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Characterization of Telmisartan-Derived PPARγ Agonists: Importance of Moiety Shift from Position 6 to 5 on Potency, Efficacy and Cofactor Recruitment

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Selective modulation of the peroxisome proliferator-activated receptor gamma (PPARγ) by direct binding of small molecules demonstrates a promising tool for treatment of insulin resistance and type 2 diabetes mellitus. Besides its blood pressure-lowering properties, the AT_1-receptor blocker telmisartan has been shown to be a partial agonist of PPARγ with beneficial metabolic effects in vitro and in mice. In our previous work, comprehensive structure–activity relationship (SAR) studies discussed the different parts of the telmisartan structure and various moieties. Based on these findings, we designed and synthesized new PPARγ ligands with a benzimidazole (agonists **4**-**5** and **4**-**6**), benzothiophene (agonists **5**-**5** and **5**-**6**) or benzofuran (agonists **6**-**5** and **6**-**6**) moiety either at position 5 or 6 of the benzimidazole core structure. Lipophilicity and EC_{50} values were improved for all new compounds compared with telmisartan. Regarding PPARγ activation, the compounds were characterized by a differentiation assay using 3T3-L1 cells and a luciferase assay with COS-7 cells transiently transfected with pGal4-hPPARgDEF, pGal5-TK-pGL3 and pRL-CMV. A decrease in both potency and efficacy was observed after the shift of either the benzothiophene or the benzofuran moiety from position 6 to position 5. Selective recruitment of the coactivators TRAP220, SRC-1 and PGC-1α, and release of corepressor NCoR1 determined by time-resolved fluorescence resonance energy transfer (TR-FRET) was detected depending on residues in position 5 or 6.

Introduction

Activation of the peroxisome proliferator-activated receptor gamma (PPARγ) exhibits beneficial effects on the regulation of genes involved in glucose and lipid metabolism.[1] Therefore, synthetic high-affinity ligands for PPARγ, known as thiazolidinediones (TZDs)/glitazones are currently used for the treatment of type 2 diabetes mellitus and insulin resistance. However, full activation of PPARγ frequently goes along with an increase in body weight, fluid retention, and an increased risk for heart failure and loss of bone mineral density.[2] Occurrence of serious side effects accelerated the interest for further investigation of favorable PPARγ ligands. The inhibition of the CDK5-mediated phosphorylation of some PPARγ ligands is currently discussed to ameliorate an anti-diabetic effect independent of the PPARγ agonism.[3] Meanwhile, nearly all of the initially approved TZDs have been withdrawn from the market.[4] During the last years, the concept of selective or "partial activation" of the receptor, which is reported to be correlated with promising metabolic efficacy without being tempered by adverse actions, resulted in the development of a new group of ligands: the selective PPARγ modulators (SPPARγMs).[5] Upon binding, these compounds induce a specific conformational change of the receptor, resulting in conformation-dependent recruitment or release of cofactors, and activation of a ligand-specific PPARγ gene-expression profile. The crucial factor is the binding mode of compounds to the receptor, influencing cofactor interaction and receptor activation. According to the current understanding, stabilizing helix 12 is required for full agonist activity,[6] while partial agonists bind via independent mechanisms of helix 12.[7] NMR studies performed by Hughes et al. revealed that a conformational disorientation, due to the ability of ligands to sample different binding modes, contributes to reduced receptor activation and therefore leads to an unfavorable conformation for cofactor binding.[8]

Beside its antihypertensive properties, the angiotensin type 1 (AT_1)-receptor blocker telmisartan has been shown to act as partial agonist of PPARγ in vitro with an excellent side-effect profile in vivo.[9] In previous studies, we focused on the analysis of structure–activity relationships (SARs) and modification of structural components of telmisartan to develop new

