB1, SRC-1, GRIP-1, CBP, p300, pCAF
2. Corepressors: NCoR and SMRT



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

Hypothesis

- Inhibit the transcriptional activity of the NRs by **blocking** this transcriptionally **critical receptor-coactivator interaction directly**, using an appropriately designed coactivator binding inhibitor (CBI).

- A proof-of-principle demonstration of the effectiveness of this approach is the observation that **peptides having the LXXLL** sequence are able to block gene transcription induced by estrogen agonists working through the estrogen receptor (ER).

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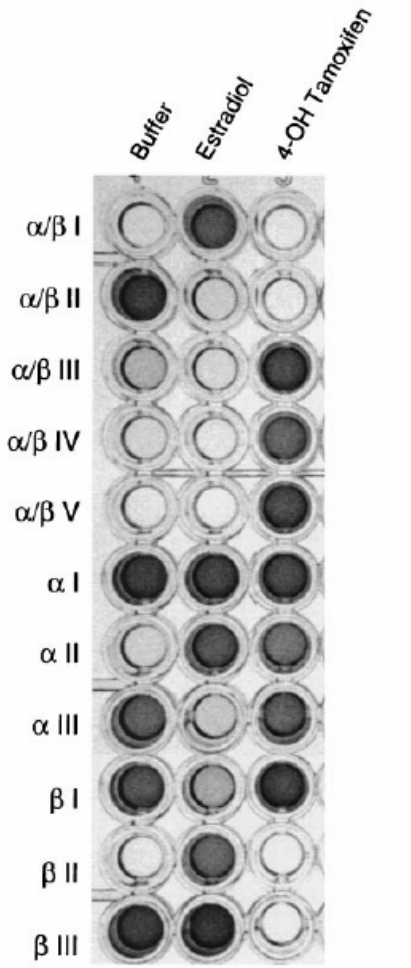
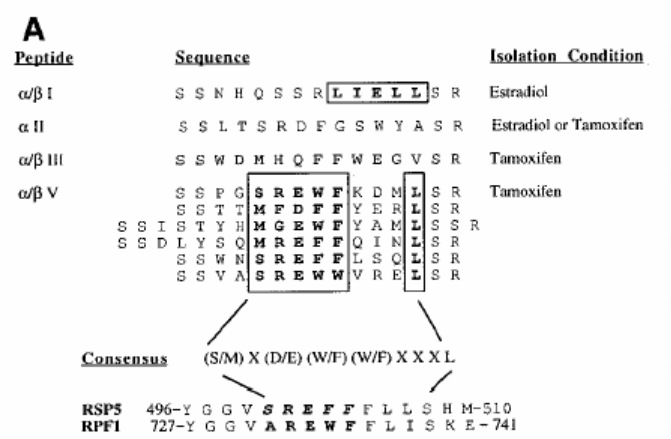
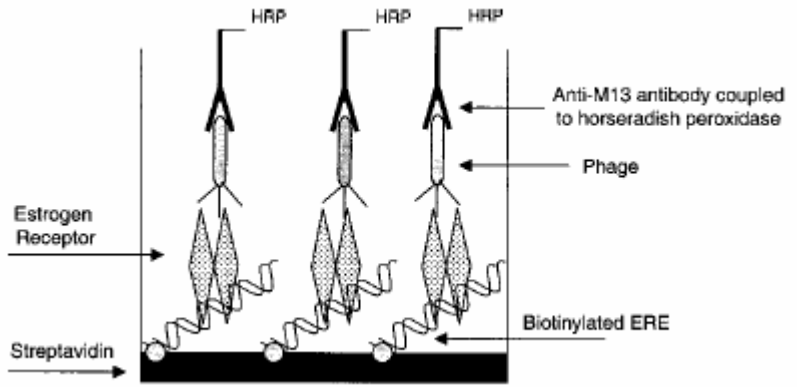
ERα-interacting peptides.







