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Different structures of the two peroxisome proliferator-activated receptor gamma (PPARγ) ligand-binding domains in homodimeric complex with partial agonist, but not full agonist

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ABSTRACT

We designed and synthesized acylsulfonamide derivative (**3**) as a human peroxisome proliferator-activated receptor gamma (hPPARγ) partial agonist by structural modification of hPPARγ full agonist **1**. Co-crystallization of **3** with hPPARγ LBD afforded a homodimeric complex, and X-ray crystallographic analysis at 2.1 Å resolution showed that one of the LBDs adopts a fully active structure identical with that in the complex of rosiglitazone, a full agonist; however, the other LBD in the complex of **3** exhibits a different (non-fully active) structure. Interestingly, the apo-homodimer contained similar LBD structures. Intrigued by these results, we surveyed reported X-ray crystal structures of partial agonists complexed with hPPARγ LBD homodimer, and identified several types of LBD structures distinct from the fully active structure. In contrast, both LBDs in the rosiglitazone complex have the fully active structure. These results suggest hPPARγ partial agonists lack the ability to induce fully active LBD. The presence of at least one non-fully active LBD in the agonist complex may be a useful criterion to distinguish hPPARγ partial agonists from full agonists.

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Human peroxisome proliferator-activated receptors (hPPARs) are ligand-dependent transcription factors belonging to the nuclear receptor (NR) superfamily.[1] Three subtypes of hPPARs, that is, hPPARα, hPPARβ/δ, and hPPARγ have been identified to date. These subtypes are expressed differentially in a tissue-specific manner,[2] and contribute to pivotal biological responses.[3] For example, hPPARγ is mainly expressed in adipocytes and macrophages,[2] but is also found in various cancer cells, including breast cancer, gastric cancer and colorectal cancer.[4] It is a master regulator of adipocyte differentiation,[5] and also plays important roles in insulin sensitivity, cell cycle regulation, differentiation, inflammation, and immune responses.[3] Therefore, modulators of hPPARγ are candidates for treatment of various diseases. For example, thiazolidinedione (TZD) class hPPARγ full agonists are widely used for the treatment of type 2 diabetes, and are also under clinical trial for the treatment of Alzheimer's disease.[6] However, TZDs possess a number of adverse effects, including significant weight gain, peripheral edema, bone loss and increased risk of congestive heart failure, which are considered to be associated with over-activation of hPPARγ.[7] Therefore, attention has turned to hPPARγ partial agonists, which activate hPPARγ less than maximally.

In this Letter, we present the design and synthesis of a hPPARγ partial agonist (**3**), as well as the results of X-ray structure determination of its complex with hPPARγ LBD homodimer. The resulting structure is compared with that of the apo-homodimer and with the reported structures of hPPARγ LBD homodimer complexes of other hPPARγ partial agonists, as well as full agonists, in order to identify the structural basis of hPPARγ partial agonist activity.

For the design of **3**, we noted that the formation of a tight hydrogen-bonding network is critical for full agonistic nature of hPPARγ agonists, and so replacement of the carboxyl group of hPPARγ agonist with other functional groups, such as acylsulfonamide, is one option to obtain hPPARγ partial agonists.[8–10] Therefore, we focused on modification of the structure of the hPPARγ–selective agonist MEKT-21 (**2**), which is structurally derived from hPPARγ–pan agonist TIPP-703 (**1**), to remove the carboxyl group and introduce a sulfonyl group, as shown in Figure 1.

The synthetic route to **3** is shown in Figure 2(A). Salicylaldehyde **10** was *n*-propylated to give **5**. Compound **5** was treated with hydroxylamine HCl, and subsequent reduction with 10% Pd on carbon afforded aminomethylbenzene derivative **6** as the hydrochloric acid salt. Compound **6** was condensed with