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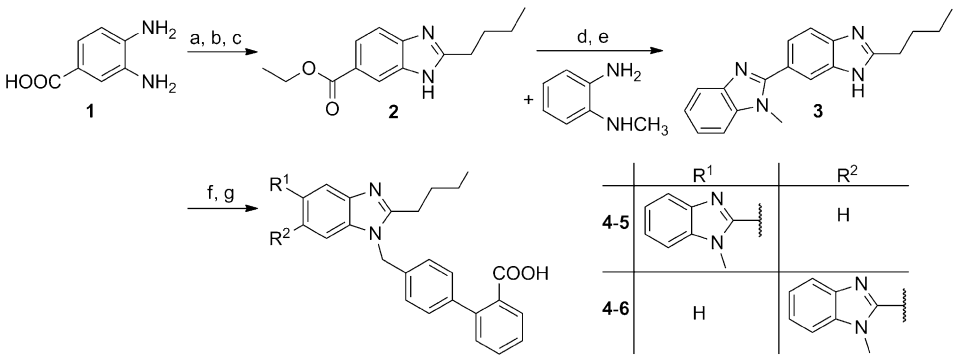
therapeutic strategies against insulin resistance.[10] We demonstrated that a "backbone" part of telmisartan containing the basic scaffold of the 1-(biphenyl-4-ylmethyl)-1*H*-benzimidazole is necessary to meet the minimum requirements for PPARγ activation.[10a] Moreover, elongation of the alkyl chain at position 2 of the benzimidazole core, especially the propyl chain, was important for potency and activation.[10b] Furthermore, changes at positions 5 and 6 of the core scaffold were shown to have a strong influence on receptor activity.[10c] Based on this knowledge, we intended to create substances with high potency, but we also wanted to gain more information about the SAR between telmisartan and PPARγ. Accordingly, we synthesized PPARγ ligands with a benzimidazole, benzothiophene or benzofuran moiety either at position 5 or 6 of the benzimidazole core. The synthesized compounds exert partial (compounds **4**-**5**, **4**-**6**, **5**-**5** and **6**-**5**) and full (compounds **5**-**6** and **6**-**6**) agonistic receptor-activation properties, and show a selective recruitment/release pattern of cofactors (thyroid-hormone-receptor-associated protein [TRAP220], steroid-receptor coactivator 1 [SRC-1], PPARγ coactivator 1α [PGC-1α], nuclear-receptor corepressor 1 [NCoR1]). Metabolic efficacy and the influence of these compounds on the AT_1 receptor need to be elucidated in the future.



Scheme 1. Synthesis of compounds **4**-**5** and **4**-**6**. *Reagents and conditions*: a) EtOH, concd H_2SO_4, reflux, 5 h; b) THF, valeryl chloride, RT, 1 h; c) toluene, pTsOH·H_2O, reflux, 3 h; d) MeOH, aq NaOH (10%), reflux, 2 h; e) polyphosphoric acid, 150 °C, 2 h; f) NaH, 4′-(bromomethyl)-2-biphenylcarbonitrile, DMF, 0 °C–RT, 5 h; g) ethylene glycol, KOH, 185 °C, 6 h.

Result and Discussion

Synthesis

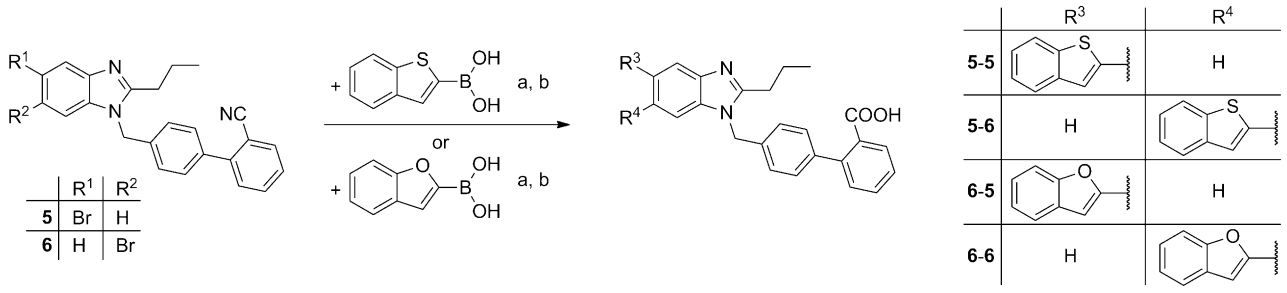
After esterification of 3,4-diaminobenzoic acid with ethanol/sulfuric acid, the free amino groups were acylated with valeryl chloride in dry tetrahydrofuran (THF) at room temperature, followed by cyclocondensation in toluene and *p*-toluenesulfonic acid to give compound **2** (Scheme 1).[11] The ester cleavage was carried out in a 1:1 mixture of methanol and aqueous sodium hydroxide solution (10%). The cyclocondensation of the free carboxyl group with *N*-methyl-1,2-benzenediamine in polyphosphoric acid at 150 °C yielded compound **3.**[12] Compounds **4**-**5** and **4**-**6** were prepared by N-alkylation of **3** with 4′-(bromomethyl)-2-biphenylcarbonitrile followed by saponification of the carbonitrile with potassium hydroxide in ethylene glycol at 185 °C.

