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# 3,3'-Disubstituted bipolar biphenyls as inhibitors of nuclear receptor coactivator binding

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# ABSTRACT

A series of bipolar biphenyl compounds was synthesized as proteomimetic analogs of the LXXLL pentapeptide motif responsible for the binding of coactivator proteins to the nuclear hormone receptor coactivator binding domain. These compounds were subjected to multiple in vitro assays to evaluate their effectiveness as competitive binding inhibitors. The results from this initial study indicate that these proteomimetics possess the ability to inhibit this protein-protein interaction.

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Modulation of nuclear receptor (NR) mediated gene transcription has exhibited great potential in the treatment and prevention of hormone responsive cancers, including breast and prostate cancers.<sup>1</sup> Traditionally, this effect has been accomplished through modulation of endogenous hormone levels or by targeting the NR via competitive inhibition of the hormone binding event at the ligand binding domain (LBD). NR antagonism at the LBD works by inducing a unique conformational change in which helix 12 rotates directly into the coactivator binding domain (CBD), blocking the binding of specific coactivator proteins that are essential to recruiting the necessary framework for gene transcription.<sup>2</sup> A number of cancer therapeutic agents, such as tamoxifen and bicalutamide, have achieved clinical success by targeting the LBD of estrogen receptor-alpha (ER $\alpha$ ) and androgen receptor (AR) respectively. However, these therapies elicit undesirable side-effects due to their inability to localize in specific tissues. Additionally, prolonged administration of these agents often results in the development of drug resistant disease strains, providing further challenges to the ongoing treatment and prevention of breast and prostate cancer.<sup>1,3</sup>

As a result, there is a recent trend toward the development of coactivator binding inhibitors (CBI) in place of traditional LBDantagonists.<sup>4</sup> CBIs concede the initial hormone binding to the LBD and instead target the protein-protein interactions of co-activator proteins and the NR-CBD.<sup>5</sup> An alpha-helical pentapeptide domain, termed the NR box, governs NR-coactivator binding and generally consists of a conserved LXXLL motif, where L represents a leucine and X represents any amino acid. When bound to the

\* Corresponding author. *E-mail address:* r.hanson@neu.edu (R.N. Hanson). ER $\alpha$ -CBD, the first and third leucine residues of the coactivator protein's NR-box protrude into the hydrophobic groove of the CBD, while the second leucine residue rests along it. Analogously, due to the enlarged hydrophobic groove characteristic of the AR-CBD, the AR generally prefers an FXXLF variant of the NR-box, where F represents the larger phenylalanine residue.<sup>6</sup> Flanking the hydrophobic groove of the CBD are charged residues (lysine and glutamic acid) which align with the intrinsic dipole of the alpha helical backbone of the NR box to create a 'charge clamp' that locks the coactivator in place.<sup>2b,5d,7</sup> Based on the ability of NRs in different tissue to select specific coactivator proteins to serve diverse biological functions, we suggest that inhibition at the CBD will enhance our capacity to control tissue selectivity, thereby preventing the further recruitment of the transcription apparatus and effectively halting tumor proliferation.

Peptide fragments have in the past been demonstrated to be capable of the NR-coactivator interaction in vitro, but such an approach presents numerous challenges with regards to bioavailability and selectivity.<sup>8</sup> Small molecule CBIs have since been synthesized and exhibit modest binding affinity.<sup>9</sup> These small molecule mimics offer a unique opportunity to disrupt NR-coactivator binding while retaining those physical properties typically inherent to effective pharmaceuticals.<sup>10</sup> In an effort to introduce conformational flexibility to this small-molecule approach, appropriately substituted biaryls have been introduced as mimics of amphipathic alpha-helical peptides, and the use of such compounds as inhibitors of alpha-helix mediated protein-protein interactions is supported by preliminary theoretical and experimental data.<sup>11</sup>

We recently described the design and synthesis of a small initial series of symmetrically substituted bipolar bis-4, 4'-oxybiphe-