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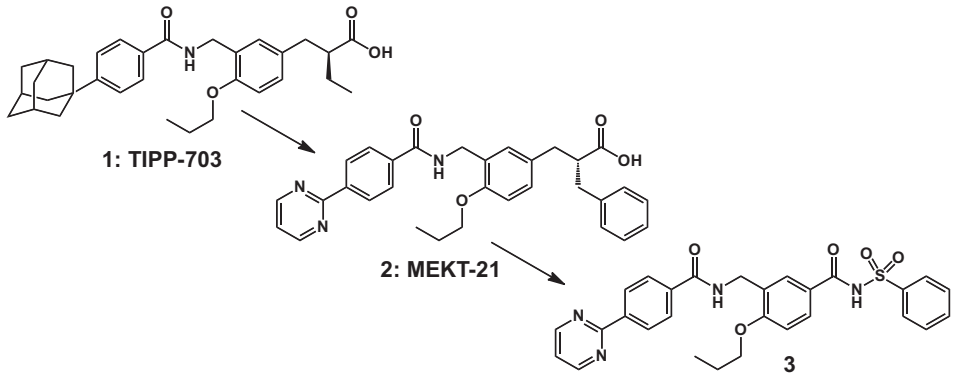

Figure 1. Structural development of hPPARγ pan agonist (**1**) to hPPARγ-selective partial agonist (**3**).

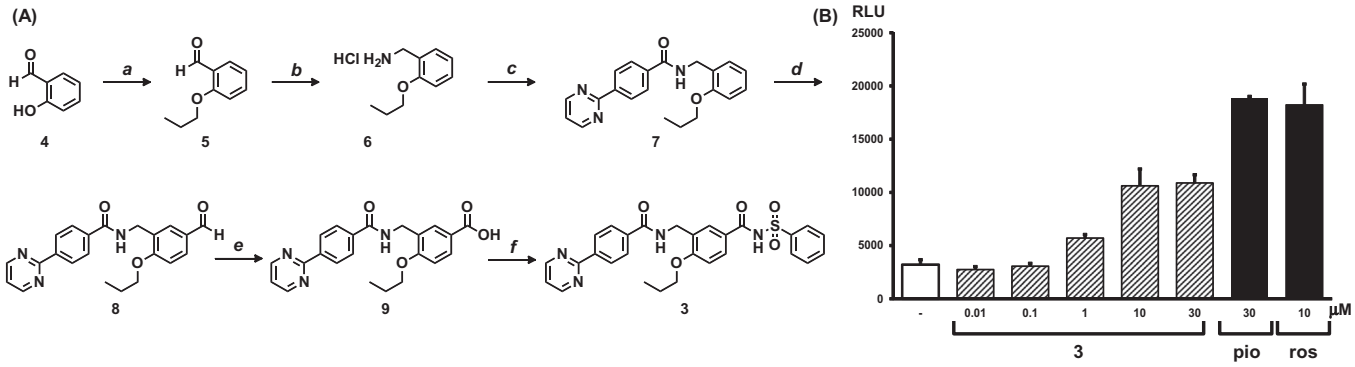

Figure 2. Synthetic routes to **3**, and dose-response relation for hPPARγ agonistic activity of **3**. Reagents and conditions: (a) *n*-PrI, K_2CO_3, DMF, 80 °C, 24 h, 89%; (b) (1) $NH_2OH{\cdot}HCl$, pyridine, EtOH, reflux, 12 h, (2) H_2, 10% Pd-C, c.HCl, EtOH, rt, 2.5 h, 89% (2 steps); (c) 4-(pyrimidine-2-yl)benzoic acid, DEPC, TEA, DMF, rt, overnight, 64%; (d) $TiCl_4$, CH_3OCHCl_2, CH_2Cl_2, 0 °C to rt, overnight, 71%; (e) $NaClO_2$, $NaHPO_4{\cdot}2H_2O$, 2-methyl-2-butene, *t*-BuOH, THF, H_2O, rt, overnight, 90%; (f) benzenesulfonamide, EDC, DMAP, DMF, rt, overnight, 27%.

4-(2-pyrimidinyl)benzoic acid and the resulting product **7** was regioselectively formylated (**8**), followed by Pinnick oxidation[11] to afford benzoic acid **9**. Compound **9** was condensed with benzenesulfonamaide in the presence of EDC and DMAP to afford the target compound **3**,[12] albeit in low yield.

To characterize the hPPARγ agonistic profile of **3**, we examined transactivation activity.[13] Compound **3** exhibited apparent transactivation activity and its maximal activity was about 50% of the maximal response of the positive controls (30 μM pioglitazone and 10 μM rosiglitazone; both are well-established hPPARγ full agonists), as shown in Figure 2(B). These results are consistent with the idea that **3** is a hPPARγ partial agonist.

