

# Amphipathic Benzenes Are Designed Inhibitors of the Estrogen Receptor $\alpha$ /Steroid Receptor Coactivator Interaction

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**ABSTRACT** We report here on the design, synthesis, and evaluation of small molecule inhibitors of the interaction between a steroid receptor coactivator and estrogen receptor  $\alpha$ . These inhibitors are based upon an amphipathic benzene scaffold whose hydrophobic face mimics the leucine-rich  $\alpha$ -helical consensus sequence on the steroid receptor coactivators that interacts with a shallow groove on estrogen receptor  $\alpha$ . Several of these molecules are among the most potent inhibitors of this interaction described to date and are active at low micromolar concentrations in both *in vitro* models of estrogen receptor action and in cell-based assays of estrogen receptor-mediated coactivator interaction and transcription.

Estrogen receptor  $\alpha$  (ER $\alpha$ ), a ligand-activated transcription factor and member of the nuclear hormone receptor superfamily, is a well-validated target for the treatment of breast cancer, osteoporosis, and other endocrine disorders. It exerts its genomic effects by binding natural or synthetic estrogens and then recruiting steroid receptor coactivators (SRCs) that regulate the magnitude of gene transcription. SRCs of the p160 class bind to the ER through “nuclear receptor (NR)-box” sequence motifs comprising two turns of an amphipathic  $\alpha$ -helix and containing an LXXLL sequence (where L is leucine and X is any amino acid, though typically a polar one, e.g., histidine, arginine); these NR boxes bind to the C-terminal activation function 2 of ER-agonist complexes in a shallow hydrophobic groove formed by hydrophobic residues from helices 3, 4, 5, and 12 (1). Conventional ER antagonists, exemplified by the selective estrogen receptor modulator (SERM) tamoxifen, block this interaction *indirectly*: the large basic side chain of tamoxifen disrupts the folding of helix 12, causing it to move so that it blocks the recruitment of SRC-NR boxes to the ER (1–3). Although many breast cancer patients benefit from tamoxifen therapy, resistance to this drug develops in the majority of cases; moreover, if tamoxifen treatment is not discontinued after onset of resistance, the drug

can actually promote, rather than inhibit, tumor growth (4).

We (5, 6) and others (7–9) have envisioned a novel strategy to overcome this problem: *directly* blocking the ER/SRC interaction with a small molecule, termed a “coactivator binding inhibitor” (CBI), that is capable of binding to the groove formed on the receptor surface. This approach is unique in that it allows for the activity of an agonist-bound ER to be inhibited directly, thereby potentially circumventing the need for SERM treatment and the risk of the development of tamoxifen resistance. Because protein–protein interactions typically occur over large surface areas, they have historically been viewed as difficult targets for inhibition by small molecules, but auspiciously, there have been a number of recent advances in the field (10–13). Because of the short, well-defined nature of the LXXLL interaction motif (14), the ER $\alpha$ –SRC interaction seems to be a promising target for small molecule therapy. Toward that goal, we report here a series of amphipathic benzene CBIs obtained from *de novo* design that are active as inhibitors in cell-based assays of ER $\alpha$ -mediated transcription.

**Design and Synthesis.** We have previously reported the trisubstituted pyrimidine **A** that inhibits the interaction of ER $\alpha$  and SRC1 NR Box II at midmicromolar potency as assayed in a fluorescence polarization assay (6). The three leucine residues of the

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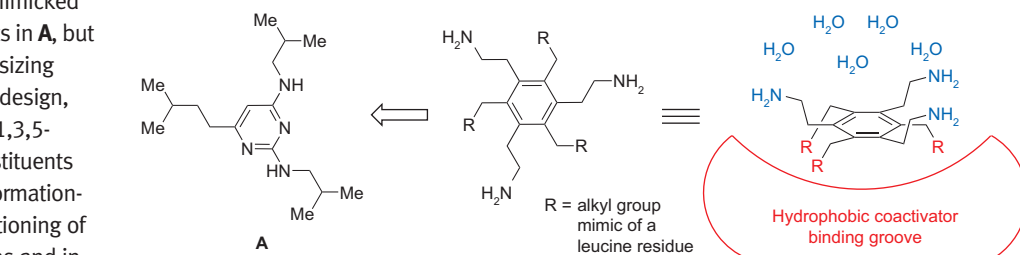
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LXXLL motif are sufficiently mimicked by the three alkyl substituents in **A**, but we were interested in synthesizing molecules of a more refined design, ones that not only keep the 1,3,5-relationship of the alkyl substituents but also are both more conformationally constrained in their positioning of leucine-mimicking side chains and incorporate structural elements that mimic the amphipathic nature of the LXXLL  $\alpha$ -helix (Figure 1).

