Insights into the mechanism of partial agonism: crystal structures of the peroxisome proliferator-activated receptor-gamma ligand-binding domain in the complex with two enantiomeric ligands

Giorgio Pochetti[1,a], Cristina Godio[2,a], Nico Mitro[2], Donatella Caruso[2], Samuele Scurati[2], Andrea Galmozzi[2], Fulvio Loiodice[3], Giuseppe Fracchiolla[3], Paolo Tortorella[3], Antonio Laghezza[3], Antonio Lavecchia[4], Ettore Novellino[4], Fernando Mazza[1,5], Maurizio Crestani[2]

From the [1]Istituto di Cristallografia, Consiglio Nazionale delle Ricerche, Montelibretti, Roma, Italia; [2]Laboratorio "Giovanni Galli" di Biochimica e Biologia Molecolare dei Lipidi e di Spettrometria di Massa, Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Milano, Italia; [3]Dipartimento Farmacochimico, Università degli Studi di Bari, Bari, Italia; [4]Dipartimento di Chimica Farmaceutica, Università degli Studi di Napoli, Napoli, Italia; [5]Dipartimento di Chimica, Ingegneria Chimica e Materiali, Università di L'Aquila, L'Aquila, Italia

ABSTRACT : The peroxisome proliferator-activated receptors (PPARs) are transcriptional regulators of glucose and lipid metabolism[1,2]. They are activated by natural ligands[3], such as fatty acids, and are also target of synthetic antidiabetic and hypolipidemic drugs[4,5].

We synthesized the two enantiomers of the novel compound, 2-(4-{2-[1,3-benzoxazol-2-yl(heptyl)amino]ethyl}phenoxy)-2-methylbutanoic acid, a conformationally constrained analogue of the well-known PPARα/γ agonist GW2331[3] (Fig. 1).

By using cell-based reporter assays, we studied the transactivation activity of the two enantiomers (Table 1, Fig.2).

In particular, we show that the *R*-enantiomer, (*R*)-1, is a full agonist of PPARγ, whereas the *S*-enantiomer, (*S*)-1, is a less potent partial agonist. These two molecules affect specifically the transcriptional activity of PPARα and γ subtypes, whereas the activity of other members of the nuclear receptor gene superfamily is not altered.

We also provide a molecular explanation for their different behavior as full and partial agonists of PPARγ by showing the crystal structures of the complexes of these new ligands with PPARγ ligand binding domain (LBD).

The analysis of the two crystal structures shows that the different degree of stabilization of the helix 12 induced by the ligand determines its behavior as full or partial agonist.

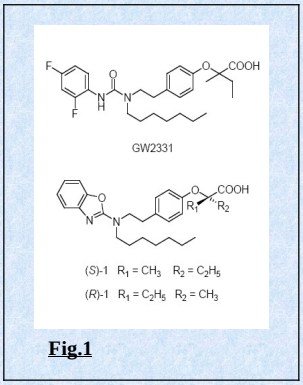

Fig.1

Table 1. Potency and efficacy of tested ligands towards PPARγ as determined in Gal4-based assays (top) and in cell-free co-activator recruitment assays (bottom).

Tested ligand	EC_{50} (nM)	Efficacy (%)
Rosiglitazone	3.8 ± 1.2	100.0 ± 10.5
(*R*)-1	73.3 ± 49.9	116.1 ± 9.0
(*S*)-1	593.0 ± 114.1	50.4 ± 5.1

Tested ligand	K_d (nM)	Δ_{max} (%)
Rosiglitazone	14.8 ± 6.6	100.0 ± 4.9
(*R*)-1	684.8 ± 106.8	70.5 ± 2.5
(*S*)-1	1.978 ± 523.5	23.3 ± 3.2

Values are mean ± SD. Efficacy values were calculated as percentage of the reference compound rosiglitazone.