To understand the structural basis of the hPPARγ partial agonist character of **3**, we solved the X-ray crystallographic structure of the complex of **3** with the hPPARγ LBD homodimer at 2.1 Å resolution. The crystal of the complex was obtained by soaking a crystal of the homodimer in ligand solution. The crystallographic data are summarized in Table 1, and the crystallographic structure is depicted in Figure 3(A–G). Each hPPARγ LBD in the homodimer binds one molecule of **3** (Fig. 3(A)). It is noteworthy that the structural folding of the LBD remains almost unchanged, except for the region from the end of helix 11 (H11) to the C-terminal H12 region (Fig. 3(B, D and E)); the dotted circle in Fig. 3(B) indicates the changed region). The bound **3** ligands in the two LBDs exhibit almost the same three-dimensional structure (Fig. 3(C)), and are positioned in the Y2 and Y3 arms of the binding pocket.[14] However, the structural folds in one part of the homodimer structure (Fig. 3(D)) are almost identical to those in the hPPARγ LBD–rosiglitazone complex (Fig. 4(G)),[15] whereas those in the other part of the homodimer structure are different (Fig. 3(E)). Therefore, we tentatively designated the former structure as the fully active form of the hPPARγ LBD.

Table 1
Crystallographic data and refinement statistics of hPPARγ-**3**

Complex	hPPARγ-**3**	Complex	hPPARγ-**3**
Data collection		*Refinement*	
Space group	*C*2	Resolution range (Å)	50.0–2.10
Unit cell constants		R_{work}/R_{free}	21.5/25.8
a (Å)	93.249	Number of atoms	
b (Å)	61.113	Protein	4165
c (Å)	119.28	Water	72
β (deg)	103.115	Ligand	76
Wavelength (Å)	1	Average B-factor (Å)	
Resolution (Å)	50.0–2.10 (2.18–2.10)	Protein	54.5
No. of total reflections	187868 (10921)	Water	60.1
No. of unique reflections	37883 (3523)	Ligand	46.4
Completeness (%)	98.8 (93.2)	r.m.s.d	
$I/\sigma(I)$	19.7 (2.7)	Bond lengths (Å)	0.008
Redundancy	5.0 (3.1)	Angles (deg)	0.99
Mosaicity	0.59–0.90	PDB code	3WMH
R_{merge} (%)	5.3(36.2)		

To understand the nature and significance of the two forms of the LBD, the binding mode of each form was investigated in detail. Figure 3(F and H) shows the fully active form of the LBD, while