Benzenes exhibiting a substitution pattern of alternating hydrophobic and hydrophilic residues give rise to facially amphipathic molecules that have been used as tripodal receptors for metals and ions (15, 16), as well as for generating supramolecular assemblies (17, 18). Owing to the amphipathic nature of the NR-box helix (e.g., solvent-exposed histidine and arginine residues in SRC1 Box II), as well as the success garnered in mimicking the NR-box leucine residues in our previously reported work in this area, we designed a series of hexasubstituted amphipathic benzenes with alternating hydrophobic groups to mimic leucine residues and with hydrophilic groups to increase solubility, to mimic the amphipathic nature of the peptide and to allow interaction with the exposed solvent.

Hydrophobic groups were added at the 1, 3, and 5 positions of benzene by Kumada coupling of 1,3,5-trichlorobenzene (**1**) and an alkyl Grignard reagent (Scheme 1). Exhaustive chloromethylation of the 1,3,5-trialkylbenzenes afforded persubstituted benzenes **2**, which were further functionalized by nucleophilic substitution with cyanide. Reduction of these intermediates gave triethylamines **3** (18).

**In Vitro CBI and Ligand Binding Assays.** We have developed a reliable time-resolved fluorescence resonance energy transfer

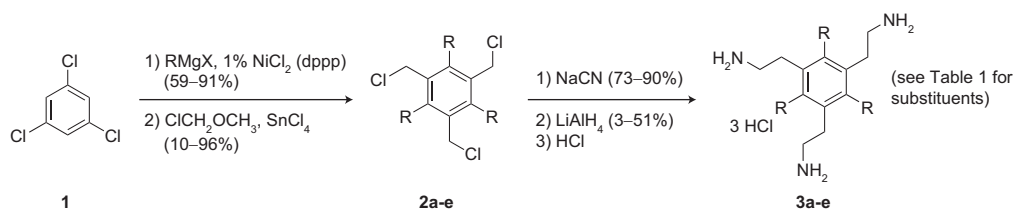


**Figure 1. Considerations in CBI design.** Amphipathic benzenes (center and right) based on the 1,3,5-substitution pattern of previously reported pyrimidine **A**. These amphipathic benzenes mimic the isobutyl side chains of the leucine residues of the amphipathic  $\alpha$ -helical LXXLL motif, and the ethyl amine groups are able to interact with the solvent and increase solubility.