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

PNAS 1999, 96, 3999–4004

Structure based design

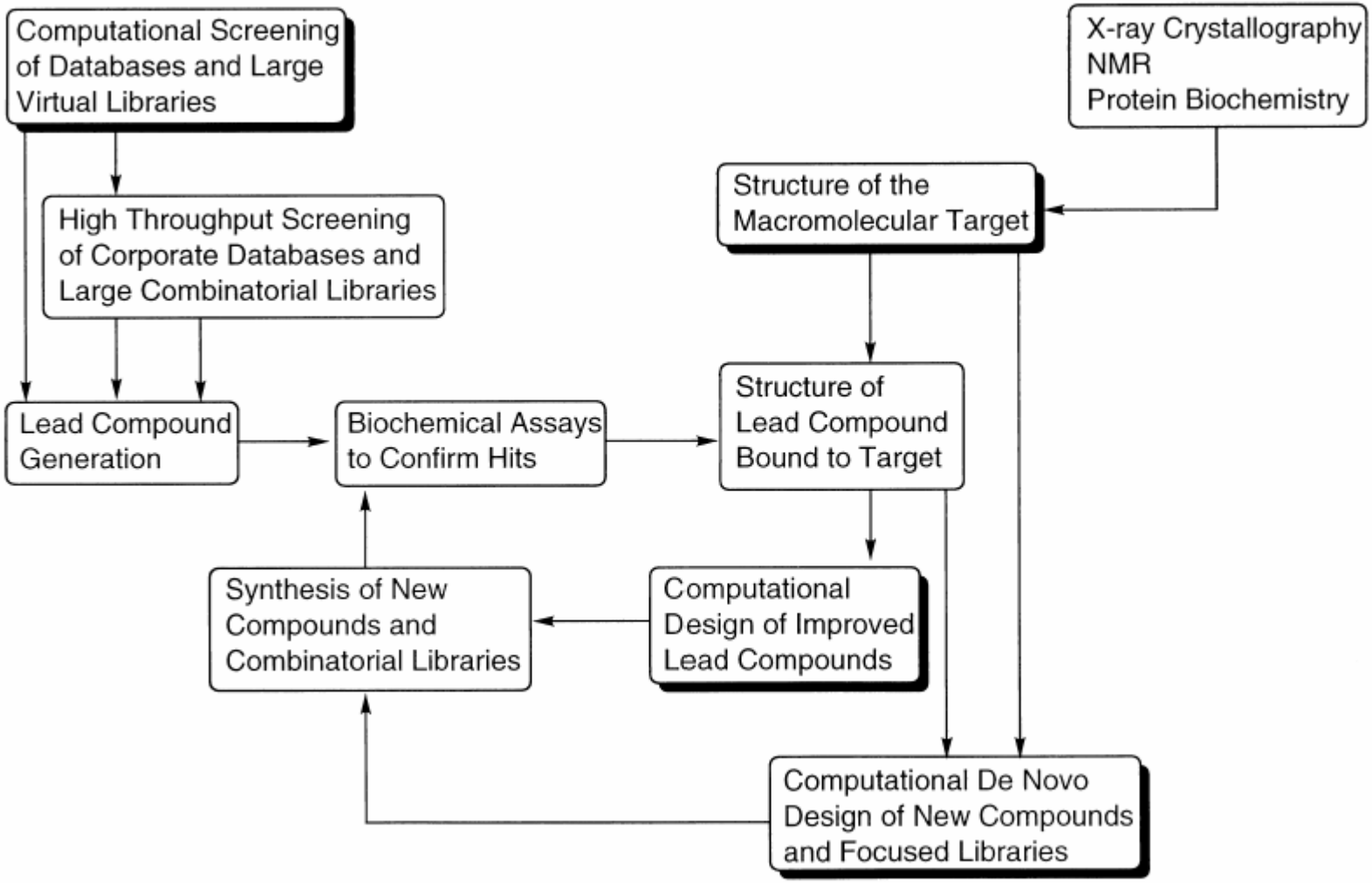


Fig. 2. Outline of the structure-based drug design process.

Table 1. Algorithms for Docking Small Molecules or Fragments against a Target

	Program	Flexible Protein?	Flexible Ligand?	Description
Virtual screening	DOCK	no	yes	docks either small molecules or fragments, includes solvent effects
	FlexX	no	yes	incremental construction
	FlexE	yes	yes	incremental construction; samples ensembles of receptor structures
	SLIDE	yes	yes	anchor fragments placed, remainder of ligand added; backbone flexibility
	Flo98	no	yes	can rapidly dock a large number of ligand molecules, graphically view results
	ADAM	no	yes	fragments aligned based on hydrogen bonding
	Hammerhead	no	yes	genetic algorithms to link tail fragments to anchor fragments
	MCSA-PCR	yes	yes	uses simulated annealing to generate conformations of target
	AUTODOCK	yes	yes	uses averaged interaction energy grid to account for receptor conformations and simulated annealing for ligand conformations
	MCDOCK	no	yes	Monte Carlo to sample ligand placement
	ProDOCK	yes	yes	Monte Carlo minimization for flexible ligand, flexible site
	ICM	yes	yes	Monte Carlo minimization for protein-ligand docking
	DockVision	no	no	Monte Carlo minimization
De novo generation of ligands	LUDI	no	yes	docks and scores fragments
	GRID	no	yes	calculates binding energies for functional groups
	MCSS	no	yes	exhaustive search of binding site for functional group minima
	SMoG	no	yes	knowledge-based scoring function; molecules built by joining rigid fragments
	CONCERTS	no	yes	fills active site with molecular fragments, links fragments
	Legend	no	yes	grows molecule atom by atom
	DLD	no	yes	saturates binding site with sp^3 carbons, later linked
	GrowMol	no	yes	builds ligands from a library of atom types
	GenStar	no	yes	builds ligands from sp^3 carbons
	GROW	no	yes	constructs a peptide by residue addition
	GroupBuild	no	yes	builds ligand from a predefined library of fragments
	HOOK	no	yes	searches database of molecular skeletons for fit to binding site; hooks two MCSS functional groups to skeleton
	SPROUT	no	yes	generates skeletons that fit site, substitutes atoms into skeleton to give molecules with correct properties
	CAVEAT	no	yes	searches database of small molecules to connect fragments

Chemistry & Biology 2003, 10, 787–797

Journal of Computer-Aided Molecular Design, **16:** 151–166, 2002.