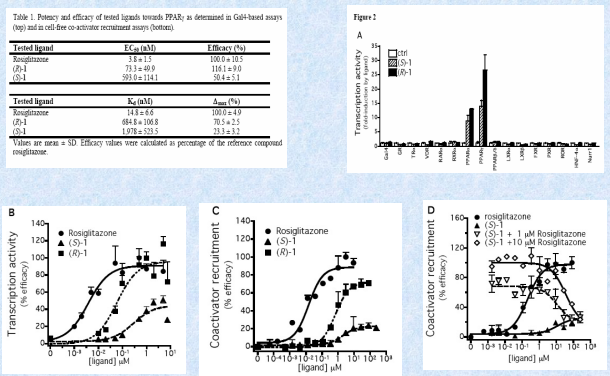

Fig. 2. (*R*)-**1** and (*S*)-**1** selectively activate PPARα and γ. The specificity of (*S*)-**1** (5 µM) and (*R*)-**1** (1 µM) was assessed in a co-transfection assay in HepG2 cells using expression vectors for Gal4-NR-LBD fusion proteins as indicated in (A). Concentration-response curves of rosiglitazone, (*S*)-**1** and (*R*)-**1** in HepG2 cells co-transfected with p5xGal4UAS reporter and pGal4-hPPARγ-LBD (B) and in co-activator recruitment assay (C). Antagonism of (*S*)-**1** (5 µM) against rosiglitazone (1 µM) in Gal4-based assay in HepG2 cells (D).

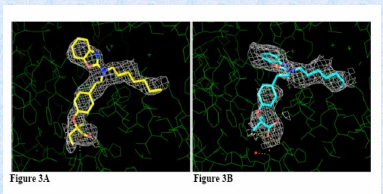

Fig. 3 (A) 2Fo-Fc electron density map calculated around the *R*-enantiomer (shown in yellow) and contoured at 1σ ; (B) 2Fo-Fc electron density map calculated around the *S*-enantiomer (shown in cyan) and contoured at 0.9σ.

Statistics of crystallographic data and refinement

	(*R*)-1	(*S*)-1
wavelength (Å)	1.0	1.0
temperature (K)	100	100
cell dimensions (Å)	93.14; 60.95; 118.11	93.54; 60.91; 118.35
beta angle (°)	103.26	103.08
resolution range (Å)	30.0-2.10 (2.17-2.10)[a]	30.0-2.10 (2.18-2.10)[a]
space group	C2	C2
no. of unique refl.	32491	36706
R_{sym} (%)	4.0	4.0
I/σ(I)	24.2 (3.1)[a]	25.8 (2.1)[a]
Completeness (%)	85.9 (77.8)[a]	96.3 (92.7)[a]
R-factor (%)	24.7	27.2
R_{free}	27.8	29.5
rmsd bonds (Å)	0.009	0.008
rmsd angles (deg)	1.335	1.619
n. of water mol.	187	116
n. of protein atoms	2165[b]	2165[b]
n. of ligand atoms	33[b]	33[b]

[a] The values in parentheses refer to the outer shell
[b] The value refers to one monomer

In figures **4-6** the principal features of the two complexes are compared, with particular regard to the factors stabilizing the helix 12.

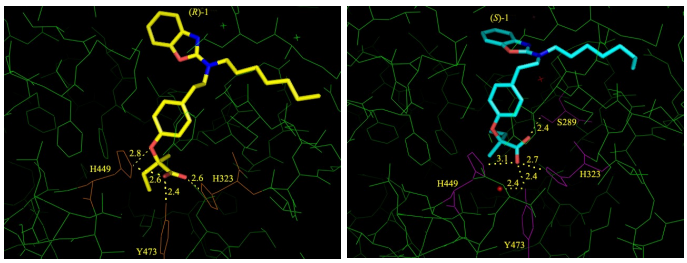

Fig. 4. **(A)** H-bonding network between the *R*-enantiomer (yellow) and the LBD of PPARγ (the triad H323, H449, Y473 is coloured in orange); **(B)** H-bonding network between the *S*-enantiomer (cyan) and the LBD of PPARγ (the triad and S289 are in purple).

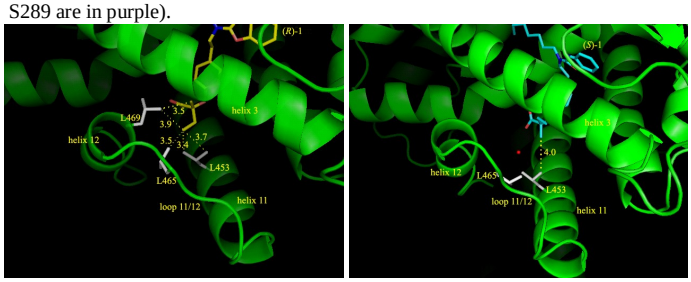

Fig. 5. **(A)** Hydrophobic interactions of the *R*-enantiomer (yellow) with Leu residues (white) of LBD; **(B)** Hydrophobic interactions of the *S*-enantiomer (cyan) with Leu residues (white) of LBD.

