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Structure–activity relationships of dimeric PPAR agonists

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Abstract—A series of dimeric PPAR agonists were designed and tested for PPAR activity in vitro. The SAR showed that dimeric ligands with a common group or full dimeric ligands had retained or even increased PPARγ potency. The dimeric agonist concept can be used to fine tune the subtype selectivity of PPAR agonists. The PPARγ potency could, at least partly, be explained using molecular modeling.

1. Introduction

The peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor super family. The marketed insulin sensitizers (rosiglitazone and pioglitazone) and the lipid lowering fibrates (e.g., fenofibrate and clofibrate) are PPARγ and α agonists, respectively. There is no drug available targeting the third PPARδ receptor, but several reports suggest PPARδ to be involved in lipid transport and metabolism.[1]

We have recently published on three novel dimeric PPAR agonists (**2**, **14**, and a regioisomer of **2**) with good activity both in vitro and in vivo.[2] A criticism in the review process of that paper was the low number of dimeric ligands excluding a proper structure–activity relationship analysis. Thus, the purpose of the present investigation was to expand the structural scope of dimeric PPAR agonists with focus on especially the PPARγ activity.[3] In vitro transactivation assays using the ligand binding domains (LBDs) of hPPARα, γ, and δ were used to evaluate the designed and synthesized dimeric ligands.[4] Docking experiments based on the crystal structure of monomeric and dimeric ligands bound to the LBD-PPARγ receptor were used to explain the obtained data.

Dimeric ligands are usually defined as molecules containing two recognition units linked covalently through either a common group or through a spacer. The SAR of the investigated ligands suggests that the concept of dimeric ligands in general can be applied to the design of PPAR agonists, if certain steric requirements are fulfilled.

2. Results and discussion

The potent PPAR agonists, **1**[2,5] and **5**, were used as starting points for the design of dimeric agonists. Dimeric ligands with a common group (**2** and **6**), full dimeric ligands (**7** and **14**), as well as truncated dimeric ligands (**3**, **4**, **11**, and **12**) were designed, synthesized[6] and tested (Figs. 1 and 2 and Table 1).

The dimeric ligands with a common group (**2** and **6**) were both approximately 10 times more potent on PPARγ than their monomeric counterparts (**1** and **5**, respectively). At the same time, the PPARα and the PPARδ potencies had changed (Table 1). The monomeric ligands **1** and **5** as well as the dimeric ligands **2** and **6** were equally potent suggesting that the allylic methyl group in **5** and **6** was of little importance. The full dimeric ligand **7** had the same PPARγ potency as **5**, whereas the rotation restricted analog **8** had lower potency. The dimeric ligands **7** and **8**, both had lower efficacy on PPARα and PPARδ compared to **5**. The truncated dimeric ligand **3** had retained the PPARγ potency, but lost PPARα and PPARδ activity, compared to **1**. Further, the short truncated dimeric analog **4**