Structure-Based Design of Coactivator Binding Inhibitors (CIBs).

- **crystal structure** of the ERα-LBD complexed with diethylstilbestrol and a 13 amino acid peptide derived from the p160 class coactivator, GRIP1 (SRC-2), containing a single NR box
- ***The interaction surface in the ER consists of 16 amino acid residues from helices 3, 4, 5, and 12***: L354, V355, I358, A361, K362 (helix 3); L372 (helix 3,4 turn); F367, V368 (helix 4); Q375, V376, L379, E380 (helix 5); and D538, L539, E542, M543 (helix 12).

Three zones of interaction are evident:

- ***L690 and L694*** of the coactivator peptide are entirely engulfed by the ER, forming a deep, strong **hydrophobic groove interaction**;
- ***I689 and L693*** of the coactivator peptide interact with the receptor on one-half of their surface, forming a weaker but apparently significant surface hydrophobic interaction;
- ***E542 and K362*** of the ER form the ***charge clamp*** properly aligned for interaction with the inherent dipole of the α-helix,
- and finally various ***amide functionalities*** on the helix backbone, locking and holding the hydrophobic side chains into position.

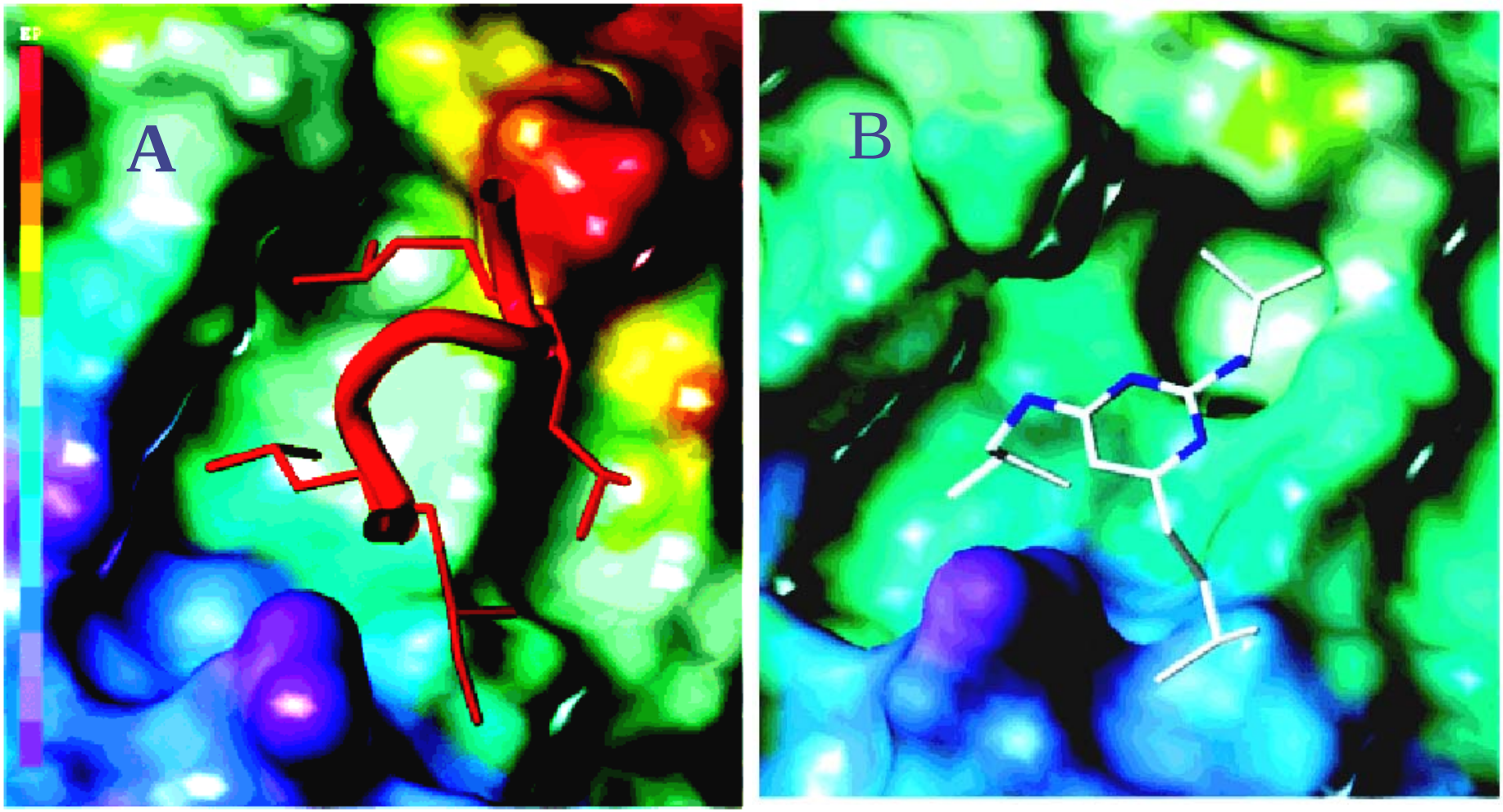
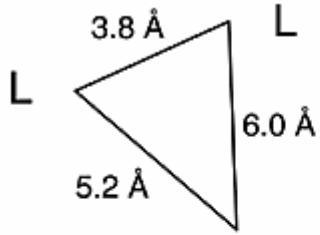