The preparation of compound **5** and **6** was described before.[10c] Compounds **5**-**5**, **5**-**6** and **6**-**5** and **6**-**6** were prepared with the respective boronic esters by a Suzuki reaction in acetonitrile and aqueous sodium carbonate (0.4 M) catalyzed by palladium complex $(Ph_3P)_4Pd$ (Scheme 2).[10c]

Separation of the regioisomers was carried out by a combination of column chromatography (silica gel) and fractional crystallization in methanol or ethanol, respectively. The assignment of the moieties to position 5 or 6 was performed by differential ^{1}H NMR nuclear Overhauser effect experiments (NOE-Diff) on the basis of a saturation transfer from the C-7-positioned proton (H-7) at the central benzimidazole to the benzylic methylene group.

In vitro SAR studies

The AT_1-receptor blocker telmisartan has been identified as a partial PPARγ agonist with low potency. In order to optimize the PPARγ-activating properties and develop more potent PPARγ agonists, we synthesized a series of new telmisartan-based compounds, and performed structure–PPARγ-activity relationship studies. Accordingly, compounds were characterized



Scheme 2. Synthesis of compounds **5**-**5**, **5**-**6**, **6**-**5** and **6**-**6**. *Reagents and conditions*: a) $(Ph_3P)_4Pd$, MeCN, aq Na_2CO_3 (0.4 M), 90 °C, 6 h; b) ethylene glycol, KOH, 185 °C, 6 h.

for PPARγ activation in vitro by luciferase transactivation assay (Figure 1). Pioglitazone, known as a full agonist, was used as positive control, and maximum activation was defined as

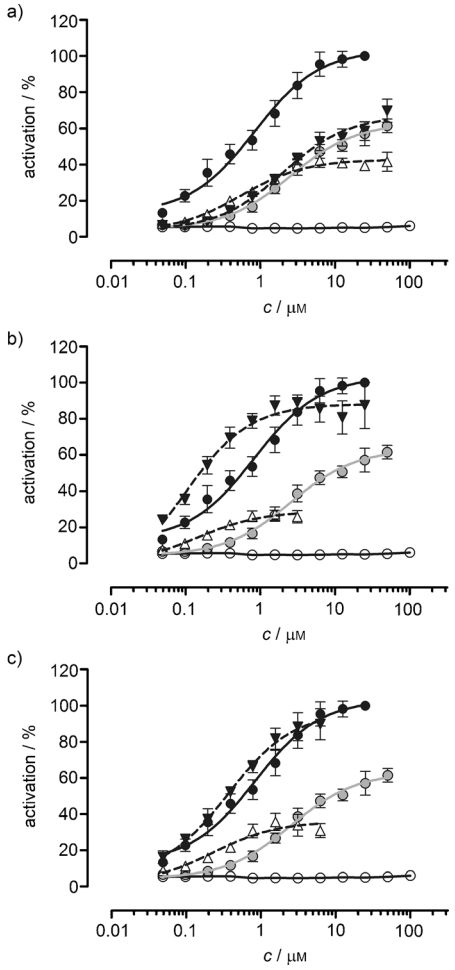


Figure 1. PPARγ activation measured with the transactivation assay. COS-7 cells transiently transfected with pGal4-hPPARγDEF and pGal5-Tk-pGL3 were stimulated with pioglitazone (●; max. activation was set to 100%), telmisartan (◉), or DMSO (○), or synthesized compounds a) **4-5** (△), **4-6** (▼); b) **5-5** (△), **5-6** (▼); and c) **6-5** (△), **6-6** (▼) in a dose-dependent manner. Firefly luciferase activity was measured after 36 h and normalized with activity of co-transfected pCMV-*Renilla*. Data points represent the mean (±SD) of threefold determination of three independent experiments.

100%. Table 1 shows an overview of lipophilicity, EC_{50} values and maximum activation of the ligand binding domain (LBD) of PPARγ. All of the newly synthesized compounds exhibit lipophilicity values of about twofold or more than that of telmisartan (clog D = 2.34). Best results were obtained with compounds **5-5** and **5-6** merged with a benzothiophene moiety (clog D = 5.39). Relative to telmisartan and even pioglitazone, EC_{50} values were improved with the modifications at position 5 or 6: telmisartan ($EC_{50} = 2.53 \pm 0.40$) > **4-6** ($EC_{50} = 1.99 \pm 0.34$); pioglitazone ($EC_{50} = 0.83 \pm 0.16$) > **4-5** ($EC_{50} = 0.55 \pm 0.12$) > **6-6** ($EC_{50} = 0.37 \pm 0.08$) > **6-5** (0.23 ± 0.12) > **5-5** ($EC_{50} = 0.14 \pm 0.07$) > **5-6** ($EC_{50} = 0.09 \pm 0.04$). Telmisartan analogous with a central imidazolpyridine published by Casimiro-Garcia et al.