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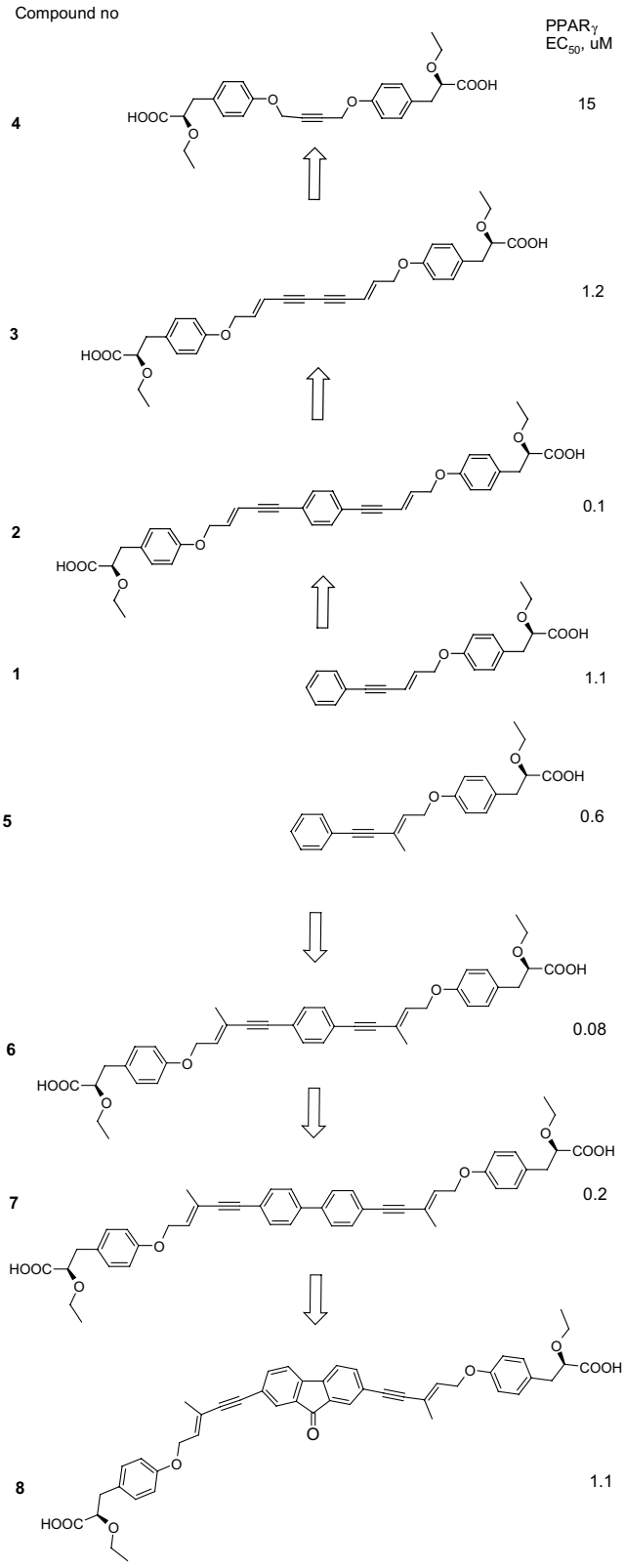

Figure 1. Graphic illustration of the design of various dimeric PPAR ligands using the monomeric PPAR agonists **1** and **5** as starting point. The PPARγ transactivation potency (EC_{50} in μM) of the compounds is also indicated.

had much lower PPAR receptor activity than the monomeric counterpart **1**.