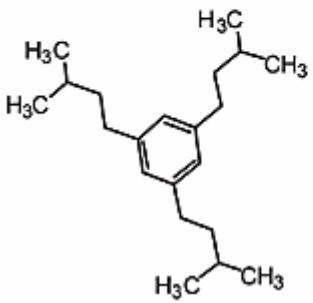
Figure 1. Electrostatic surface rendering of (A) the crystal structure of GRIP1 peptide bound to ERα and (B) pyrimidine CBI

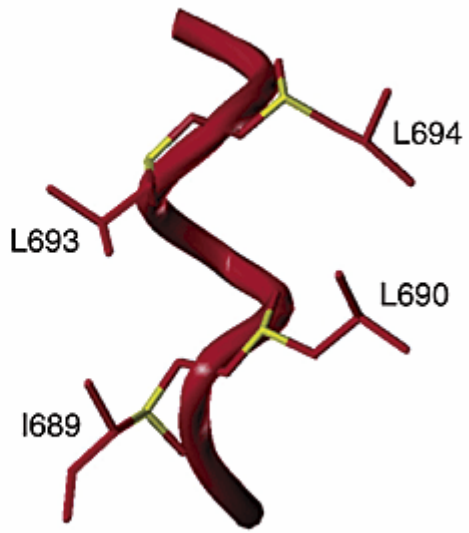
12a docked into ERα. The receptor is shown as a continuous surface where red coloring indicates positive charge and blue coloring indicates negative charge. **12a** is colored by atom type, whereas the coactivator peptide is depicted as a red tube.

Class I Design

- Four scaffolds - candidate cores for the class I CBIs: ***triazene, pyrimidine, trithiane, and cyclohexane.***

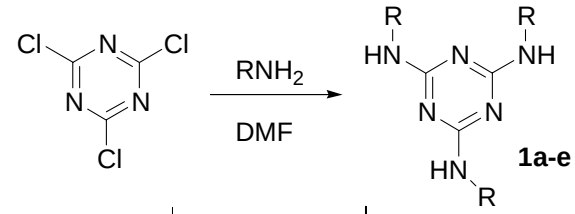




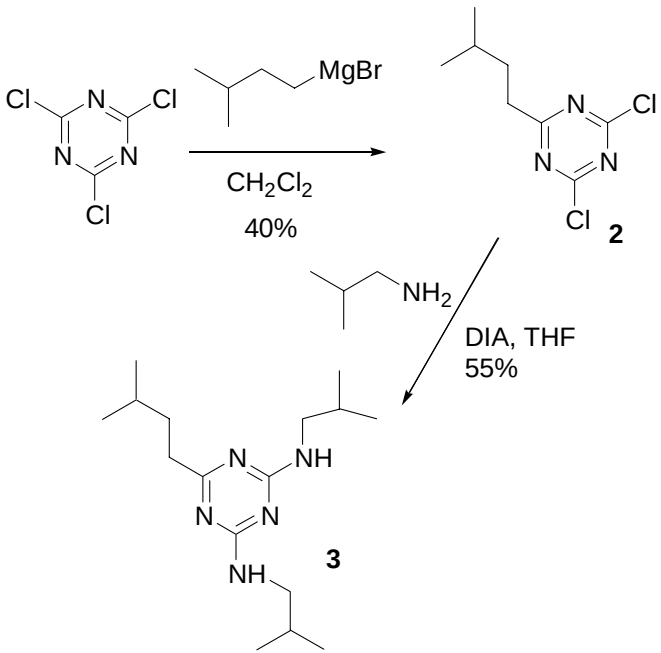


- ***"outside-in" approach.***
- A head-on view of the coactivator peptide - positions of the three leucine residues in the LXXLL signature sequence motif - an equilateral triangle,
- and then hydrophobic substituents were attached in a manner that mimics-in a topologically faithful fashion-the positions of the three leucine residues of the coactivator peptide.
- **These CBIs** are said to follow an *outside-in* design, because they **begin with a mimic for the helix backbone**, which is ***outside of the hydrophobic groove***, and **then proceed *inward* by adding residue elements.**