Table 1. clog D, EC_{50} and maximum activation of the hPPARγ-LBD in transient transfection.

Compd	clog D[a] (pH 7.4)	EC_{50}[b,d] [μM]	A_{max}[c,d] [%]
Pioglitazone	1.97	0.83 ± 0.16	98 ± 4
Telmisartan	2.34	2.53 ± 0.40	62 ± 4
4-5	4.51	0.55 ± 0.12	42 ± 5
4-6	4.51	1.99 ± 0.34	70 ± 6
5-5	5.39	0.14 ± 0.07	27 ± 4
5-6	5.39	0.09 ± 0.04	87 ± 13
6-5	4.46	0.23 ± 0.12	34 ± 6
6-6	4.46	0.37 ± 0.08	90 ± 9

[a] Calculated log D (clog D) values were determined with MarvinSketch 5.4.0.0. [b] EC_{50} values were calculated by the use of the dose–response curves (Figure 1). [c] The maximal activation shows the highest relative PPARγ-activation triggered by the compounds in comparison to pioglitazone (100%). [d] Data points represent the mean (±SD) of threefold determination of three independent experiments.

had similar potency for PPARγ as our compounds presented in this publication.[13] However, replacement of the central benzimidazole with an indole dramatically increased potency to the picomolar range under partial receptor activation, but completely abolished AT_1 antagonism.[14] Previous studies by our group (data not published), and also other groups[13] showed a negative correlation between PPARγ activation and AT_1 antagonism. Conclusively, agonists with low efficacy for PPARγ might still be able to block the AT_1 receptor.

The shift of a benzimidazole, benzothiophene or benzofuran moiety from position 6 to position 5 decreased potency and efficacy (Figure 1). While compounds **4-5** and **4-6** are both partial agonists, the relocation of the benzothiophene or benzofuran substituent from position 6 to 5 transformed a full agonist with higher potency than pioglitazone into a partial agonist. Both, compounds **5-5** and **6-5** showed reduced maximum activation values (27 ± 4% for **5-5** and 34 ± 6% for **6-5**) and demonstrated improved potency relative to telmisartan (Table 1). Compounds corresponding to agonists **4-5** and **4-6** with an *n*-propyl chain also resulted in a decrease in efficacy, as published by Goebel et al.[10a] Moreover and also shown by Goebel et al., a one-carbon extension at position 2 is able to increase potency and efficacy.[10b] In order to generate more potent agonists, compounds **4-5** and **4-6** were synthesized with an *n*-butyl chain. Although compounds **4-5** and **4-6** with an *n*-propyl or *n*-butyl chain were not analyzed in the same experimental approach, the extension of the alkyl chain did not seem to increase potency.

The adipocyte-differentiation assay represents a well-established assay for the assessment of cellular PPARγ activation.[15] Thus, the degree of differentiation closely correlates with PPARγ activity. The differentiation grade of 3T3-L1 cells was analyzed by Oil-Red O staining and results are shown in Figure 2. Oil-Red O staining confirmed the results of the luciferase assay. Absence of triglyceride accumulation in 3T3-L1 cells after dimethyl sulfoxide (DMSO) treatment confirmed that activation is only triggered by pioglitazone, telmisartan or the new compounds. In accordance with results from the PPARγ-LBD activi-

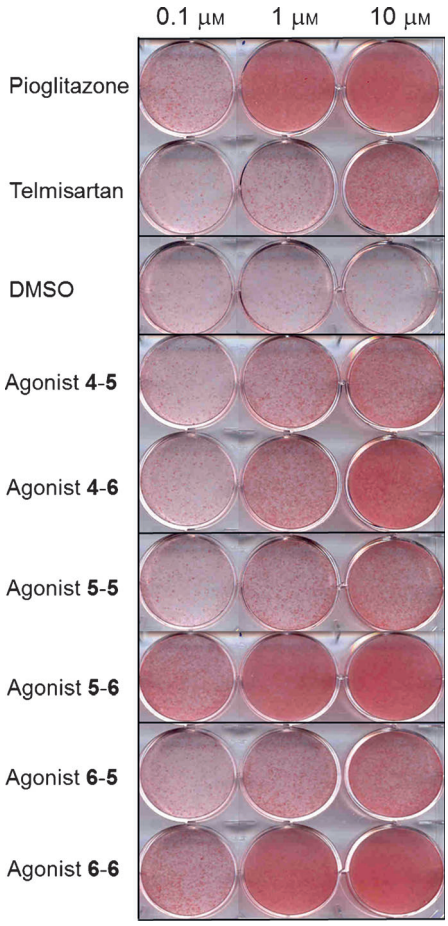


Figure 2. PPARγ–ligand-dependent adipocyte differentiation. 3T3-L1 cells were differentiated with the indicated compounds at 0.1 μM, 1 μM and 10 μM. Pioglitazone and telmisartan served as positive controls and DMSO as vehicle. Oil-Red O staining was performed after 9 days. Pictures are representative of three independent experiments.