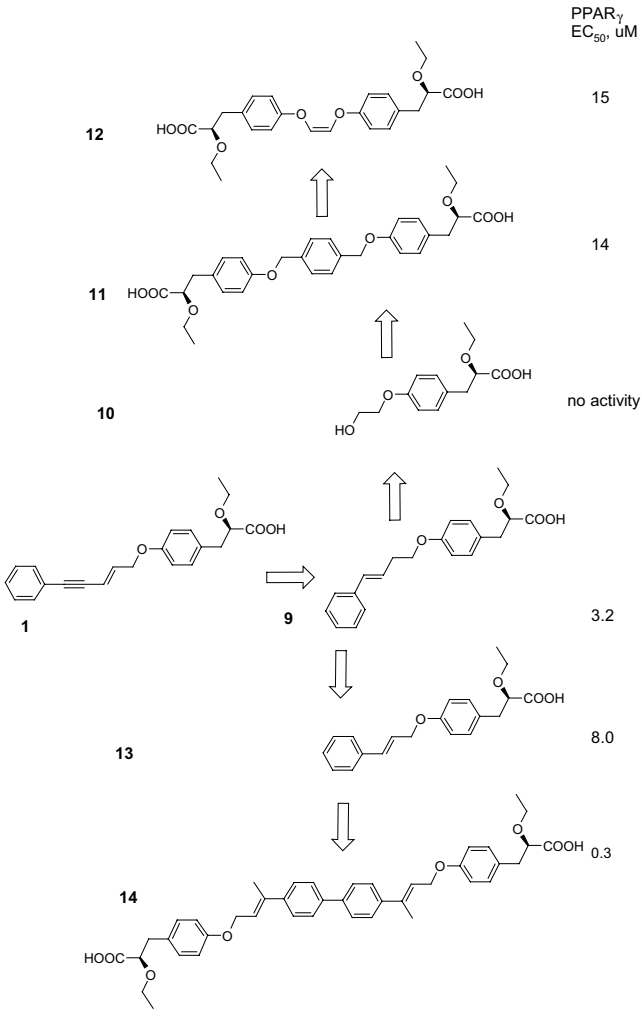

Figure 2. Design of additional monomeric and dimeric PPAR ligands. See also Figure 1.

In order to investigate if the above obtained SAR was of a more general nature a second set of monomeric and dimeric ligands were designed (Fig. 2). The conjugated triple–double bonds in **1** could be replaced by the double–single bonds, **9**, without significant loss of PPARγ activity. Removal of the lipophilic phenyl group, **10**, resulted however in lack of PPAR activity. Again, the short truncated dimeric analogs **11** and **12** had lower PPAR activity than **9**, and the full dimeric ligand **14** was more potent than **13**.

The obtained data indicated that the lipophilic part of the molecule was necessary (**10** was inactive) and this part could not be replaced by any lipophilic/hydrophilic group as in the short truncated dimeric ligands (**4**, **11**, and **12**), although the large size of the ligand binding pocket might indicate so.[1]

To investigate the SAR at the receptor level representative dimeric ligands (**3**, **4**, **6**, and **7**) were docked into the crystal structure of the LBD of the hPPARγ receptor crystallized with **1** (Fig. 3). FlexX[7–10] was used as docking tool.

In the crystal structure of PPARγ in complex with **1**, His323, His449, Tyr473, and Ser289 were found within

Table 1. hPPARα, hPPARγ, and hPPARδ transactivation data on compounds **1**–**14** and standard compounds

Compounds	hPPARα		hPPARγ		hPPARδ	
	EC_{50}, μM[a]	% max[b]	EC_{50}, μM	% max[c]	EC_{50}, μM	% max[d]
1	0.07	123	1.1	98	6.9	173
2	0.7	173	0.1	103	2.2	238
3	3.7	95	1.2	89	15	70
4	9.6	82	15	37	—	0
5	0.3	191	0.6	101	11	150
6	1.2	65	0.08	119	1.8	184
7	1.8	38	0.2	93	4.1	25
8	2.3	88	1.1	91	—	18
9	1.1	112	3.2	83	19	205
10	—	17	—	1	—	1
11	15	56	14	71	nt	nt
12	—	2	15	56	—	0
13	1.6	169	7.9	96	18	177
14	6.7	247	0.3	162	2.1	314
WY14643	13	100	29	22	—	6
Rosiglitazone	4.1	43	0.2	100	—	7
Carbacycline	1.0	79	8.0	24	1.9	100

[a] Compounds were tested in five concentrations ranging from 0.01 to 30 μM in three independent experiments. Data represent mean, with SEM ± 15%. EC_{50}s were not calculated for compounds producing transactivation lower than 25% at 30 μM.
[b] Fold activation relative to maximum activation obtained with WY14643 (approx. 20-fold corresponded to 100%).
[c] Fold activation relative to maximum activation obtained with rosiglitazone (approx. 120-fold corresponded to 100%).
[d] Fold activation relative to maximum activation obtained with carbacyclin (approx. 250 fold corresponded to 100%).