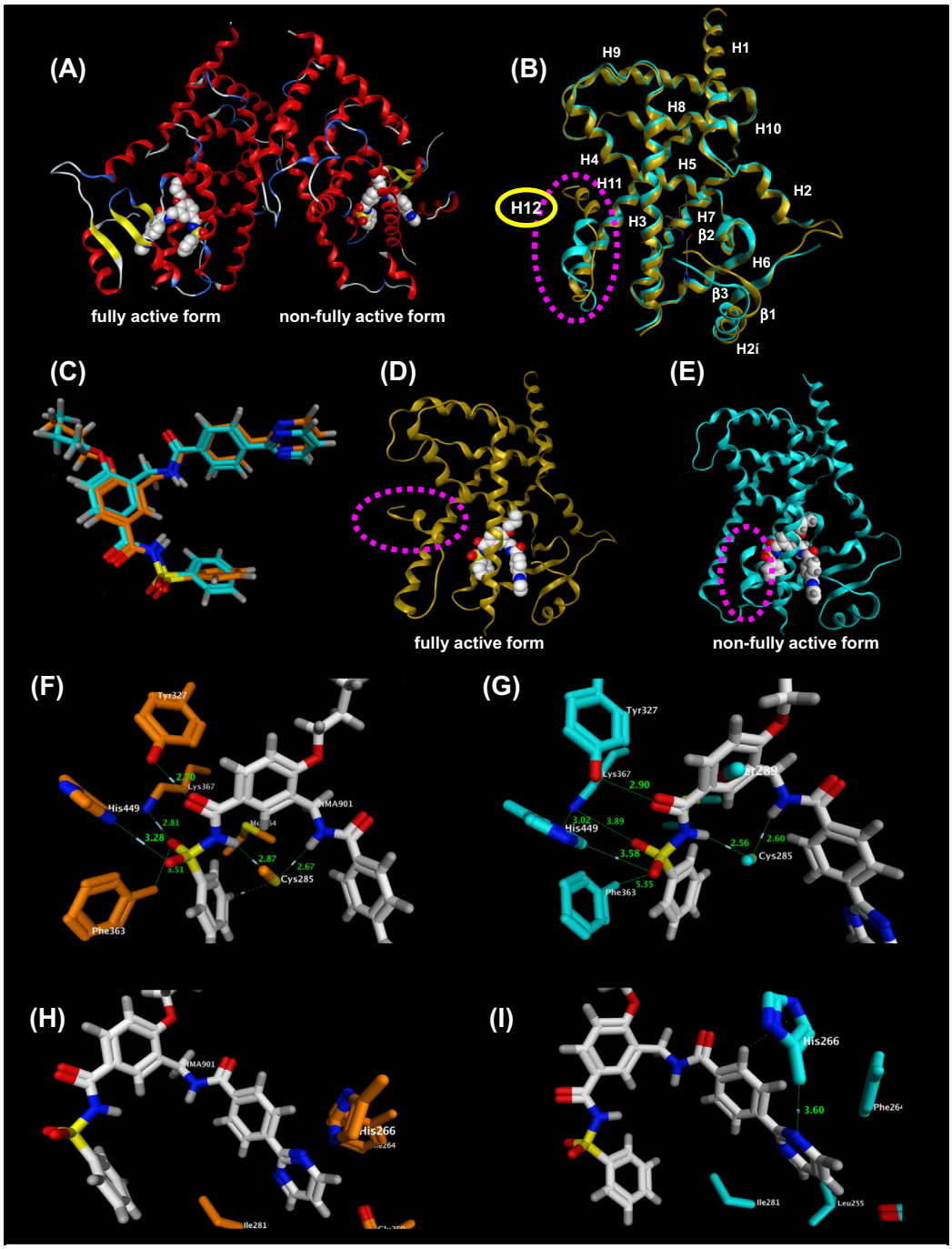

Figure 3. (A)–(I) Crystal structures of hPPARγ LBD-**3** homodimer (PDB: 3WMH). (A) Whole structure of hPPARγ LBD-**3** homodimer. Protein is represented as a red ribbon model and **3** is depicted as a Wan Der Waals model. (B) The superimposed structures of both homodimer partners. The numbering of the second structure is also depicted. The nomenclature of the helices is based on the RXR-α crystal structure. (C) Zoomed view of aligned **3** in both LBDs. Compound **3** is depicted as a cylinder model. (D) Whole structure of the full-agonist form LBD of hPPARγ LBD–**3** crystal. (E) Whole structure of the non-full-agonist form LBD of hPPARγ LBD-**3** crystal. (F) and (H) Zoomed views of the binding mode of **3** in the full-agonist form LBD of hPPARγ LBD-**3** crystal. (G) and (I) Zoomed views of the binding mode of **3** in the non-full-agonist form LBD of hPPARγ LBD-**3** crystal.

Figure 3(G and I) shows the other form, which we tentatively designate as the non-fully active form.

In the fully active form, compound **3** takes a U-shaped structure with hydrogen-bonding interactions between (1) the sulfonylamino group hydrogen of **3** and sulfur atom of Cys285, and (2) the amide group hydrogen of **3** and sulfur atom of Cys285. The acylsulfonamide group of **3** is positioned near H11, and interacts with the side-chains of Ser289, Tyr327, Lys367, His449 and Phe363. A hydrogen-bonding network involving five amino acids, Ser289, His323, Tyr327, His449 and Tyr473, is reported to be formed in the interaction of full agonists with the LDB.[16] Thus, three of these five amino acids interactions are conserved at one LDB in the complex with **3**, and this appears to be sufficient to support the fully active LDB structure. However, in the non-fully active LDB (Fig. 3(G and I)), another interaction was noted: the side chain imidazole group of His266 is located close to the (pyrimidin-2-yl)phenyl group of **3**, resulting in hydrophobic interaction between the imidazole side chain of His266 and phenyl group, and between