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**Figure 1.** Reagents and conditions: (a) tetrabutylammoniumtribromide, CHCl<sub>3</sub>, rt, 100%; (b) PdCl<sub>2</sub>(dppf), B<sub>2</sub>pin<sub>2</sub>, dioxane, 80 °C, 95%; (c) 2-Etoxycarbonylethyl bromide, NaH, THF, rt, 92%; (d) PdCl = (PPh<sub>3</sub>)<sub>2</sub>, PPh<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub>, THF/H<sub>20</sub> (4:1), reflux, 65%; (e) 2-chloro-*N*,*N*-dimethlethlamine hydrochloride, K<sub>2</sub>CO<sub>3</sub>, acetone, reflux, 60%; (f) 1 N NaOH, EtOH or MeOH, 40 °C; (g) 4 N HCl, EtOAc or dioxane, rt, 85%; <sup>a</sup> **Ga** was analogously prepared using commercially available 4-iodophenol and 4-hydroxyphenylboronic acid as starting material.

nyl proteomimetics (Fig. 1) as novel ERa coactivator binding inhibitors.<sup>12</sup> A combinatorial approach was used to allow for synthesis of a large, diverse library from relatively few starting materials. Commercially available 2-substituted phenols (2b-e), substituted with isopropyl, sec-butyl, tert-butyl and benzyl, were selectively brominated using tetrabutylammonium tribromide to yield the substituted 4-bromophenols (3b-e) in excellent yields. These important intermediates were then converted to (4b-e) and (5b-e) via Miyaura and Williamson ether conditions respectively. The biphenyl phenolic-ester series (6a-e) was synthesized from the coupling of identically substituted 4 bromobenzene and **5** arylboronic ester under Suzuki conditions. utilizing PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub> as a catalyst. A tertiary amine functionality was subsequently introduced via Williamson ether synthesis of the biphenyl phenolic-esters with *N*,*N*-dimethylaminoethylchloride to yield biphenyl amino-esters 7a-e. Finally, the C-terminal functionality was varied by hydrolyzing the ethyl ester to the corresponding carboxylic acid and the resultant biphenyl amino-acid was isolated as the hydrochloride salt (1a-e). Initial biological studies probed the effect of these compounds on antagonizing the ERa-coactivator interaction and demonstrated that some of the final amino-carboxy products **1a–e** were significant inhibitors at the ER $\alpha$ -CBD. Compound 1c emerged as a lead compound from this initial analysis, demonstrating low micromolar affinity for ERa in TR-FRET binding assays and the capability to disrupt NR-coactivator binding interactions when evaluated in  $ER\alpha$  reporter gene assays in Hec-1 cells. This evaluation clearly demonstrated that the identity of hydrophobic substituents plays an integral role in influencing binding affinity, however, further analysis remained necessary to investigate the significance of the terminal functionalities.

In this study we evaluated the significance of the terminal groups in competitively inhibiting coactivator binding to the ER $\alpha$ -CBD and the AR-CBD. These polar termini were designed to interact with polar residues in the CBD, mimicking the charge clamp typically observed between the CBD and backbone residues surrounding the NR box. Analyzing the biological effect of truncating the amino terminus or esterifying the carboxyl terminus would provide a basis for determining their contribution to affinity and selectivity. For this reason we evaluated the phenolic-esters **6a–e** and the amino-esters **7a–e** in addition to the final amino-acids **1a–e**.