Synthesis of Class I CBIs



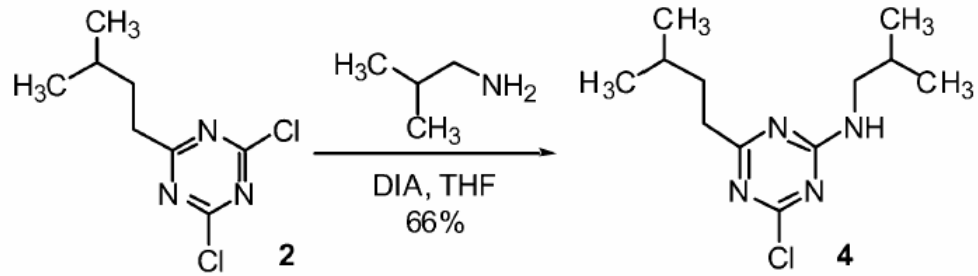
1	R	Yields
a	$CH_2CH(CH_3)_2$	43%
b	$CH_2CH_2CH_3$	27%
c	$CH_2C_6H_5$	23%
d	$CH_2C_6H_{13}$	2.3%
e	$CH_2C_{10}H_7$	49%

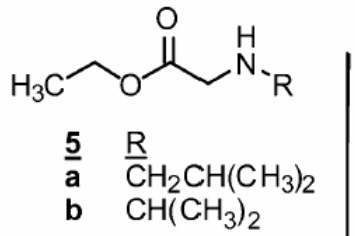


Scheme 1. Synthesis of Triazene-Core CBIs

- **Synthesis. Triazenes.** Triaminotriazenes were synthesized in a single step from commercially available cyanuric chloride (Scheme 1). Triazene **1a** was synthesized by reacting cyanuric chloride with isobutylamine to produce a compound having substituents that directly mimic the three branched leucine amino acids. Triazenes **1b-e** were synthesized by reacting cyanuric chloride with a variety of alkyl- and arylamines containing simple straight-chain and cyclic alkyl and aryl substituents.

Scheme 2. Synthesis of Other Triazene-Core CBIs

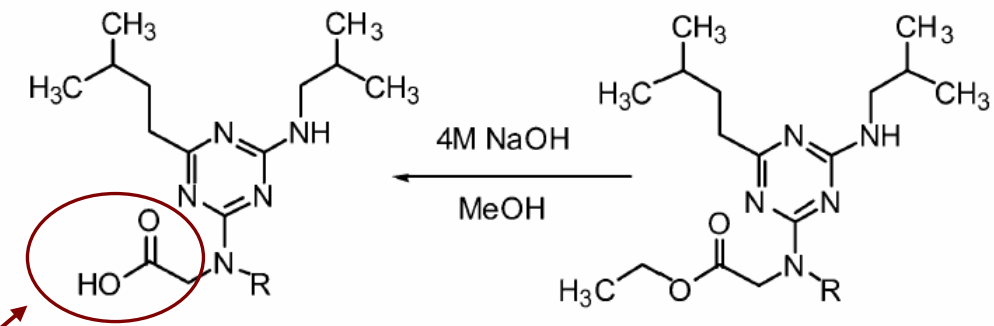




5

	R
a	$CH_2CH(CH_3)_2$
b	$CH(CH_3)_2$

TEA, THF



7

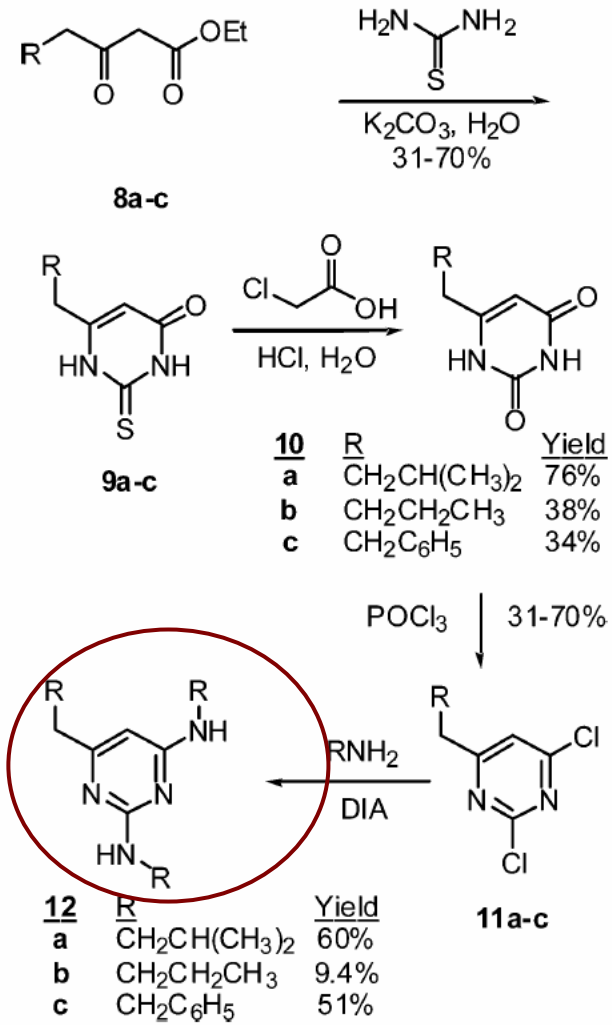
	R	Yield
a	$CH_2CH(CH_3)_2$	84%
b	$CH(CH_3)_2$	49%