ty assay, cells treated with the full agonists **5-6** and **6-6** exhibited the highest degree of adipocyte differentiation. All other partial agonists showed a similar differentiation pattern to the one of telmisartan.

As full PPARy agonism and strong adipocyte differentiation has been associated with body weight gain in clinical studies,[16] one may speculate that compound **5-6** and **6-6** behave in the same manner. However, the mechanisms underlying TZD-induced weight gain are not completely understood. TZDs also increase weight via fluid retention resulting in edema formation.[17] These processes could, at least partially, be explained by activation of PPARγ in the collecting duct of the kidney, followed by up regulation of the sodium transporter, ENaC, or water channels (aquaporins), and subsequent fluid retention.[18] The effect of the new compounds on fluid retention remains unclear and has to be addressed in future in vivo studies.

In vitro cofactor interaction

For further characterization of compounds **4-5** and **4-6**, **5-5** and **5-6**, and **6-5** and **6-6**, nuclear cofactor–PPARγ-LBD binding studies using time-resolved fluorescence resonance energy transfer (FRET) were performed with the corepressor NCoR1 and co-activators SRC1, PGC-1α and 220TRAP220. Binding of the indicated compounds to the PPARγ-LBD consistently resulted in dose-dependent release of the corepressor (Figure 3) and selective coactivator recruitment (Figure 4). Furthermore, PPARγ activity seems to have a strong impact on the degree of cofactor recruitment/release. Compounds with strong PPARγ activation properties, like **5-6** and **6-6**, showed a strong dose-dependent release of NCoR1, and recruitment of SRC1, PGC-1α and TRAP220, while partial PPARγ activators (compounds **5-5** and **6-5**) hardly interacted with the indicated cofactors (Figure 3 and Figure 4). Compounds with a benzimidazole moiety at position 5 or 6 (**4-5** and **4-6**) and with a similar degree of PPARγ activity as telmisartan also showed a telmisartan-like cofactor-recruitment/release pattern (Figure 3a and Figure 4a, d, and g). These findings suggest a different binding mode of compound **4-6** compared with compounds **5-6** and **6-6**.

Development of selective modulators targeting PPARγ for therapeutic purposes without significant side effects still represents a pharmacological challenge. Nevertheless, selective binding of compounds in a different way compared with TZDs could result in distinct receptor confirmation followed by selective cofactor release/recruitment. Such selective PPARγ modu-

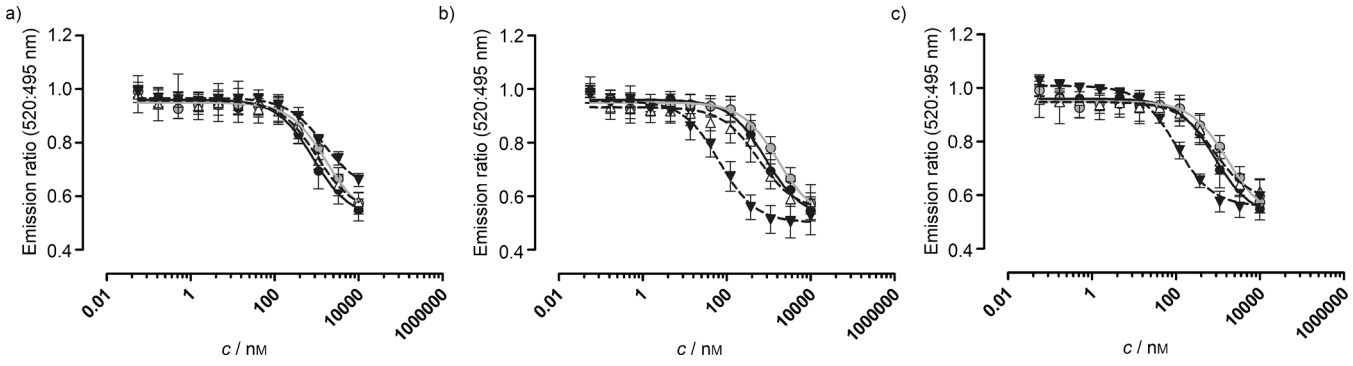


Figure 3. Corepressor release by TR-FRET. Fluorescein-labeled corepressor NCoR1 was incubated with PPARγ-LBD protein labeled with terbium in the presence of increasing concentrations of pioglitazone (●), telmisartan (●), or synthesized compounds a) **4-5** (△), **4-6** (▼); b) **5-5** (△), **5-6** (▼); and c) **6-5** (△), **6-6** (▼). FRET signal was calculated as a ratio of 520 nm (fluorescein) to 495 nm (terbium). Data points represent the mean (±SD) of threefold determination of three independent experiments.