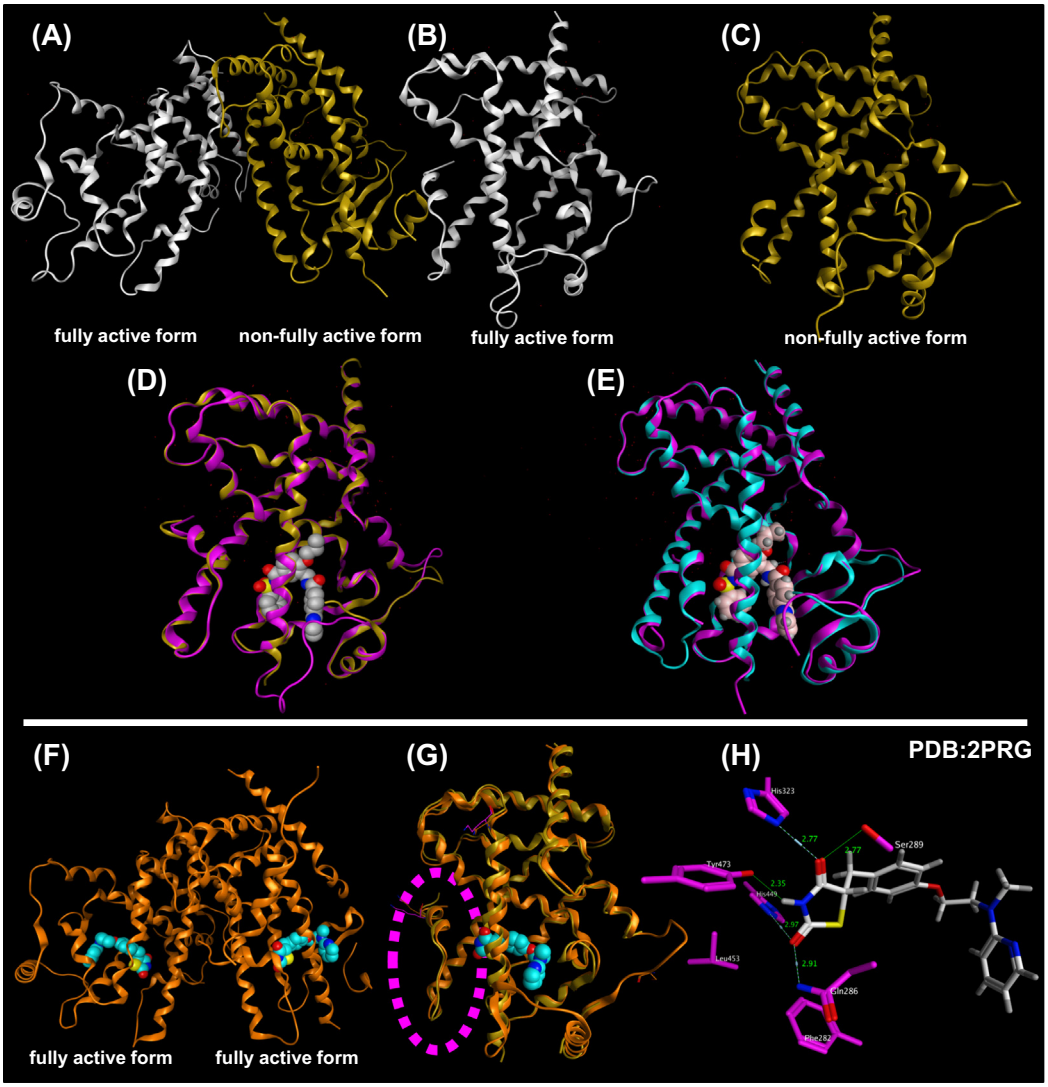

Figure 4. (A)–(H) Crystal structures of apo form of hPPARγ LBD homodimer. (A) Whole structure of apo form of hPPARγ LBD homodimer. Protein is represented as a ribbon model. (B) Whole structure of full-agonist form LBD of apo-hPPARγ homodimer. (C) Whole structure of the non-full-agonist form LBD of apo-hPPARγ homodimer. (D) The superimposed structures of the full-agonist form LBDs of apo-hPPARγ and hPPARγ LBD-**3** homodimer crystals. (E) The superimposed structures of the non-full-agonist form LBD of apo-hPPARγ and non-full-agonist form LBD of hPPARγ LBD-**3** homodimer crystals. (F) Whole structure of apo-hPPARγ LBD-rosiglitazone homodimer (PDB: 2PRG). (G) The superimposed structures of both LBDs of hPPARγ LBD-rosiglitazone homodimer. (H) Zoomed view of the binding mode of rosiglitazone in the full-agonist form LBD in hPPARγ LBD-rosiglitazone homodimer crystal.

the imidazole side chain of His266 and the pyrimidinyl group. These interactions cause the bound **3** to move to the right, so that the distance from the phenylsulfonylaminocarbonyl group of **3** to the side chains of Ser289, Tyr327, Lys367, His449 and Phe363 becomes longer. As a result, the hydrogen-bonding network is weaker in the case of the non-fully active LBD, and this may mean that the H12 region is not restricted to the appropriate location for full activity.