To complement our previously reported preliminary biological analysis of the amino-acid series (1a-e), we subjected the same series to a mammalian two-hybrid competitive binding assay employing the estrogen receptor (VP16-ER $\alpha$ ) and the co-activator peptide pM-GRIP1 LxxLL2 (Table 1). This assay was used to assess whether the test compounds interrupt the interaction between ERα and the LXXLL2 peptide derived from the coactivator GRIP1. VP16-ERa and pM-GRIP1-LXXLL2 were transfected into HepG2 cells together with the reporter gene 5xGal4-Luc3 and a normalization control pCMV-  $\beta$  -gal as described.<sup>13</sup> Cells were then treated with serial twofold dilutions of test compounds +/-1 nM estradiol and incubated for 40 h before assaving. As these results illustrate, structural changes on the biphenyl core significantly affect the activity of the compounds. 1c and 1e, which have the largest substituents, inhibit the E2-induced association of the peptide with the receptor, which is consistent with our previously reported analysis which identified 1c as the most effective inhibitor of ERα-coactivator interactions, as assessed by both TR-FRET and reporter gene assays. Additionally, 1d illustrates agonist activity in the absence of E2 and inhibitory activity in the presence of E2, indicating that this compound is likely binding inside of the LBD. The unsubstituted biphenyl amino-ester 1a not only did not inhibit peptide binding in the presence of E2, but also was observed to promote association in the absence of E2. This is consistent with our previous finding that **1a** possesses low but observable affinity for the ER $\alpha$ -LBD. Additionally, compound **1b**, where *R* = isopropyl, has little effect on either association or inhibition. The most effective compounds in the competitive binding assays were the phenolic-esters **6b** and **6c**, where *R* = isopropyl and sec-butyl, respectively. The amino-ester series (7a-e) had little to no effect on binding.

A subsequent cellular assay was employed to evaluate the in vitro activity of the amino-acids to inhibit gene transcription in MCF-7 (ER+) human breast cancer cells (Table 2). MCF-7 cells were pre-treated with 20  $\mu$ M solutions of each compound for 2 h, followed by exposure to either vehicle or estradiol for 14 h. RNA was harvested and quantitative-PCR was used to determine the effects of these compounds on the expression of the known ER $\alpha$  target genes SDF1, PR, and PS2. IDH3A, which is not an ER $\alpha$  target gene, was used as a negative control to demonstrate the selectivity of these compounds.

**Table 1** ERα/GRIP1 LXXLL2 Interaction assay

Compd	Vehicle	1 nm Estradiol
6a	+	0
6b	0	-
6c	0	-
6d	+	0
6e	0	0
7a	+	0
7b	0	0
7c	+ <sup>a</sup>	_ <sup>a</sup>
7d	0 <sup>b</sup>	0 <sup>b</sup>
7e	+++	-
1a	+++	0
1b	0	0
1c	0	-
1d	+++	_
1e	0	_b
OH-tam	0	-

(+) Denotes a greater than 50% increase in reporter gene activity at compound concentration <20  $\mu M.$ 

(++) Denotes a greater than 50% increase in reporter gene activity at compound concentration between 20–50  $\mu M.$ 

(+++) Denotes a greater than 50% increase in reporter gene activity at compound concentration between >50  $\mu$ M.

(-) Denotes a greater than 50% decrease in reporter gene activity at compound concentration >50  $\mu M.$ 

(-) Denotes a greater than 50% decrease in reporter gene activity at compound concentration <between 20–50 µM.

(–) Denotes a greater than 50% decrease in reporter gene activity at compound concentration <20  $\mu M.$ 

(0) Denotes a less than 20% change in reporter gene activity. 4-Hydroxytamoxifen was used as an antagonist control.

<sup>a</sup> Denotes cell death at concentration greater than 50 μM.

<sup>b</sup> Denotes precipitation of compound from solution.

#### Table 2

qPCR analysis of endogenous ER target genes

Compd	SDF1 -/+ E2	PS2 -/+ E2	PR -/+ E2	IDH3A -/+ E2
1a	+/	+/0	+/	0/0
1b	0/-	0/0	0/-	0/0
1c	0/-	0/-	0/-	0/0
1d	+/	+/+	+/+	0/0
1e	0/-	0/0	0/-	0/0

(+) Denotes an >20% increase in target gene expression compared to 1 nM E2, (-) denotes a >20% inhibition of 1 nM E2 induced target gene expression, and (0) denotes no change in gene expression from vehicle.

