



In Vitro Identification of Subtype-selective Peroxisome Proliferator-activated Receptor (PPAR) Ligands using PPAR Reporter Cell Lines.

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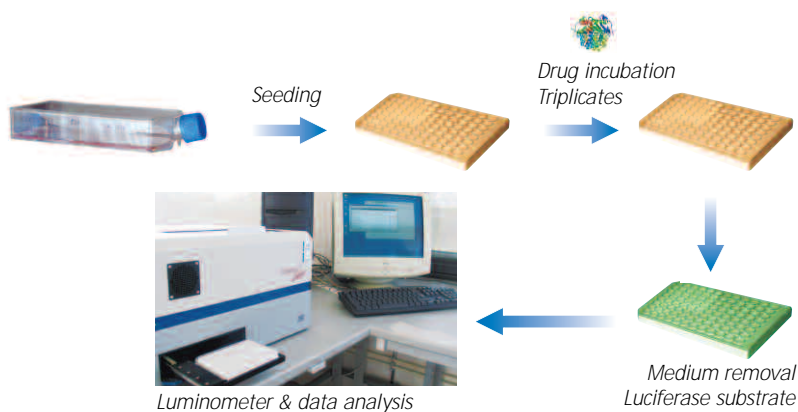
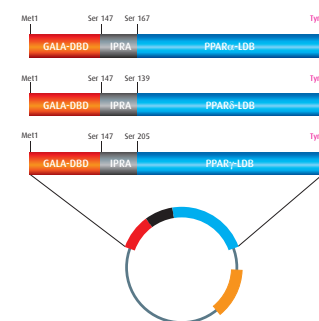
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Introduction & Purpose

The peroxisome proliferator-activated receptors (PPARs) are involved in many critical physiological and pathological functions. Therefore a cell-based assay for the identification of high-affinity ligands would be a useful tool for studying the role of PPAR in mammalian biology. To characterize PPAR specificity of synthetic ligands, we are using HeLa-derived stable reporter cell lines in which PPAR α , PPAR δ , and PPAR γ agonists induce luciferase activity.

Methods

Stable reporter cell lines were generated by Seimandi *et al.*, 2005. They were designed to express a chimeric protein containing the ligand binding domain (LBD) of either human PPAR α , human PPAR δ , or human PPAR γ fused to the yeast transactivator GAL4 DNA binding domain (DBD). The luciferase reporter gene was driven by a pentamer of the GAL4 recognition sequence in front of the β -globin promoter. A reference drug that lead to maximal activity has been established for each cell line, i.e. GW7647, L-165041 and BRL49653 for PPAR α , PPAR δ and PPAR γ , respectively.



Dose-response studies were carried out on each PPAR cell line as follows. The cells were seeded in 96-well plates and incubated the following day with tested compounds for 24h. At the end of the incubation, the luciferase activity was measured using a BMG LUMistar Galaxy luminometer. Results expressed as relative light units (RLU) were obtained from experiments performed in triplicate for each tested compounds and for each tested concentration. Data were shown as means and standard deviations. Values were expressed as percentages of luciferase activity with the 100% activity set for the reference drug at 1 μ M for the respective cell line.

Results

We observed that the expression of PPAR isotypes modulated differentially the reporter gene basal activity and provided interesting information on the recruitment of HeLa-specific coregulators by PPARs. Using specific PPAR agonists and antagonists, we demonstrated that these stable cell lines allow specific and sensitive measurement of PPAR ligand activities. They provide a powerful high-throughput cell-based screening tool for identifying and characterizing PPAR ligands.

The tested compounds were classified for each PPAR subtype as non agonist, partial agonist and total agonist by comparison to the known drug references and the solvent vehicle (0,1% DMSO) as a negative control (Table 1 & figure 1).