Next, we considered whether these structural differences in LBD folding might be induced by binding of **3**. To examine this question, we determined the X-ray crystallographic structure of hPPARγ LBD apo-form (without ligand). As depicted in Figure 4, hPPARγ LBD apo-form also forms a homodimer (Fig. 4(A)) in which one LDB is in the fully active form (Fig. 4(B)), and the other is in a non-fully active form (Fig. 4(C)). These results indicate that two types of hPPARγ LBD structures are present in the crystal state, irrespective of the presence or absence of agonist. Indeed, the apo-form hPPARγ LBD homodimer and ligand-bound hPPARγ LBD homodimer did not show apparent structural differences in each type of LDB (Fig. 4(D and E)), suggesting that partial agonists lack the ability to induce fully active LBD.

On the other hand, the hPPARγ LBD-rosiglitazone (a hPPARγ full agonist) complex also formed a homodimer in the crystal, but each LBD was present in fully active form (Fig. 4(F) and (G)).[14] This result indicates that full agonists do induce structural change of non-fully active hPPARγ LBD to fully active LBD, presumably by facilitating a tight hydrogen-bonding network of the LBD, especially to the C-terminal region (H12) (Fig. 4(H)).

In order to ascertain the generality of this conclusion, we surveyed the PDB database. Representative hPPARγ LBD homodimer–partial agonist structures are depicted in Figure 5((A and B): PDB: 2I4Z,[17] (C and D): PDB: 2Q5S,[18] (E and F): PDB: 2Q6R[18]). In the case of PDB: 2I4Z, the homodimer contains a fully active LBD and a non-fully active LBD, and the phenoxyacetic acid-type ligand is bound to the fully active LBD. In the case of PDB: 2Q5S, the homodimer also contains the two forms of LBD, and the indole-2-carboxylic acid-type ligands are bound to both LBDs, as in our case. In the case of PDB: 2Q6R, the homodimer contains