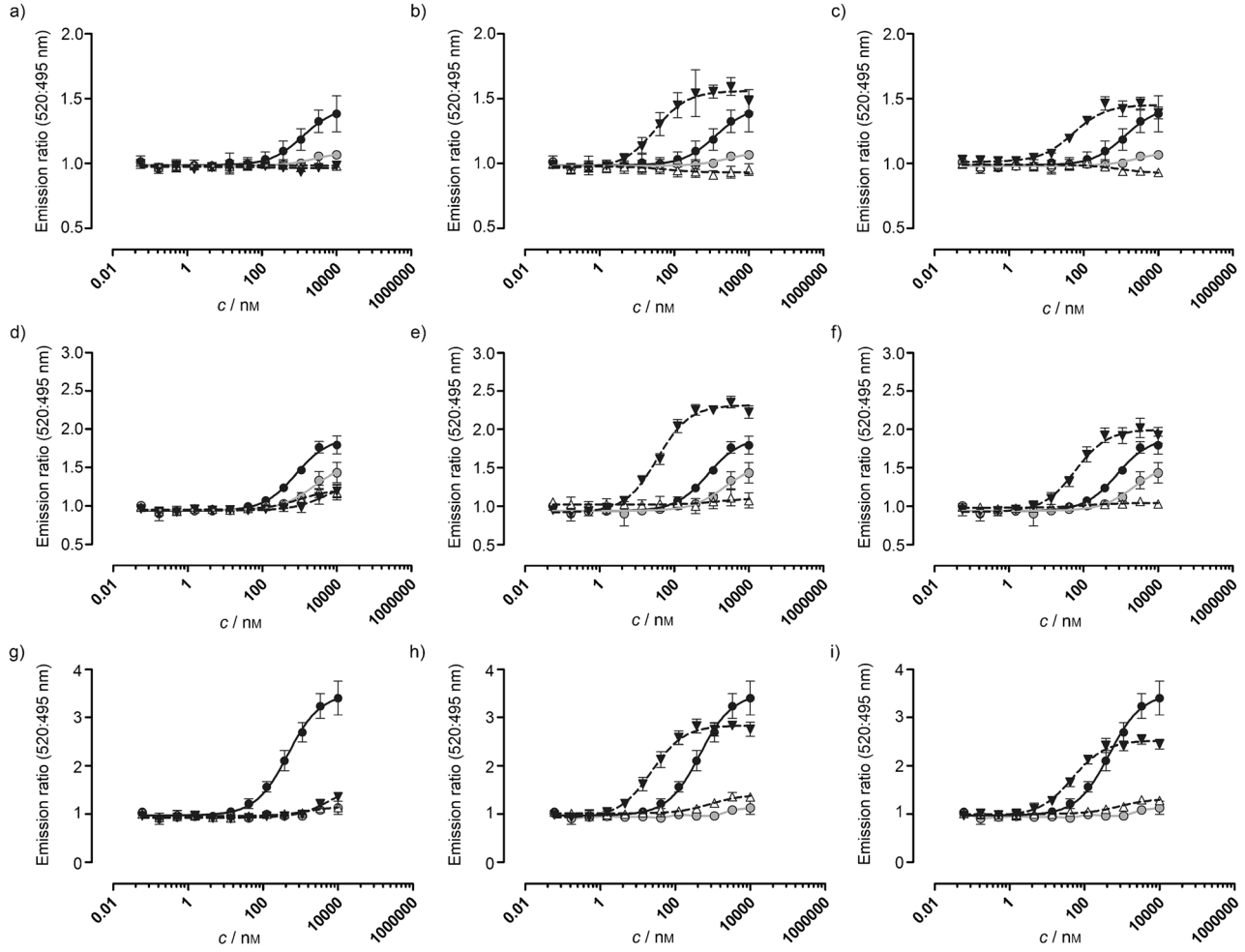


Figure 4. Coactivator recruitment by TR-FRET. Fluorescein-labeled coactivators a–c) SRC1, d–f) PGC-1α and g–i) TRAP220 were incubated with PPARγ-LBD protein labeled with terbium in the presence of increasing concentrations of pioglitazone (●), telmisartan (●), or synthesized compounds a, d, g) **4**-**5** (△), **4**-**6** (▼); b, e, h) **5**-**5** (△), **5**-**6** (▼); and c, f, i) **6**-**5** (△), **6**-**6** (▼). FRET signal increases when terbium and fluorescein are in close proximity. Recruitment of coactivators was calculated as a ratio of 520 nm (fluorescein) to 495 nm (terbium). Data points represent the mean (±SD) of threefold determination of three independent experiments.

