α Helix Mimetics

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Helix Mimetics as Inhibitors of the Interaction of the Estrogen Receptor with Coactivator Peptides**

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The estrogen receptor (ER) is a transcription factor that mediates the expression of estrogen-activated genes. The ER has been associated with a variety of diseases including breast cancer, osteoporosis, and cardiovascular disease and is therefore an important target for therapeutic intervention.[1] Binding of an estrogen molecule to the ligand binding domain (LBD) of the ER ultimately leads to interaction with specific DNA promoters and recruitment of coactivator proteins. These coactivator proteins mediate the assembly of the transcriptional machinery and are therefore essential for expression of the ER-regulated genes. Traditionally, inhibition of the ER has been attempted by using antagonist molecules that bind to the LBD and trigger a conformational change that prevents the ER from recruiting the coactivator proteins.[2] An alternative and underexploited approach involves the small-molecule inhibition of the interaction between the estrogen-activated ER and the coactivator proteins.[3a,b] Importantly, it has been shown that an analogous strategy can be used to target other nuclear receptors.[3c]

The coactivator proteins possess multiple copies of a conserved LXXLL motif, also known as nuclear receptor box (where L is leucine and X is any amino acid including leucine). Extensive studies have shown that this short LXXLL sequence is necessary and sufficient for binding to the ER.[5] The X-ray structure of the ligand-bound ER and a fragment of the coactivator peptide, glutamate receptor interacting protein 1 (GRIP1; Figure 1), shows that the LXXLL peptide adopts an α-helical conformation where the leucine side chains in positions i and $i+4$ are projected into a hydrophobic groove on the ER surface while that in the $i+3$ position projects into a hydrophobic pocket (Figure 1b).[4] Additionally, the crystal structure suggests that interactions between the coactivator peptide backbone and the charged residues that flank the binding groove on the ER further stabilize the complex.

In the search for inhibitors of this interaction, various short peptide derivatives based on the LXXLL sequence have been shown to disrupt the ER–coactivator interaction.[6]

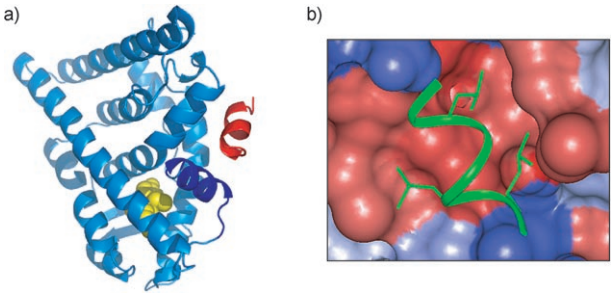

Figure 1. a) X-ray structure of ER LBD (blue) bound to GRIP1 coactivator peptide (red) and an agonist molecule (yellow). b) Magnified view of the GRIP1 peptide (green) on the surface of the ER (red = hydrophobic, blue = hydrophilic).[4]

However, there have been only two reports of small-molecule inhibitors with only one of them (with an inhibition constant (K_i) of 29 μM) designed to bind to this surface region of the ER and block the coactivator's approach.[3]

We have previously reported a broad strategy for the disruption of α-helix–protein interactions that involves the design of rigid scaffolds from which groups mimicking the surface functionality of an α helix can be projected.[7] For example, 2,3′,3″-trisubstituted terphenyls can mimic the i, $i+4$, and $i+7$ residues of two turns of an α helix and lead to potent inhibitors of protein–helix contacts such as those between Bcl-xL/Bak and MDM2/p53.[8] In the case of the coactivator LXXLL motif, a modified approach is needed to incorporate the features of the $i+3$ leucine. We and others have shown that this can be simply achieved by placing a second *ortho* substituent on a biaryl scaffold.[9] Separation of the elements of the $i+3$ side chain by a single methylene (as in Figure 2a) allows the adoption of a relative side chain conformation on the biaryl that closely mimics the distances and angular projections of the i, $i+3$, and $i+4$ groups of an α helix.

We synthesized a series of substituted pyridylpyridone derivatives as shown in Figure 2b, with the expectation that the bis-heteroaryl scaffold will have improved water solubility

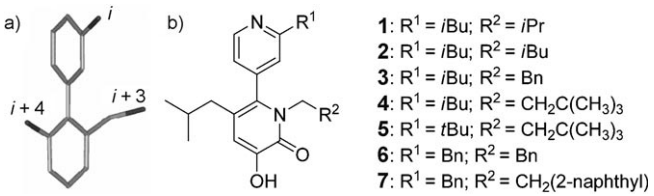

Figure 2. a) Trisubstituted biaryl scaffold mimicking the i, $i+3$, and $i+4$ residues of an α helix. b) Structure of the pyridylpyridone derivatives **1**–**7**.