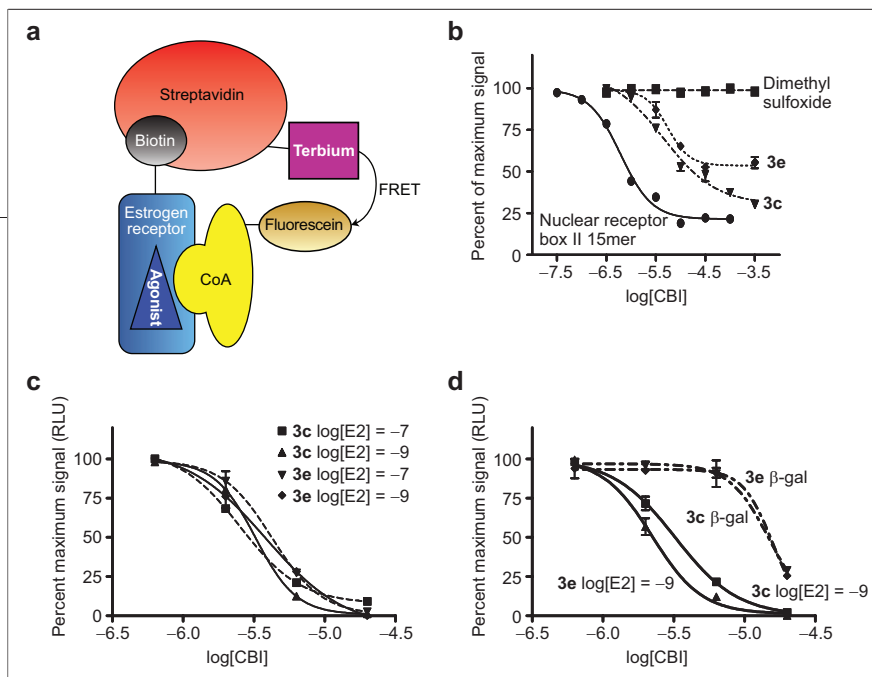
(TR-FRET) assay (Figure 2, panels a and b) to measure coactivator binding inhibition. Briefly, the ligand binding domain of ER $\alpha$  (residues 304–554; N-terminally His-tagged, with previously described C381,530S mutations that do not affect activity but leave one reactive cysteine) is site-specifically labeled (Cys417) (19) with biotin while bound to a nickel column during protein purification and subsequently tagged with a streptavidin-terbium complex. The SRC-3 nuclear receptor domain (NRD) (residues 627–829, which include all three NR-boxes) is nonspecifically labeled through the four available cysteines using 5-iodoacetamidofluorescein. When the ER is bound with a high concentration of the agonist estradiol, fluorescein-SRC3 (FRET acceptor,  $\lambda_{em}$  = 520 nm) is recruited to the terbium/estrogen receptor complex (FRET donor,  $\lambda_{em}$  = 495 nm), and a high FRET signal is produced. Coactivator binding inhibitor activity is assayed by the ability of increasing concentrations of compound to compete for ER-SRC binding and

disrupt the energy transfer. As shown in Table 1, the tri-ethylamines **3c** and **3e** exhibit, respectively,  $K_i$ 's of 1.7 and 2.1  $\mu$ M, among the lowest reported for such inhibitors. Interestingly, *n*-pentyl-substituted **3c** shows a more complete blockade of SRC-NRD binding than does the *neo*-hexyl-substituted **3e** in this assay.

It is important to establish that these compounds act as coactivator binding inhibitors rather than conventional antagonists. When assayed in a radiometric competitive ligand binding assay with [ $^3$ H]estradiol, **3e** binds to the ligand binding pocket of ER $\alpha$  with an affinity approximately 1/1000 that of estradiol. Since the estradiol concentration in the FRET assays is 1  $\mu$ M, the concentration of CBI needed to displace estradiol from its pocket and exert conventional antagonistic effects would be 1 mM or roughly 550 times greater than the  $K_i$  measured for inhibition of coactivator binding by **3e**. This provides strong evidence that this compound is, in fact, working through a CBI mechanism.



**Scheme 1.**



**Figure 2.** FRET and transfection assays. **a)** Schematic representation of the TR-FRET assay. In the absence of a CBI, coactivator is recruited to the ER/streptavidin/Tb complex and FRET is observed; when a CBI binds to ER, the coactivator is displaced and FRET is lost. **b)** Time-resolved FRET assay of displacement of SRC3-NRD-Fl by positive control peptide (NR-box II 15mer) and aminoethyl-substituted CBIs (**3c** and **3e**). **c)** Dose–response of CBI inhibition of ER-mediated reporter gene activity, at two different concentrations of estradiol. **d)** Dose–response of CBI inhibition of ER interaction with SRC-3 in a mammalian two-hybrid assay. In both assays, a constitutively active  $\beta$ -galactosidase reporter is used to monitor any cell toxicity.

**Cell-Based Assays of Coactivator Binding and Transcription Inhibition.** To further establish their activity, we have assayed these compounds in cell-based assays of ER-mediated transcription. In the first, a human endometrial cancer (HEC-1) cell line that expresses nuclear receptor coactivators but contains no endogenous ER $\alpha$  was transfected with a full-length ER $\alpha$  expression vector, an estrogen-responsive luciferase reporter gene plasmid (2ERE Luc), and pCMV  $\beta$ -galactosidase ( $\beta$ -gal; internal control). The transfected cells were incubated with two different concentrations of estradiol and increasing concentrations of CBI; luciferase activity was then measured.