PPAR alpha						
10 ⁻⁶ M	Mean RLU	Standard deviation	% of activity	Non agonist	Partial agonist	Total agonist
ciglitazone	881	26	10,8	X		
GW9662	3304	220	40,5		X	
MCC-555	1300	36	15,9	X		
GW501516	2470	142	30,3		X	
GW7647	8162	210	100,0			X
L165041	1380	45	16,9	X		
BRL49653	714	23	8,7	X		
pioglitazone	955	102	11,7	X		
troglitazone	639	68	7,8	X		
DMSO	642	11	7,9	X		

PPAR gamma						
10 ⁻⁶ M	Mean RLU	Standard deviation	% of activity	Non agonist	Partial agonist	Total agonist
ciglitazone	4803	496	41,8		X	
GW9662	3345	307	29,1	X		
MCC-555	8491	1002	73,9		X	
GW501516	4179	90	36,4		X	
GW7647	10066	221	87,6	X		
L165041	5019	256	43,7		X	
BRL49653	11495	670	100,0			X
pioglitazone	12162	651	105,8			X
troglitazone	6350	390	55,2		X	
DMSO	2215	139	19,3	X		

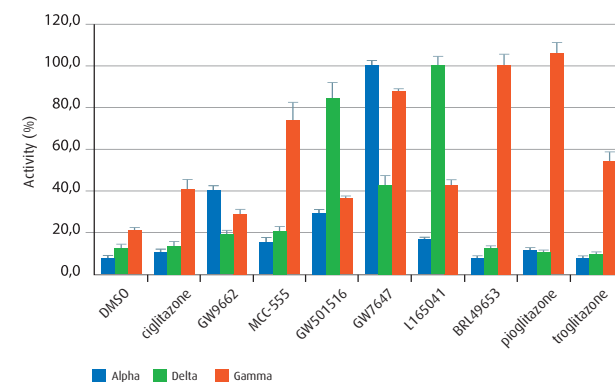


Figure 1: Percentage of luciferase activity of various ligands obtained with PPAR α , PPAR δ and PPAR γ cell lines.

PPAR delta						
10 ⁻⁶ M	Mean RLU	Standard deviation	% of activity	Non agonist	Partial agonist	Total agonist
ciglitazone	803	58	14,1	X		
GW9662	1103	80	19,4	X		
MCC-555	1201	111	21,1		X	
GW501516	4839	392	85,2		X	
GW7647	2452	232	43,2		X	
L165041	5678	260	100,0			X
BRL49653	723	53	12,7	X		
pioglitazone	651	84	11,5	X		
troglitazone	582	30	10,3	X		
DMSO	569	7	10,0	X		

Testing one concentration of a compound offers the possibility to define PPAR selectivity, but also, when compared to reference compounds, to classify your test article as non agonist, partial agonist or total agonist. A fast screening method on one point dilution!

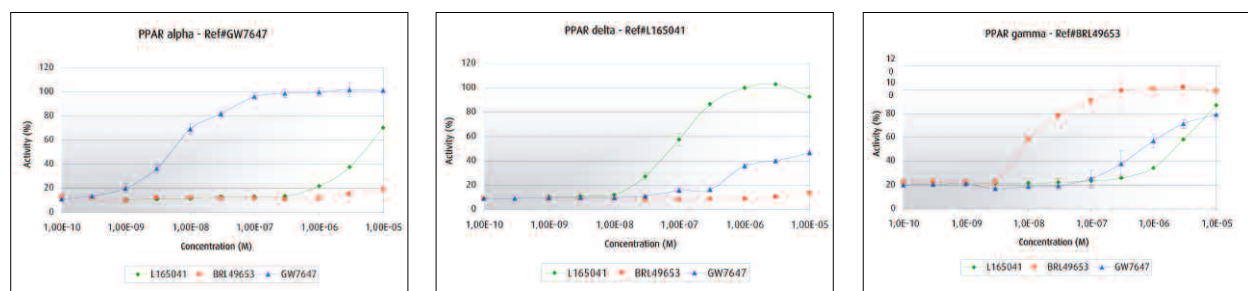


Figure 2: Dose-response curves of specific-subtype agonists in PPAR α , PPAR δ and PPAR γ cell lines.

The dose-response curves demonstrated the selectivity of activity for each PPAR isotype (Figure 2). Moreover, competition assays allow the characterization of subtype-specific antagonist activities (data not shown).

Conclusions

To characterise PPAR specificity of synthetic ligands, we are offering stable HeLa-derived reporter assays in which PPAR α , PPAR δ and PPAR γ agonists induce luciferase activity. This service is available via [tebu-bio services@tebu-bio.com](mailto:services@tebu-bio.com)

Reference: Seimandi *et al.*, Analytical Biochemistry (2005) 344, 8-15.