Supplementary information for

“Activation of RXR/PPAR heterodimers by organotin environmental endocrine disruptors”

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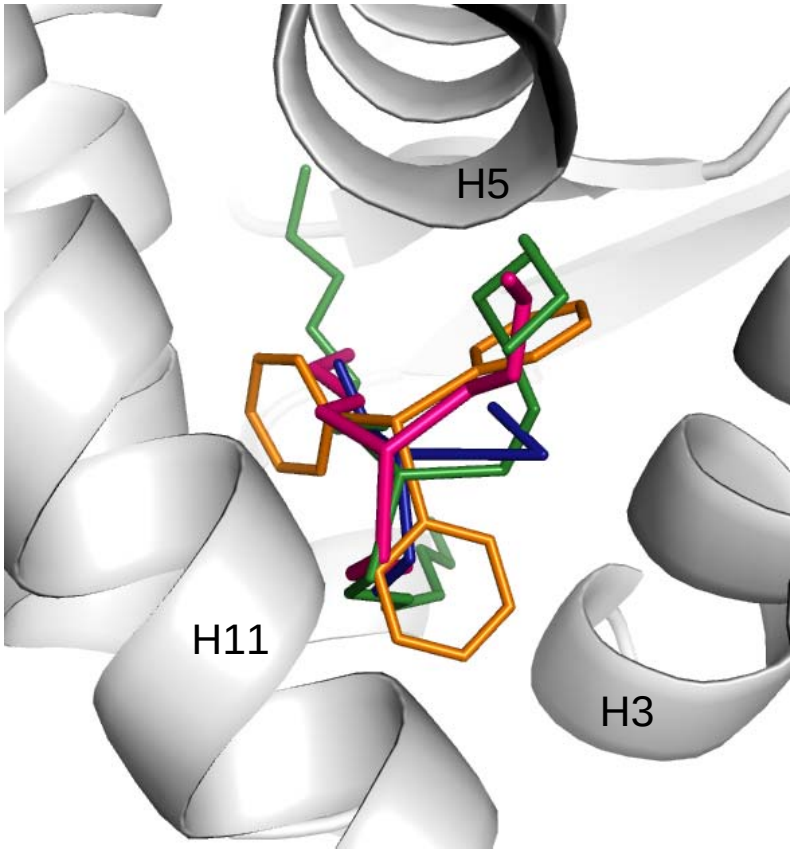

Fig S1 Comparison of the interaction between RXRα LBD and organotins. Superposition of TBT-A (pink), TPT (triphenyltin, orange), TET (triethyltin, blue) and TOT (trioctyltin, green) in stick representation in the TBT binding pocket shown in white.