If the compound acts by a coactivator binding inhibition mechanism, that is, by directly competing with the SRCs, then a change in the concentration of estradiol should have no effect on the IC<sub>50</sub> of the compound. As seen in Figure 2, panel c and Table 1, both **3c** and **3e** have IC<sub>50</sub> values near 3  $\mu$ M in the presence of either 1 nM or 100 nM estradiol, indicating that the inhibition of transcription is, in fact, occurring by a coactivator binding inhibition mechanism. The internal control  $\beta$ -galactosidase is unaffected except at the highest concentrations of **3c** and **3e**, indicating that the suppressive effect of the CBIs on transcription is not due to cell toxicity.

We also measured the effect of the CBIs in a mammalian two-hybrid assay in which HEC-1 cells were transfected with pFR luciferase, pCMV  $\beta$ -gal, Gal4-SRC-1<sub>NRD</sub>, and ER $\alpha_{DEF}$ -VP16. The cells were then treated with estradiol and CBI and assayed for luciferase activity. As shown in Figure 2, panel d and Table 1, **3c** and **3e** inhibit the interaction between ER $\alpha$  and SRC-1 with an IC<sub>50</sub> of 2–3  $\mu$ M. The  $\beta$ -galactosidase controls, carried out as above, also support a mechanism not invoked by general cellular toxicity (see Figure 2, panel d). A 100-fold increase in estradiol concentration also resulted in superimposable inhibition curves for the two compounds (data not shown).

In conclusion, we have synthesized a series of amphipathic benzenes that directly inhibit the interaction of ER $\alpha$  and steroid receptor coactivators in both *in vitro* and cell-based experiments. These molecules build on and further establish the relatively simple 1,3,5-trisubstituted pharmacophore that we have previously described (6) and which could be useful in designing inhibitors of other helix–groove interactions. To date, these compounds are among the most potent inhibitors and the few known to work in a cellular context. Their extensive evaluation in transient transfection assays confirms their ability to interrupt estrogen receptor/coactivator interactions in a cellular context and paves the way for more advanced biological assays using cellular breast cancer models. Thus, they are prom-

**TABLE 1.** Summary of binding affinity and inhibitory potency data for aminoethyl-substituted CBIs

	R	TR-FRET assay ( $\mu$ M)	Reporter gene assay ( $\mu$ M)	Mammalian two-hybrid assay ( $\mu$ M)
NR Box II 15mer	n/a	0.23	n/a	n/a
<b>3a</b>	propyl	>50	>50	>50
<b>3b</b>	butyl	1.8	8.2	10.1
<b>3c</b>	pentyl	1.7	3.2	3.2
<b>3d</b>	hexyl	2.0	3.6	3.4
<b>3e</b>	neo-hexyl	2.1	3.8	2.2

using probes of an alternative mechanism of estrogen action that could be used to explore new directions for controlling hormone-dependent breast cancer.

## METHODS

**Luciferase Reporter Gene Assay.** Human endometrial cancer (HEC-1) cells were maintained in culture as described previously and transfected in 24-well plates (20). A mixture of Hank's balanced salt solution (HBSS, 50  $\mu$ L/well), Holo-transferrin (Sigma T1408) (20  $\mu$ L/well), and lipofectin (Invitrogen no. 18292-011) (5  $\mu$ L/well) were incubated at RT for 5 min. The DNA mixture was made by adding 200 ng of pCMV $\beta$ -galactosidase as internal control, 500 ng of the estrogen-responsive reporter gene plasmid 2ERE Luc, and 100 ng of full-length ER $\alpha$  expression vector with 75  $\mu$ L of HBSS per well and, after addition to the first mixture, allowed to incubate for 20 min at RT. The cell media was changed to Opti-MEM (350  $\mu$ L/well), and 150  $\mu$ L of the transfection mixture was added to each well. The cells were incubated at 37 °C in an incubator containing 5% CO<sub>2</sub> for 6 h. The medium was then replaced with fresh medium containing 5% charcoal-dextran-treated calf serum and the desired concentrations of ligands. Reporter gene activity was assayed at 24 h after ligand addition. Luciferase activity, normalized for the internal control  $\beta$ -galactosidase activity, was assayed as described previously (20).