6

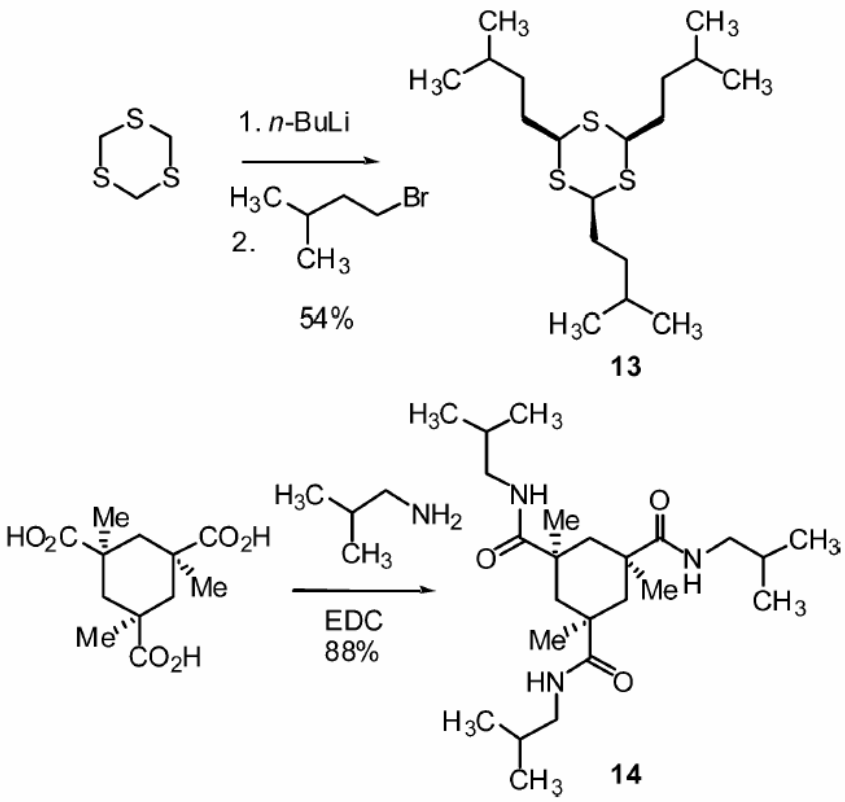
	R	Yield
a	$CH_2CH(CH_3)_2$	84%
b	$CH(CH_3)_2$	60%

To incorporate an acid functionality for interaction with K362 involved in the ***charge clamp***, intermediate **2** was reacted with a single equivalent of isobutylamine to give disubstituted triazene **4** (Scheme 2).