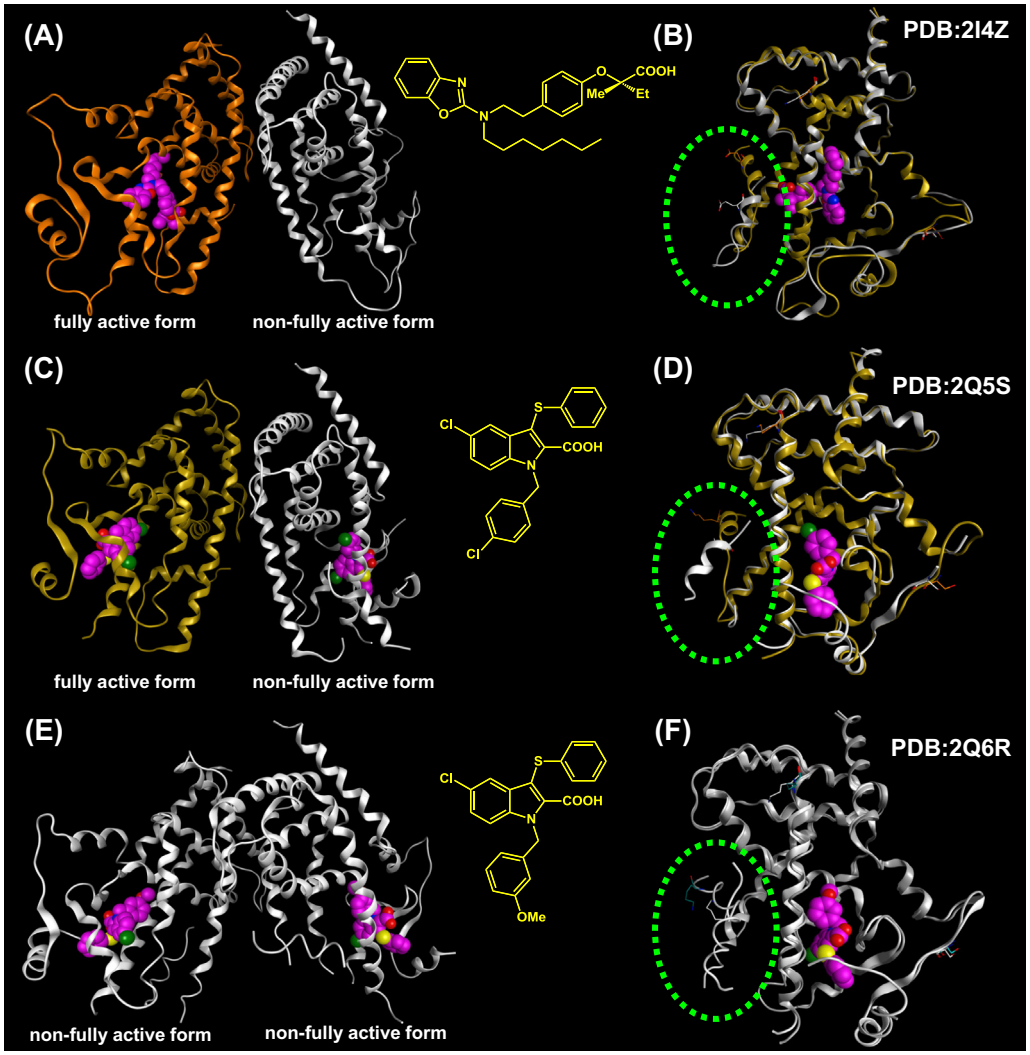

Figure 5. (A)-(F) Crystal structures of hPPARγ LBD-hPPARγ partial-agonist homodimers (PDB: 2I4Z, 2Q5S. 2Q6R). (A) Whole structure of PDB: 2I4Z. The bound ligand structure is also depicted. (B) The superimposed structures of each LBD of PDB: 2I4Z. (C) Whole structure of PDB: 2Q5S. The bound ligand structure is also depicted. (D) The superimposed structures of each LBD of PDB: 2Q5S. (E) Whole structure of PDB: 2Q6R. The bound ligand structure is also depicted. (F) The superimposed structures of each LBD of PDB: 2Q6R.

two non-fully active LBDs, and the indole-2-carboxylic acid-type ligands are bound to both LBDs. Based on these data, we hypothesize that hPPARγ partial agonists lack the ability to induce the fully active LBD. At the cellular level, binding of an agonist to hPPARγ leads to heterodimerization with another nuclear receptor, retinoid X receptor (RXR), not homodimerization. Therefore, the hPPARγ LBD homodimer structure might not necessarily reflect the real functional structure of hPPARγ, and to establish whether a similar situation exists in the heterodimer. Nevertheless, our results at least suggest that the presence of a non-fully active LBD in the agonist complex may be a useful criterion to distinguish hPPARγ partial agonists from full agonists.

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12. *Physico-chemical properties of compound* ***3***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (d, J = 5.2 Hz, 2H), 8.45 (d, J = 8.4 Hz, 2H), 8.10 (d, J = 8.4 Hz, 2H), 7.87 (d, J = 2.4 Hz, 1H), 7.82–7.78 (m, 3H), 7.58 (t, J = 7.4 Hz, 1H), 7.49 (t, J = 7.6 Hz, 2H), 7.23 (t, J = 4.8 Hz, 1H), 6.90 (t, J = 6.0 Hz, 1H), 6.87 (d, J = 8.4 Hz, 1H), 4.63 (d, J = 6.0 Hz, 2H), 4.02 (t, J = 6.6 Hz, 2H), 1.91–1.82 (m, 2H), 1.06 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.87, 162.57, 160.04, 157.87, 139.64, 136.14, 133.42, 129.28, 129.02, 128.20, 127.78, 127.55, 127.20, 120.38, 110.82, 69.60, 37.75, 21.96, 10.43. HRMS (FAB, MH^+) Calcd for $C_{28}H_{27}N_4O_5S$: 531.1702, Found: 531.1717. HPLC purity was estimated to be 97.9% by means of reversed-phase HPLC, using a Pegasil ODS sp100 column (4.6 × 250 mm, Senshu Chemical, Japan) fitted on a JASCO HPLC system, with CH_3CN: 0.1% TFA = 2:1 v/v as the eluent and detection at 254 nm.
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