In the initial screen, antagonist activity was determined at four concentrations, ranging from 20 to 0.6  $\mu$ M, in the presence of 10<sup>-9</sup> M estradiol (E<sub>2</sub>). Upon validation that compounds do act as antagonists, mechanism of action was examined by repeating the compound titration in the presence of both 10<sup>-7</sup> and 10<sup>-9</sup> M E<sub>2</sub>. Changing the concentration of E<sub>2</sub> 100-fold should not change the coactivator binding inhibitor IC<sub>50</sub> determined in the reporter gene assay, because its mode of action is through direct displacement of coactivator, not competition for estradiol binding.

**Mammalian Two-Hybrid Assay.** The plasmid pM-SRC-1<sub>NRD</sub> was constructed by releasing the insert from pVP16-SRC-1<sub>NRD</sub> with *EcoRI* and *HindIII* and subcloning into the pM vector that contains the Gal4 DNA binding domain (Clontech) between *EcoRI* and *HindIII* sites. ER<sub>DEF</sub> was subcloned into pVP16 vector (Clontech) by digestion with *EcoRI* and *MluI* of pM-ER<sub>DEF</sub> and insertion into pVP16 vector between *EcoRI* and *MluI* sites (21).

Human endometrial cancer (HEC-1) cells were maintained in culture as described and transfected in 24-well plates using lipofectin (20). HEC-1 cells were plated at 2  $\times$  10<sup>4</sup> per well in 24-well plates and transfected 24 h later with 1  $\mu$ g of pFR-Luc (Stratagene), 0.2  $\mu$ g of pCMV $\beta$ -Gal, 0.2  $\mu$ g of pM-SRC-1<sub>NRD</sub>, and 0.2  $\mu$ g of pVP16-ER<sub>DEF</sub>. At 8 h after transfection, cells were treated with ligand or control vehicle. Cells were harvested 24 h after ligand treatment, and cell extracts were prepared.  $\beta$ -Galactosidase activity and luciferase activity were assayed as described previously (20).

**TR-FRET CBI Assay.** Purified biotin-ER $\alpha$ -417 and fluorescein-SRC3-NRD were used in the TR-FRET assays. A portion (5  $\mu$ L) of a stock solution of ER $\alpha$ -417 (8 nM), estradiol (4  $\mu$ M), and LanthaScreen streptavidin-terbium (Invitrogen) (2 nM) in TR-FRET buffer (20 mM Tris, pH 7.5, 0.01% NP40, 50 mM NaCl) was placed in separate wells of a black 96-well Molecular Devices HE high efficiency microplate (Molecular Devices, Inc.). In a second 96-well Nunc polypropylene plate (Nalge Nunc International), a 0.02 M solution of the coactivator binding inhibitor was serially diluted in a 1:10 fashion into DMF. Each concentration of coactivator binding inhibitor was then diluted 1:10 into TR-FRET buffer, and 10  $\mu$ L of this solution or vehicle was added to the stock estrogen receptor  $\alpha$  solution in the 96-well plate. After a 2-min incubation, 5  $\mu$ L of 200 nM fluorescein-SRC3-NRD was added to each well. This mixture was allowed to incubate for 1 h at RT in the dark. TR-FRET was measured using an excitation filter at 340/10 nm, and emission filters for terbium and fluorescein at 495/20 and 520/25 nm, respectively. The final concentrations of the reagents were as follows: ER $\alpha$ -417 (2 nM), streptavidin-terbium (0.5 nM), estradiol (1  $\mu$ M), coactivator binding inhibitor (0–1 mM), SRC3-NRD (50 nM) (6).

**General Procedure for the Preparation of 1,3,5-Trialkylbenzenes.** 1,3,5-Trichlorobenzene and [1,3-bis(diphenylphosphino)propane]-dichloronickel(II) were combined with diethyl ether. The alkyl Grignard reagent was added, and the solution began to reflux and turned from orange to brown. The reaction was maintained at reflux overnight. After being cooled to RT, the reaction mixture was poured into cold 1 M HCl. The aqueous layer was exhaustively extracted with ether. The combined organic extracts were dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo* to an oil, which was purified through a short silica gel column, with hexanes as the eluant.