Scheme 3. Synthesis of Pyrimidine-Core CBIs

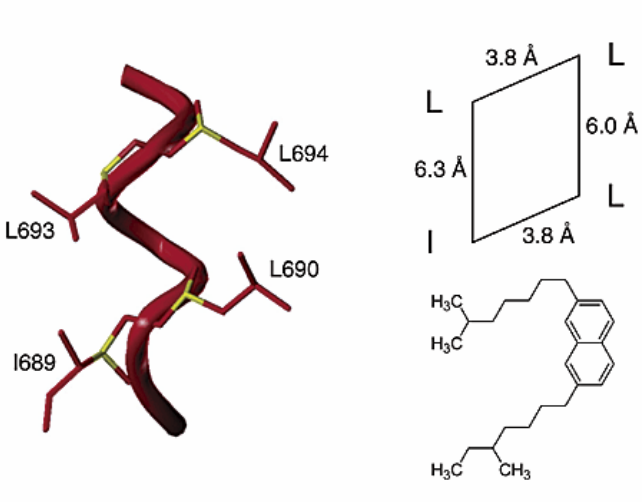


Scheme 4. Synthesis of Trithiane- and Cyclohexane-Core CBIs

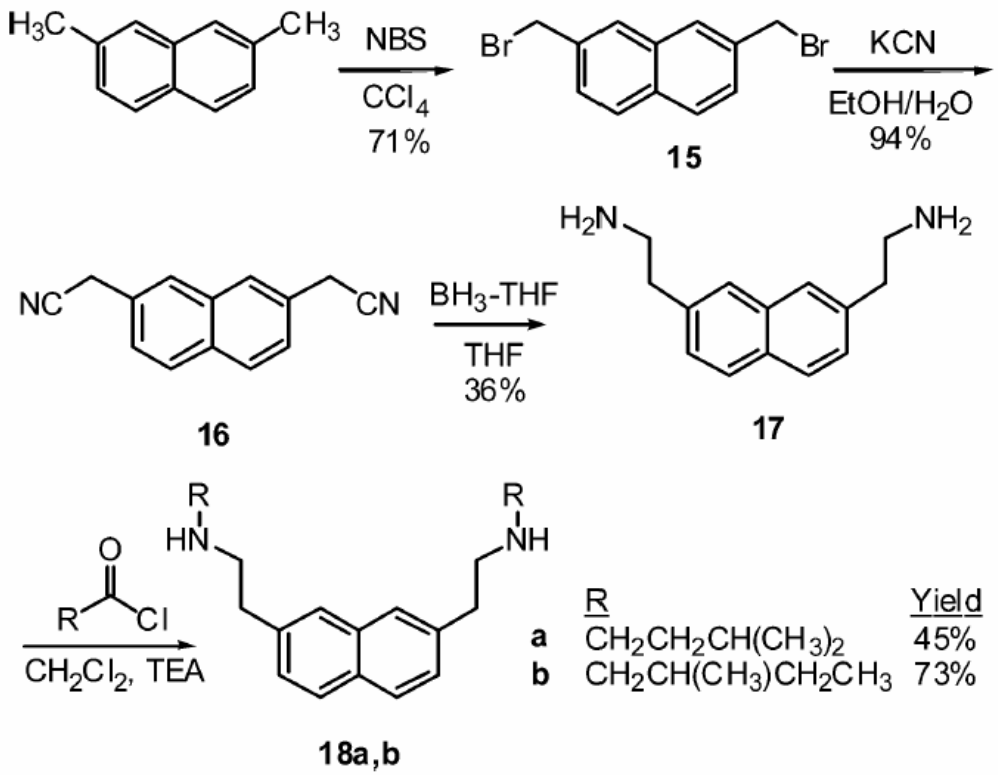


Pyrimidine compounds were examined because they have greater torsional flexibility than the triazene compounds.

Class II Design.

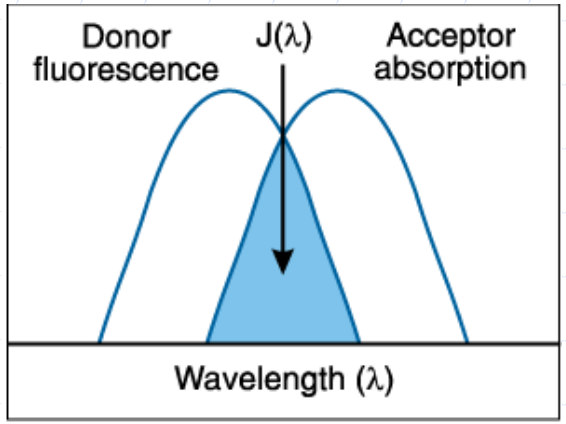


Scheme 5. Synthesis of Naphthalene-Core CBIs



- ***"inside-out" design.***
- From the face of the α-helix, the four hydrophobic residues of the NR box approximate the shape of a parallelogram
- hydrophobic small molecule to mimic both of the most deeply buried *groove* residues, L690 and L694, which are considered most crucial for stabilizing the ER α -coactivator interface.

Biochemical evaluation – FRET-
Fluorescence Resonance Energy Transfer



- Fluorescence resonance energy transfer (FRET) is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule *without emission of a photon*.
- The efficiency of FRET is dependent on the inverse sixth power of the intermolecular separation, making it useful over distances comparable with the dimensions of biological macromolecules.
- **Primary Conditions for FRET**
  - Donor and acceptor molecules must be in close proximity (typically 10–100 Å).
  - The absorption spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor
  - Donor and acceptor transition dipole orientations must be approximately parallel.

Methods, 1999, 19, 222-233
http://www.probes.com/handbook/boxes/0422.html

Biochemical evaluation – FRET

- Purified ERαligand binding domain and **tetramethylrhodamine-labeled NR box peptide (*ILRKLLQE)** were used for the fluorescence anisotropy assays.