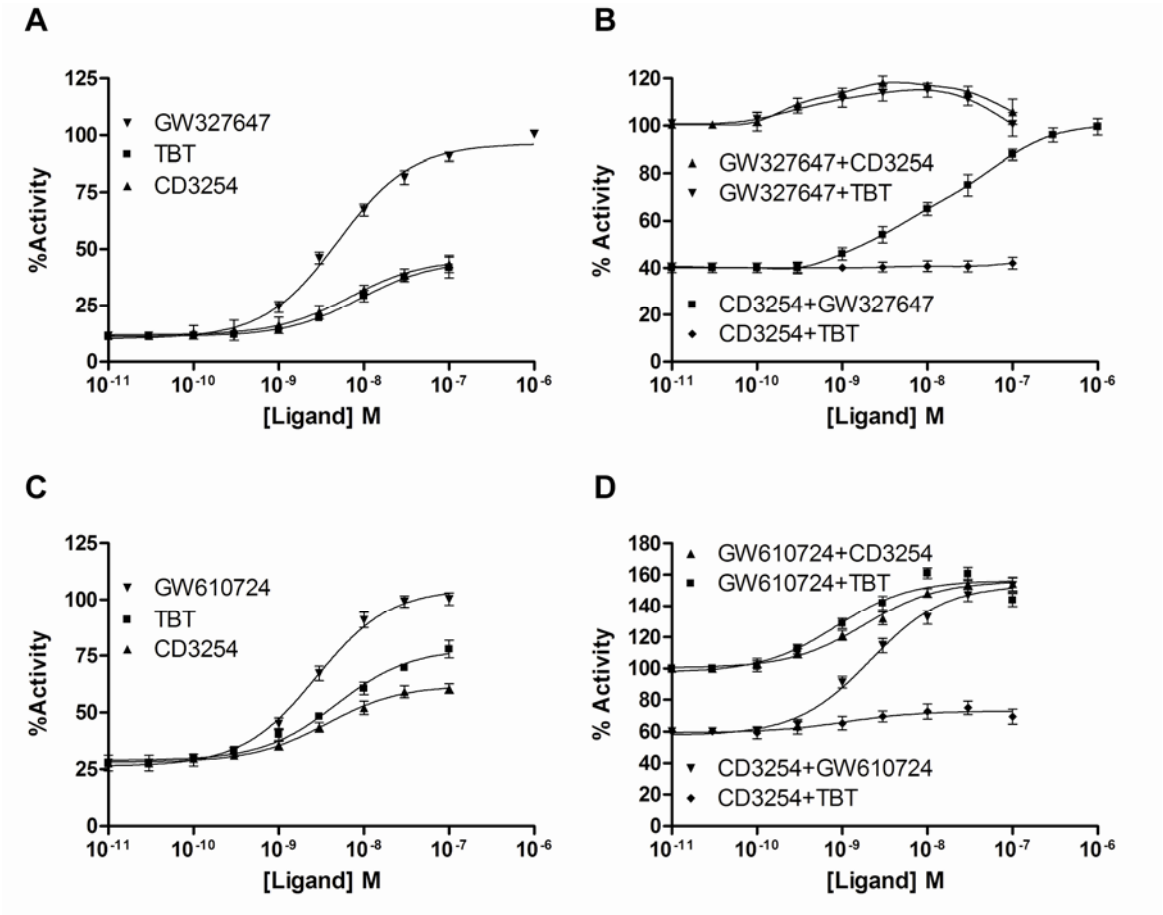

Fig S2 TBT activates RXR/PPARα and RXR/PPARδ heterodimers. (**A**) Stably transfected HELN PPARα cells were incubated with increasing concentrations of GW327647, CD3254 or TBT to measure their effect on the transcriptional activity of the heterodimer formed by Gal4-PPARα and endogenous RXR. (**B**) To measure the specific effect of the organotin on PPARα and RXR, GW327647, CD3254 or TBT were also tested in the presence of saturating concentrations of RXR (CD3254; 0.1 μM) or PPARα (GW327647; 1 μM) specific ligands. 100% activity corresponds to the activity obtained with 1 μM GW327647. (**C**) and (**D**) Same experiments with HELN PPARδ cells and GW610724 as the PPARδ specific agonist. To measure the specific effect of the organotin on PPARδ and RXR, GW610724, CD3254 or TBT were tested in the presence of saturating concentrations of RXR (CD3254; 0.1 μM) or PPARδ (GW610724; 0.1 μM) specific ligands.

Table S1 Data collection and refinement statistics

Data collection	
Space group	P43212
Cell dimensions a, b, c (Å)	64.03, 64.03, 111.88
Resolution range (Å)	30.77-1.90 (2.00-1.90) *
R_{sym}	0.062 (0.455) *
I/sI	25.9 (4.3) *
Completeness, %	98.0 (97.2) *
Redundancy	8.8 (9.2) *
Refinement	
Resolution range (Å)	28.63-1.90
Reflections used in refinement	17, 579
R (%) /R_{free} (%)	19.0 / 22.5
Total number of atoms	1,960
No. protein atoms	1,676
No. ligand atoms	26
No. water molecules	166
Average *B*-factor (Å^2)	25.2
Protein *B*-factor (Å^2)	24.2
Ligand *B*-factor (Å^2)	21.6
Water *B*-factor (Å^2)	33.2
Rmsd from ideality	
Bond lengths (Å)	0.011
Bond angles (°)	1.206
Ramachandran plot	
Most-favored regions (%)	95.4
Additionally allowed regions (%)	4.1
Generously allowed regions (%)	0.5
Disallowed regions (%)	0.0

*Values in parentheses are for highest resolution shell.

Table S2 Comparison of the binding pocket properties in RXRα-agonists (9cis-RA and CD3254) and RXRα-organotins (TBT, TPT, TET and TOT) complexes and comparison of the agonist ligands and organotin compounds ability to bind and activate RXRα. Ligand volumes were calculated using Vega ZZ (Pedretti et al., 2004). Organotins were manually docked in the TBT binding pocket and the complexes were energy-minimized using REFMAC5 from the CCP4 suite (CCP4, 1994). Protein ligand contacts (cutoff 4.2Å) were evaluated using CONTACT (CCP4, 1994).

Ligand	Ligand volume ($Å^3$)	Total contact numbers	Type of contacts (covalent/vdw /polar)	Activation and Binding	
				EC_{50} of GAL-RXR activity (nM)	IC_{50} for competition with $^{[3H]}$9cis-RA (μM)
TBT[a]	233	49	1 / 49 / 0	14.4 [e]	0.468 [e]
9cis-RA[b]	319	74	0 / 69 / 5	10.2 [e]	0.107 [e]
CD3254[c]	352	107	0 / 100 / 7	10.0[f]	-
TPT[d]	245	*82*	*1 / 81 / 0*	13.9 [e]	0.527 [e]
TET[d]	131	*21*	*(1) / 20 / 0*	not detectable [e]	6.76 [e]
TOT[d]	436	*103*	*(1) / 102 / 0*	not detectable [e]	>10 [e]

[a] The RXRα-TBT complex is described in this study.
[b] PDB code 1FBY (Egea et al., 2000).
[c] The structure of RXRα-CD3254 has been solved but not yet published (our results).
[d] These complexes were modeled from the structure of RXRα-TBT complex. Number and type of contacts are in italics to emphasize that they are only estimations.
[e] Nakanishi et al., 2005
[f] Nahoum et al., 2007

Supplementary METHODS

Preparation of stable PPAR cell lines. HGELN hPPARα, hPPARδ and hPPARγ cell lines were generated in two steps. Firstly, we generated the HGELN cell line by transfecting HeLa cells with the pGAL4RE-ERE-βGlob-Luc-SVNeo plasmid. Secondly, we transfected HGELN cells with pGAL4-PPAR-puro plasmids. pGAL4RE-ERE-βGlob-Luc-SVNeo contains a luciferase gene driven by a yeast activator GAL4 and an estrogen receptor binding site in front of the β-globin promoter and a neomycin phosphotransferase gene under the control of the SV40 promoter. In pGAL4-PPAR-puro (Seimandi et al, 2005), the encoded chimeric GAL4-PPAR proteins consists of 1-147 GAL4 amino acids followed by the human PPAR LBDs. (Puromycin N-acetyl transferase selection marker expression confers resistance to puromycin.

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