lators (SPPARγMs) have already been tested in animal models demonstrating promising metabolic effects in vivo.[19]

Conclusions

We gained knowledge about the structure–PPARγ-activity relationship for telmisartan-like PPARγ ligands after modification of position 5 or 6 of the central 1-(biphenyl-4-ylmethyl)-1*H*-benzimidazole core of telmisartan. The shift of a substituent from position 6 to 5 showed a strong influence on potency and efficacy, and induced a selective recruitment/release pattern of cofactors. The effect was more pronounced with a benzothiophene or benzofuran moiety. Thus, these new PPARγ ligands act like SPPARMs. In vivo function and side effect profile of our compounds has to be elucidated in the future.

Experimental Section

Chemistry

General: All reagents and solvents were purchased from Acros Organics, Sigma–Aldrich, Alfa Aesar or Merck (Darmstadt, Germany). All reactions were monitored by thin-layer chromatography (TLC), performed on silica gel plates 60 F_{254} (Merck). Visualization on TLC was achieved using UV light. Column chromatography was performed with silica gel 60H, grain size <0.063 mm, 230 mesh ASTM (Merck). Melting points were determined using a B 545 Büchi capillary melting point apparatus (Flawil, Switzerland). ^{1}H NMR measurements were performed on Avance DPX-400 spectrometer (Bruker, Karlsruhe, Germany) at 400 MHz (internal standard: TMS). Elemental analyses were performed in a microlaboratory of the Freie Universität Berlin (Germany) with elemental analyzer Vario EL (Elementar, Hanau). The analytical results are within 0.4% of the theoretical values. Electron ionization (EI) mass spectra were collected using a CH-7-A-Varian MAT, 70 eV (Melbourne, Australia). A microplate reader FLASHScan S12 (Analytik Jena AG, Jena, Germany) and a

microlumat Victor 2 1420 multilabel counter (Wallac, PerkinElmer, Life sciences, Turku, Finnland) were used.

General procedure for N-alkylation with 4′-(bromomethyl)biphenyl-2-carbonitrile: NaH (2 mmol) was added to a stirred solution of the appropriate secondary amine (1 mmol) in dry *N*,*N*-dimethylformamide (DMF; 3 mL) at 0 °C (ice cooling). After approx. 30 min (or after no more visible emergence of hydrogen), 4′-(bromomethyl)biphenyl-2-carbonitrile (0.3 g, 1.1 mmol) was added slowly and first stirred for 1 h at 0 °C and then 2–5 h at RT. The reaction mixture was poured into 6 N HCl (1 mL) with crushed ice (25 g) and extracted with $CHCl_3$ (3×15 mL). The organic layers were combined and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo, and the resulting crude product was purified by column chromatography (SiO_2) with stepwise gradient elution (CH_2Cl_2/MeOH, 99:1, 98:2, 95:5).

General procedure for saponification of carbonitriles: A solution of the respective carbonitrile (1 mmol), KOH (5 mmol) in H_2O (1 mmol), and ethylene glycol (4 mL) was stirred and heated to 185 °C. Every hour of the reaction time period H_2O (1 mmol) has to be added cautiously. After 5–6 h, the reaction mixture was cooled to 100 °C, and H_2O (8 mL) was added. The solution was acidified with HCl (6 N, pH 5–6) and stirred for 15 min to complete precipitation. The obtained solid was purified by column chromatography (SiO_2) with stepwise gradient elution (CH_2Cl_2/MeOH, 95:5, 9:1, 8:2) and recrystallization from MeOH.