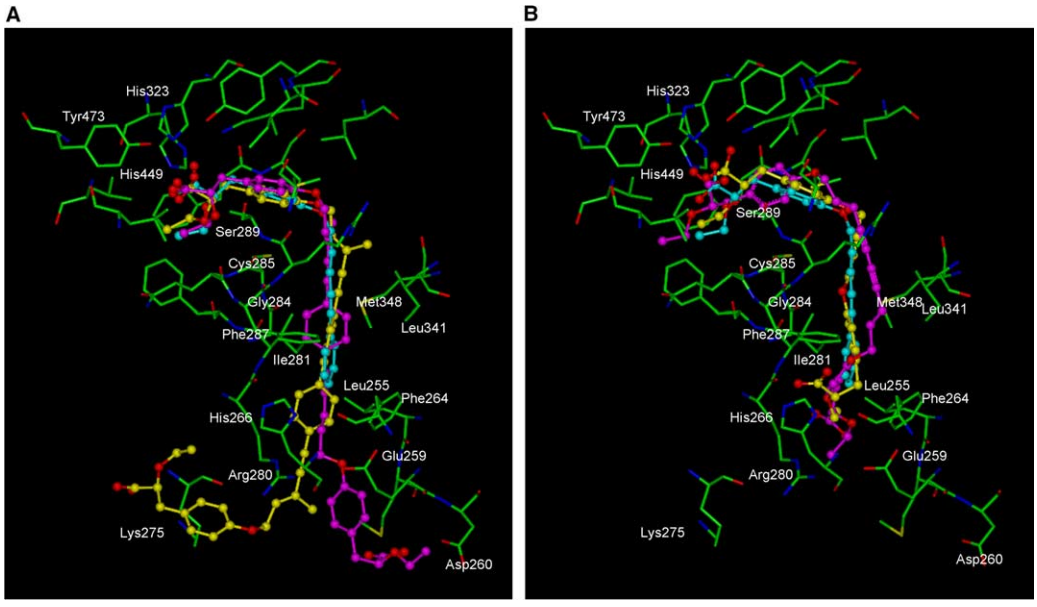

Figure 3. FlexX docking in the active site of the X-ray structure of the ligand binding domain of the hPPARγ receptor crystallized with **1** (light blue) of A: **6** (magenta) and **7** (yellow); B: **3** (magenta), and **4** (yellow).

4.0 Å from the 2-ethoxy-propionic acid part of the ligand. The hydrophobic pocket around the phenyl-pentenyn part of the ligand was created by Gly284, Cys285, Leu341, Ile281, Met348, Phe287, His266, Phe264, and Leu255. Initially compound **1** was docked into the active site in order to validate the predicted binding mode. The root mean square deviation between the crystallized ligand and the docked ligand was 1.7 Å. Then **6** was docked into the active site. In the predicted binding mode the carboxylic acid made hydrogen bond interactions with His323 and His449 (Fig. 3A) and the oxygen atom in the ethoxy group made a hydrogen bond with Ser289. Likewise, in the predicted binding mode of **7** the carboxylic acid made hydrogen bonds to His323 and His449 (Fig. 3A). Both **6** and **7** made interactions with the amino acids in the hydrophobic pocket in which the phenyl-pentenyne part of ligand **1** is positioned. An explanation for the higher potency for **6** and **7**, compared to **1**, could be the higher number of interactions between the compounds and the amino acids further away from **1** (Lys275, Arg280, Glu259, Asp260).

The truncated dimer **3** was also docked into the active site. In the predicted binding mode the 2-ethoxy-prop-

ionic acid made hydrogen bonding interactions with His449, Tyr473, and Ser289 (Fig. 3B). The compound was positioned in a pocket built up by the same amino acids as for **1** and had the same PPARγ activity. The carboxylic acid group in the short truncated dimeric ligand **4** also made hydrogen bond interactions with His449 and Tyr473 (Fig. 3B). Although the phenyl ring was positioned close to the phenyl ring in the phenylpentenyne part of **1**, the carboxylic acid attached to this phenyl ring, was positioned in the hydrophobic pocket close to Phe264. The penalty in solvation energy combined with binding in a hydrophobic pocket could explain the loss of activity for compound **4**.

In conclusion, dimeric ligands with a common group or 'full' dimeric ligands gave PPARγ agonists with retained or increased potency. The dimeric agonists had altered PPAR subtype profile compared to the monomeric counterparts, suggesting that the dimeric design concept can be used to fine tune the subtype selectivity of PPAR agonists. The PPARγ potency of the tested ligands could (at least partly) be explained using molecular receptor modeling.

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