**General Procedure for the Preparation of 1,3,5-Tris(chloromethyl)-2,4,6-trialkylbenzene Derivatives.** 1,3,5-Trialkylbenzene (0.60 mmol) was dissolved in methylene chloride (2.3 mL). Upon cooling to 0 °C, chloromethyl methyl ether (420  $\mu$ L, Aldrich) was added, followed by the slow addition of tin(IV) chloride (1 mL). The reaction was then stirred at 0 °C for 3 h and then overnight at RT. After quenching with water at 0 °C and extracting exhaustively with chloroform, the organic layer was dried with sodium sulfate, filtered, and then concentrated to give a green foam. The product was recrystallized from absolute ethanol.

**General Procedure for the Preparation of 1,3,5-Tris(cyanomethyl)-2,4,6-trialkylbenzene Derivatives.** Chloromethyl intermediate **2** (0.21 mmol) was dissolved in acetone (5.6 mL). Sodium cyanide (0.94 mmol) was added, followed by water (2.3 mL). To the thick mixture was added acetone (0.3 mL). The reaction was stirred at reflux for 17 h. After cooling to RT, the reaction mixture was poured over ice and filtered to isolate the product.

**General Procedure for the Preparation of 1,3,5-Tris(aminoethyl)-2,4,6-trialkylbenzene Derivatives.** Nitrile intermediate (0.22 mmol) was dissolved in 3 mL of THF. Lithium aluminum hydride (1 mL of 1 M in THF, 1.00 mmol) was added, and the solution was refluxed overnight. As the reaction progressed, the color changed from yellow to red. The THF was removed under vacuum, and 3 mL of ether was added in its place. The reaction was quenched by adding sequentially 40  $\mu$ L of water, 40  $\mu$ L of 3 M NaOH, and 120  $\mu$ L of water. The slurry was filtered, and the filtrate was dried over MgSO<sub>4</sub>, filtered, and concentrated to a yellow semisolid. The amine was taken up in dry CH<sub>2</sub>Cl<sub>2</sub> and was converted to the HCl salt by the addition of 1 M HCl in ether. The precipitated product was collected by filtration after cooling to -15 °C.

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**Supporting Information Available:** This material is free of charge via the Internet.

## REFERENCES

- Shiau, A. K., Barstad, D., Loria, P. M., Cheng, L., Kushner, P. J., Agard, D. A., and Greene, G. L. (1998) The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen, *Cell* 95, 927–937.
- Brzozowski, A. M., Pike, A. C. W., Dauter, Z., Hubbard, R. E., Bonn, T., Engstrom, O., Ohman, L., Greene, G. L., Gustafsson, J. A., and Carlquist, M. (1997) Molecular basis of agonism and antagonism in the oestrogen receptor, *Nature* 389, 753–758.
- Pike, A. C. W., Brzozowski, A. M., Walton, J., Hubbard, R. E., Thorsell, A. G., Li, Y. L., Gustafsson, J. A., and Carlquist, M. (2001) Structural insights into the mode of action of a pure antiestrogen, *Structure* 9, 145–153.
- Schiff, R., Massarweh, S. A., Shou, J., Bharwani, L., Arpino, G., Rimawi, M., and Osborne, C. K. (2005) Advanced concepts in estrogen receptor biology and breast cancer endocrine resistance: implicated role of growth factor signaling and estrogen receptor coregulators, *Cancer Chemother. Pharmacol.* 56, 10–20.
- Zhou, H. B., Collins, M. L., Gunther, J. R., Comminos, J. S., and Katzenellenbogen, J. A. (2007) Bicyclo[2.2.2]octanes: close structural mimics of the nuclear receptor-binding motif of steroid receptor coactivators, *Bioorg. Med. Chem. Lett.* 17, 4118–4122.
- Rodríguez, A. L., Tamrazi, A., Collins, M. L., and Katzenellenbogen, J. A. (2004) Design, synthesis, and *in vitro* biological evaluation of small molecule inhibitors of estrogen receptor  $\alpha$  coactivator binding, *J. Med. Chem.* 47, 600–611.