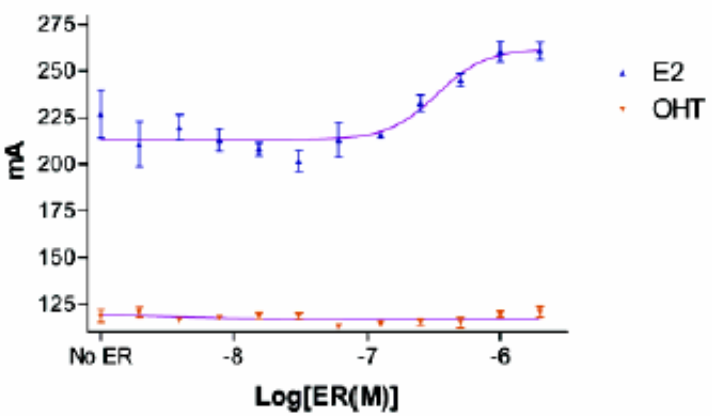


Figure 3. Recruitment of TMR-labeled NR box peptide to the ER as followed by fluorescence anisotropy. Estradiol-bound ER (E2) recruits the peptide as demonstrated by an increase in anisotropy, whereas hydroxytamoxifen-bound ER (OHT) does not recruit the peptide.

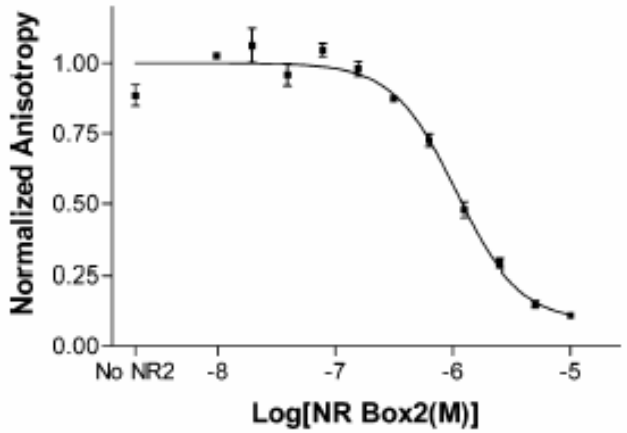


Figure 4. Displacement of TMR-labeled NR box peptide by unlabeled NR box 2, as shown by fluorescence anisotropy.

Biochemical evaluation – FRET

- The Class I compounds containing a triazene core showed ***weak inhibition of coactivator peptide binding***
- The most potent triazene was **7b**, which showed a minimal decrease in anisotropy. Addition of polar functionalities improved the binding slightly in the case of **7b**, but did not improve binding in any other case

Inhibitors of ERα Coactivator Binding

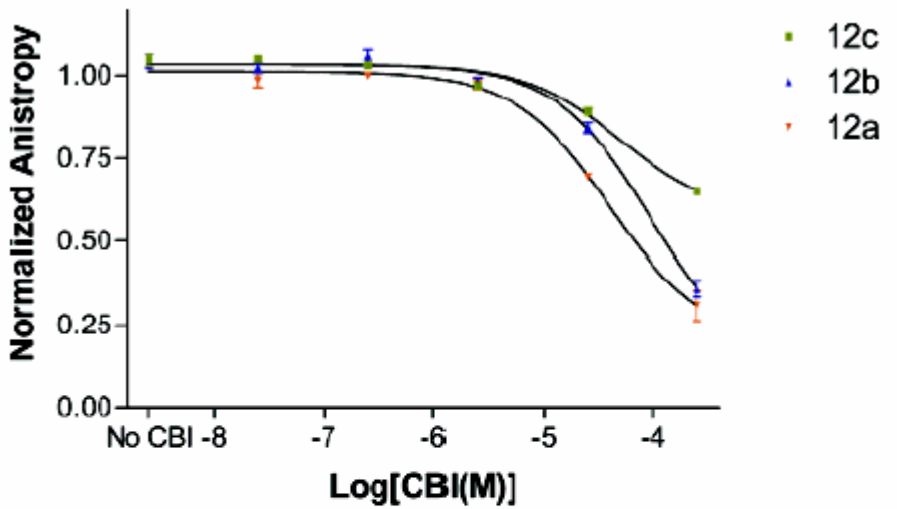


Figure 5. Displacement of TMR-labeled peptide by pyrimidine CBIs.

Table 1. Summary of Binding Affinity Data for Triazene, Pyrimidine, Trithiane, Cyclohexane and Naphthalene CBIs

CBI	K_i (μM)	CBI	K_i (μM)	CBI	K_i (μM)
1a	590	**3**	650	**12c**	49
1b	290	**7a**	790	**13**	>1000
1c	240	**7b**	410	**14**	>1000
1d	290	**12a**	29	**18a**	>1000
1e	290	**12b**	32	**18b**	>1000

Conclusions

- Inhibition of protein-protein interactions with small molecules is a relatively young field with a number of unique challenges.
- Often, protein-protein binding occurs over a relatively large surface area, which makes it difficult to focus on essential, high-affinity interactions that might be blocked with low molecular weight organic molecules. Sometimes, the binding surface between two proteins is relatively "smooth", lacking grooves or pockets that make good sites for small molecule binding.

Take home message

- Protein-protein interactions are hard to transfer-small molecule interactions
- Modeling is always good start.
- Cannot replace “wet” lab!

Thank you!