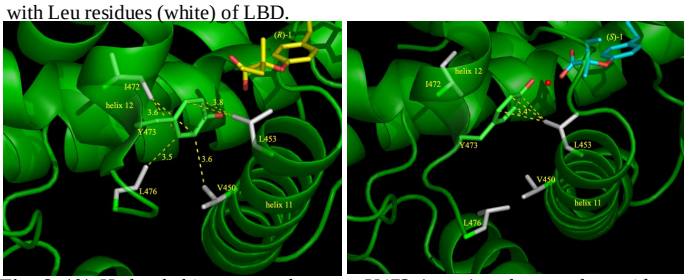

Fig. 6. **(A)** Hydrophobic contacts between Y473 (green) and non-polar residues (white) of the protein complex with the *R*-enantiomer (yellow); **(B)** Hydrophobic contacts between Y473 (green) belonging to H12 and apolar residues (white) of the protein complex with the *S*-enantiomer (cyan).

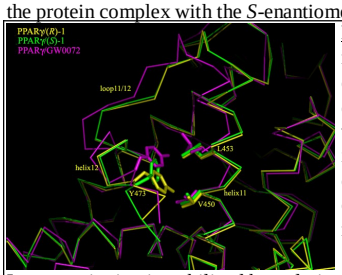

Fig. 8 represents a superposition of the receptor backbones adopted in the crystal complexes with our *R*- and *S*-enantiomers and with GW0072[6], a weak partial agonist, which does not interact at all with helix 12. There, it can be noted a progressive reorientation of the side-chain Y473, a critical residue belonging to the helix 12.

Its aromatic ring is stabilized by polarization interactions with both V450 and L453 residues in the complex with the almost full agonist *R*-enantiomer, whereas it faces only the L453 residue in the complex with the partial agonist *S*-enantiomer and the weak partial agonist GW0072. In the complex with GW0072 there is also an evident displacement of helix 11, which further destabilizes H12. The three crystal complexes can represent different states of stability of the helix 12, with the PPARγ/GW0072 clearly representing the less stable one.

References :

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In the crystal complex PPARγ/(*R*)-1 the active conformation of H12 is stabilized by the following interactions: a) both carboxylate oxygens of the ligand engage canonical H-bonds with the three residues H323, H449 and Y473 involved in the receptor activation (Figure 4A); b) the appropriate position of the Y473 aromatic side-chain is ensured by polarization interactions with I472 and L476 on one side, and with V450 and L453 on the other side (Figure 6A); c) the ligand methyl and ethyl groups form several favorable hydrophobic interactions with Leu residues of H11, H12 and the loop 11/12 (Figure 5A). Thus, the potency of the *R*-enantiomer is a direct consequence of a very effective stabilization of the helix 12, through hydrophobic and electrostatic interactions. Moreover, helix 12 is here stabilized in the proper conformation to recruit the coactivator, the same observed in other crystal structures of complexes with full agonists

In the complex with the *S*-enantiomer a 1 Å shift of the ligand away from helix 12 is observed. This is probably caused by a steric clash between the ligand ethyl group and the Q286 backbone of helix 3 (Figure 7). Even if the H12 conformation only slightly differs from that observed in the complex with the *R*-enantiomer, its stability appears completely different for the following aspects: a) only one of the carboxylate oxygens of the ligand engages H- bonds with the three residues H323, H449 and Y473 (Figure 4B); b) the 1 Å shift of the ligand reduces favorable hydrophobic contacts with helix 12 to only one (Figure 5B); c) a water molecule, situated between the ligand and hydrophobic residues of the loop 11/12, prevents further productive ligand-receptor binding interactions (Figure 7); d) the Y473 aromatic ring adopts a different orientation forming van der Waals interactions only with L453 of H11 (Figure 6B).

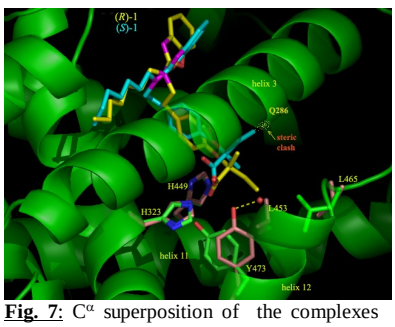

Fig. 7: C^{α} superposition of the complexes with the *R*- and the *S*-enantiomer (in yellow and cyan, respectively). Protein side-chains of the complex with the *R*-enantiomer are shown in green; the correspondent side-chains are in pink for the complex with the *S*-enantiomer.

Concluding remarks - **In the present work we argue that the partial agonist behavior of the *S*-enantiomer could be ascribed to a destabilization of the active conformation of helix 12. A suboptimal conformation of this helix was observed in the complex with the partial agonist, suggesting the coexistence in solution of transcriptionally active and inactive forms and probably explaining the dramatic lack of efficacy in co-activator recruitment and in transactivation activity.**