7. Becerril, J., and Hamilton, A. D. (2007) Helix mimetics as inhibitors of the interaction of the estrogen receptor with coactivator peptides, *Angew. Chem., Int. Ed.* **46**, 4471–3.
8. Shao, D., Berrodin, T. J., Manas, E., Hauze, D., Powers, R., Bapat, A., Gonder, D., Winneker, R. C., and Frail, D. E. (2004) Identification of novel estrogen receptor  $\alpha$  antagonists, *J. Steroid Biochem. Mol. Biol.* **88**, 351–360.
9. Norris, J. D., Paige, L. A., Christensen, D. J., Chang, C.-Y., Huacani, M. R., Fan, D., Hamilton, P. T., Fowlkes, D. M., and McDonnell, D. P. (1999) Peptide antagonists of the human estrogen receptor, *Science* **285**, 744–746.
10. Arkin, M. R., and Wells, J. A. (2004) Small-molecule inhibitors of protein-protein interactions: progressing towards the dream, *Nat. Rev. Drug Discovery* **3**, 301–317.
11. Pagliaro, L., Felding, J., Audouze, K., Nielsen, S. J., Terry, R. B., Krog-Jensen, C., and Butcher, S. (2004) Emerging classes of protein-protein interaction inhibitors and new tools for their development, *Curr. Opin. Chem. Biol.* **8**, 442–449.
12. Yin, H., and Hamilton, A. D. (2005) Strategies for targeting protein-protein interactions with synthetic agents, *Angew. Chem., Int. Ed.* **44**, 4130–4163.
13. Wang, S., Yang, D., and Lippman, M. E. (2003) Targeting Bcl-2 and Bcl-XL with nonpeptidic small-molecule antagonists, *Semin. Oncol* **30**, 133–142.
14. Heery, D. M., Kalkhoven, E., Hoare, S., and Parker, M. G. (1997) A signature motif in transcriptional coactivators mediates binding to nuclear receptors, *Nature* **387**, 733–736.
15. Voo, J. K., Lam, K. C., Rheingold, A. L., and Riordan, C. G. (2001) Synthesis, characterization and reactivity of copper(I) imidazole complexes based on a cavitand ligand design, *J. Chem. Soc., Dalton Trans.* 1803–1805.
16. Yun, S. G., Kim, Y. O., Kim, D., Kim, H. G., Ihm, H., Kim, J. K., Lee, C. W., Lee, W. J., Yoon, J., Oh, K. S., Yoon, J., Park, S. M., and Kim, K. S. (2003) Rational design of biologically important chemosensors: a novel receptor for selective recognition of acetylcholine over ammonium cations, *Org. Lett.* **5**, 471–474.
17. Grawe, T., Schrader, T., Zadmand, R., and Kraft, A. (2002) Self-assembly of ball-shaped molecular complexes in water, *J. Org. Chem.* **67**, 3755–3763.
18. Kilway, K. V., and Siegel, J. S. (2001) Control of functional group proximity and direction by conformational networks: synthesis and stereodynamics of persubstituted arenes, *Tetrahedron* **57**, 3615–3627.
19. Tamrazi, A., Carlson, K. E., Daniels, J. R., Hurth, K. M., and Katzenellenbogen, J. A. (2002) Estrogen receptor dimerization: ligand binding regulates dimer affinity and dimer dissociation rate, *Mol. Endocrinol.* **16**, 2706–2719.
20. Sun, J., Meyers, M. J., Fink, B. E., Rajendran, R., Katzenellenbogen, J. A., and Katzenellenbogen, B. S. (1999) Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor- $\alpha$  or estrogen receptor- $\beta$ , *Endocrinology* **140**, 800–4.
21. Martini, P. G., Delage-Mourroux, R., Kraichely, D. M., and Katzenellenbogen, B. S. (2000) Prothymosin  $\alpha$  selectively enhances estrogen receptor transcriptional activity by interacting with a repressor of estrogen receptor activity, *Mol. Cell. Biol.* **20**, 6224–32.