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and bioavailability while allowing the ready introduction of substituents into the 2-pyridyl and 1,5-pyridone positions. Compounds **1**–**7** were synthesized through radical monoalkylation of *p*-cyanopyridine followed by the addition of a Grignard reagent to obtain the alkylpyridylketone derivative.[10] Imine formation followed by reaction with benzyloxyacetyl chloride and subsequent cyclization yielded the appropriately substituted pyridylpyridone.[11]

The crystal structure of **5** shows a nonplanar conformation (Figure 3a) with an aryl–aryl dihedral angle of 82° and distances of 5.6, 5.4, and 5.7 Å between the atoms that mimic

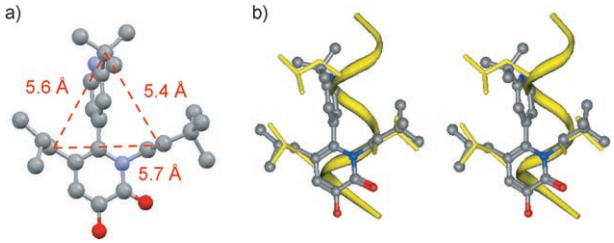

Figure 3. a) X-ray structure of **5**. b) Stereoview of the X-ray structure of **5** superimposed on the α-helical LXXLL motif of the GRIP1 peptide. Hydrogen atoms and nonrelevant amino acid side chains have been omitted for clarity.

the β carbons of the key amino acid residues. These distances are similar to those found in α-helical LXXLL motifs. The β-carbon atoms of the *i*, *i*+3, and *i*+4 leucine residues of the GRIP1 peptide superimposed with the corresponding carbon atoms of **5** showed good matching with a root-mean-square (rms) deviation of 0.36 Å (Figure 3b).

To test the effectiveness of these derivatives in blocking the ER–coactivator interaction, we used a fluorescent polarization (FP) assay based on rhodamine-labeled peptide D22 (Rho-LPYEGSLLLKLLRAPVEEV-COOH), which contains a single LXXLL motif.[5b] Displacement of this fluorescent peptide from the surface of estradiol-activated ER leads to a decrease in the polarization value. A control peptide that mirrors the second NR box of the coactivator protein SRC-1 (SRC-1 NR II) gave a K_i of 0.95 μM (Figure 4), which is comparable with the reported value of 1 μM.[3,12]

Evaluation of the binding affinities of the pyridylpyridone compounds showed that most were able to inhibit the ER–coactivator interaction with low micromolar activity (Table 1). As expected, all the compounds were soluble under assay conditions. Compound **2** most closely mimics the LXXLL sequence and showed good binding with $K_i = 16$ μM.

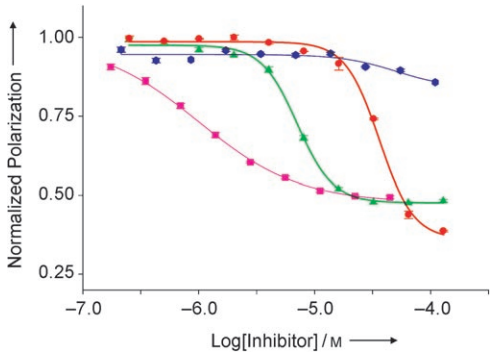

Figure 4. Titration curves for compounds **1** (red), **5** (blue), **6** (green), and control peptide SRC-1 NR II (pink).

Table 1: Results of the fluorescence polarization assay.[a]

Compound	K_i [μM]	Compound	K_i [μM]
SRC-1 NR II	1.0 (0.3)	**4**	>50
1	34 (3)	**5**	>50
2	16 (3)	**6**	6.5 (0.5)
3	9.4 (2.0)	**7**	4.2 (0.5)

[a] Active compounds were tested in triplicate in at least three independent experiments. The values in brackets are the corresponding standard deviations.

Shortening of the *i*+3-mimicking *N*-alkyl side chain by one methylene, as in **1**, led to a twofold decrease in affinity, supporting the conformational requirements for optimal *i*+3 and *i*+4 mimicry. Compounds **3** and **6** containing one and two benzyl groups, respectively, in the *i* and *i*+3 positions were based on previously reported peptidomimetics and gave K_i values of 9.4 and 6.5 μM, respectively.[6d] Introduction of a naphthyl group in **7** led to a further improvement with a K_i value of 4.2 μM. Finally, steric constrain of the hydrophobic pockets on the ER could explain the weak affinity of **4** and **5** in which one or both of the *i* and the *i*+3 groups were converted to bulkier *tert*-butyl and neopentyl groups.

Inhibition of coactivator binding by a small molecule could involve either direct competition with the coactivator peptide for the ER surface or binding of the small molecule to the ligand binding site as an antagonist, deactivating the ER and preventing coactivator recruitment.

To rule out the latter mechanism, a competitive radioligand assay with [^{3}H]estradiol was carried out to specifically measure the affinity of **3** for the estradiol binding site. According to the radioligand assay, **3** binds to this site with an affinity less than 0.001% of the affinity of 17β-estradiol. As the concentration of estradiol in the FP assays is kept constant at 2 μM, we estimated that compound **3** should have a K_i value of 300 mM, which is six orders of magnitude higher than the K_i value found in the FP assay (9.4 μM). From this, we conclude that our helix mimetics prevent the coactivator association through direct competition for its binding site on the ER surface.

In conclusion, a new α-helix mimetic based on a pyridylpyridone scaffold was designed to mimic the surface functionality of an α-helical LXXLL motif. Results from FP indicate that most compounds bind with K_i values in the low micromolar range. The most potent inhibitors had comparable affinity to that of the control SRC-1 NR II; a peptide that mirrors the second LXXLL motif of the natural SRC-1 coactivator. Extensive structure–activity relationship studies to improve the affinity of this scaffold as well as other structurally related molecules are currently underway.

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