

PPAR α were determined by chemical-mediated fluorescence energy transfer assays using the AlphaScreen Technology from Packard BioScience³⁰. The experiments were conducted with 5 nM PPAR α LBD of biotinylated peptide containing individual motifs (Fig. 3a), following the manufacturer's instructions for the hexahistidine detection kit in a buffer containing 50 mM MOPS, pH 7.4, 50 mM NaF, 0.05 mM CHAPS, 0.1 mg ml⁻¹ bovine serum albumin, and 10 mM dithiothreitol (DTT). The binding signals were detected with the increasing concentrations of GW6471, and the results from four repeated experiments were normalized as a percentage of the binding in the absence of GW6471.

The effects of GW6471 on the affinity of the SMRT or N-CoR peptides with purified PPAR α LBD were determined by fluorescence polarization in a buffer containing 10 mM HEPES, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% polysorbate-20, 5 mM DTT and 2.5% DMSO. Varied concentration of PPAR α LBD in the presence or absence of 40 μ M GW6471 were incubated at room temperature with 10 nM of a fluorescein-labelled peptide of N-CoR2 or SMRT2 (Fig. 3a). The fluorescence polarization values for each concentration of receptor were determined using a BMG PolarStar Galaxy fluorescence reader with 485 nm excitation and 520 nm emission filters. The apparent dissociation constant (K_d) values were determined by the binding curves derived from a nonlinear least-squares-fit of the data for a simple 1:1 interaction.

Mutational analysis of the SMRT co-repressor motif interaction with the PPAR α and TR β LBDs was also performed by fluorescence polarization. To determine the importance of each amino acid in the SMRT motif for binding to nuclear receptors, SMRT peptides with alanine substitution at each position were added to inhibit the binding of 1 μ M TR β LBD or 2 μ M PPAR α to the fluorescent N-CoR2 peptide. For the PPAR α experiments we added 10 μ M GW6471. The inhibition curves were constructed and IC₅₀ values were determined by nonlinear least-squares-fit of the data to a simple 1:1 interaction.

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Competing interests statement

The authors declare that they have no competing financial interests.

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(e-mail: ex11957@gs.k.com). The Protein Data Bank code for the PPAR α /GW6471/SMRT complex and the PPAR α /GW409544/SRC-1 complex is 1KQQ and 1K7L, respectively.

correction

Autonomic healing of polymer composites

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In this Letter, the middle infrared spectrum in Fig. 3b, corresponding to an authentic sample of poly(DCPD) prepared with Grubbs' catalyst and DCPD monomer, was a duplicate of the top spectrum owing to a formatting error. The corrected spectra are shown below. □