At the concentration of 20  $\mu$ M, **1b** (iPr), **1e** (Bn) and **1c** (s-Bu) showed subtle inhibition of the expression of SDF1 and PR, further supporting evidence that these compounds are modest inhibitors of ER $\alpha$ -coactivators interactions, as observed in the competitive binding assay reported above as well as the preliminary biological analysis previously reported. None of the three compounds induced responses in the absence of estradiol. As would be predicted from the mammalian two-hybrid assays described above, compounds **1a** (H) and **1d** (t-Bu) functioned as agonists with respect to ER $\alpha$  target genes, further enhancing induction of PR and PS2.

We were interested in the effect of these compounds ason other NRs, in addition to ER $\alpha$ . The amino-acids **1a–e**, the corresponding amino-esters **7a–e** and the phenolic-esters **6a–e** were therefore evaluated for NR-CBD antagonism in an MMTV-Luc reporter gene assay in the CV-1 cell line transfected with exogenous AR (Table 3). Bicalutamide (Casodex<sup>®</sup>), a non-steroidal antiandrogen used to treat prostate cancer, was used as an antagonist control and R1881, a potent anabolic steroid, was used as an agonist to activate AR. CV-1 cells were transfected with MMTV-Luc, SG5-AR and pCMV- $\beta$ -gal then treated with test compounds in

Tabla	2	
Table	3	

AR responsive MMTV-Luc reporter gene assay in CV-1 cells

Compd Vehicle	1 nM R1881
<b>6a</b> 0	0
<b>6b</b> 0	0 <sup>a</sup>
6c +	0 <sup>a</sup>
6d +	0
<b>6e</b> 0	0
<b>7a</b> 0	0
<b>7b</b> 0	_
<b>7c</b> 0	_a
<b>7d</b> 0	_a
<b>7e</b> 0	_a
<b>1a</b> 0	0
<b>1b</b> 0	0
1c 0	0
<b>1d</b> 0	0
<b>1e</b> 0	0
Casodex 0	_
R1818 +	0

(+) Denotes an >20% activation of reporter gene activation compared to 1 nM R1881, (-) denotes a decrease in reporter gene activation and (0) denotes no change. Casodex was used as an antagonist control and R1881 was used as a positive control. All compounds are tested at <= 40  $\mu$ M.

<sup>a</sup> Denotes cell death at concentration greater than 50  $\mu$ M.

the presence or absence of 1 nM R1881 for 40 h. Luciferase assays were performed as described.<sup>14</sup> In the absence of R1881, most of the tested compounds did not induce reporter gene activation, but the phenolic-esters **6c** (s-Bu) and **6d** (t-Bu) showed slight gene activation, implying direct ligand binding to the AR-LBD. While the amino-acids **1a–1e**, and phenolic-esters **6a**, **6b**, **6d**, **6e**, were ineffective in blocking R1881-induced gene transfection, the amino-esters **7b–e** demonstrated significant inhibition at the doses tested, although in some cases toxicity was noted at the higher doses. The effectiveness of the amino-ester derivatives in comparison with the amino-acids and the phenolic-esters highlights the importance of further optimization of the design of our core scaffold itself, in addition to further optimizing hydrophobic substitution.

In summary, we've analyzed the phenolic-esters, amino-esters and amino-acid derivatives in this series of 3, 3'-biphenyls as ER $\alpha$ and AR CBIs. The phenolic-esters and the amino-acids containing larger substituents emerged as effective inhibitors of the ER $\alpha$ / GRIP1 interaction, while the amino-esters did not exhibit substantial activity. The amino-acids were additionally examined in MCF-7 cells and exhibited slight inhibition of ER $\alpha$  regulated genes. When evaluated in the AR responsive MMTV-Luc reporter gene assay, the amino-esters containing larger substituents exhibited the greatest inhibitory activity. The amino-acids performed poorly in this cell-based assay, in comparison with the amino-esters, highlighting the the importance of further optimizing the nature of the core scaffold and demonstrating the existence of subtle variations between the binding modes of the coactivator binding domains of ER $\alpha$  and AR.

This study provides evidence that this bipolar biphenyl scaffold is capable of disrupting the interactions of NRs and their respective coactivator proteins. Future optimization of this compound series is currently underway and favors the inclusion of non-ionized polar termini and large hydrophobic substituents.

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# Supplementary data

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Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09. 007.

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