Ethyl-2-butyl-1*H*-benzo[*d*]imidazole-6-carboxylate (2): A solution of 3,4-diaminobenzoic acid (1 g, 6.57 mmol) in dry EtOH (20 mL) and concd H_2SO_4 (0.35 mL, 6.6 mmol) was heated under reflux. After 5–10 h, the reaction was cooled, poured into a $NaHCO_3$ solution (5%, 40 mL) and extracted with $CHCl_3$ (3×25 mL). The aqueous phase was kept at pH 8 to solely extract the desired ethyl-3,4-diaminobenzoate. The organic layers were combined and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo, and the resulting crude product (1.05 g, 5.8 mmol, 89%) was dissolved in tetrahydrofuran (THF; 10 mL). Valeryl chloride (1.38 mL, 11.6 mmol) was added dropwise and stirred for 1 h at RT. The reaction was poured into a $NaHCO_3$ solution (5%, 15 mL) and extracted with $CHCl_3$ (3×20 mL). The organic layers were combined and dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure, and the resulting crude product (ethyl-3,4-dipentanamidobenzoate, 1.92 g, 5.51 mmol, 95%) was dissolved in a suspension of toluene (55 mL) and *p*-toluenesulfonic acid monohydrate (2.1 g, 11 mmol), and refluxed for 3 h. The reaction work up was performed as described in the previous step, and the crude product was purified by column chromatography (SiO_2) with stepwise gradient elution (CH_2Cl_2/MeOH, 98:2, 95:5, 9:1) to give the compound **2** as a colorless solid (93%; 78% overall yield): ^{1}H NMR ([D$_6$]DMSO): δ = 12.50 (s, 1H), 8.05 (s, 1H), 7.75 (dd, J = 8.4, 1.2 Hz, 1H), 7.52 (s, 1H), 4.30 (quintet, J = 7.1 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 1.74 (sextet, J = 7.4 Hz, 2H), 1.32 (m, 5H), 0.90 ppm (t, J = 7.4 Hz, 3H); MS (EI, 150 °C): *m/z* (%): 246 (43) $[M]^{+\bullet}$, 217 (17), 202 (100), 188 (14).

2-Butyl-1′-methyl-1′*H*,3*H*-2′,5-bibenzo[*d*]imidazole (3): After ester cleavage of **2** (1 g, 4.06 mmol) in MeOH/aq NaOH (10%) (1:1, 18 mL) at reflux temperature for 2 h, the resulting 2-butyl-1*H*-benzimidazole-6-carboxylic acid (0.8 g, 3.7 mmol, 91%) was dissolved in polyphosphoric acid (16 g) at 150 °C, and *N*-methyl-benzene-1,2-diamine (0.5 g, 4.09 mmol) was added in small portions. After stirring at 150 °C for 24 h, the mixture was allowed to cool and H_2O was added in small portions. The solution was adjusted to pH 9 by addition of concd NH_3 under ice cooling. The precipitated solid was removed by filtration, and the soution was recrystallized from CH_2Cl_2/MeOH to give compound **3** as colorless crystals (34%; 31% overall yield): ^{1}H NMR ([D$_6$]DMSO): δ = 12.4 (s, 1H), 7.9 (d, 1H), 7.63 (m, 4H), 7.26 (m, 2H), 3.9 (s, 3H), 2.87 (t, J = 7.5 Hz, 2H), 1.79 (quintet, J = 7.5 Hz, 2H), 1.39 (sextet, J = 7.4 Hz, 2H), 0.94 ppm (t, J = 7.4 Hz, 3H); MS (EI, 175 °C): *m/z* (%): 304 (89) $[M]^{+\bullet}$, 275 (28), 262 (100).

4′-[(1′-Methyl-2-propyl-2′,5-bi-1*H*-benzo[*d*]imidazole]-1-yl)methyl]biphenyl-2-carboxylic acid (4-5) and 4′-[(1′-Methyl-2-butyl-2′,6-bi-1*H*-benzo[*d*]imidazole]-1-yl)methyl]biphenyl-2-carboxylic acid (4-6): Compound **3** (0.38 g, 1.26 mmol) was N-alkylated by a general procedure with 4′-(bromomethyl)biphenyl-2-carbonitrile (0.38 g, 1.38 mmol). The resulting isomeric mixture of 4′-[(1′-methyl-2-propyl-2′,5-/6-bi-1*H*-benzo[*d*]imidazole]-1-yl)methyl]biphenyl-2-carbonitrile (89%, 0.56 g, 1.12 mmol) was treated with KOH (0.31 g, 5.6 mmol) as described for the general procedure for saponification to give a colorless solid. Separation of the 5- and 6-regioisomers was carried out by a combination of column chromatography (SiO_2, CH_2Cl_2/MeOH, 95:5) and fractional crystallization.

Compound **4-5** was isolated (CH_2Cl_2/MeOH, 9:1) as a colorless solid (34%): mp: 159 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.73 (s, 1H), 8.07 (m, 1H), 7.72–7.67 (m, 4H), 7.61 (d, J = 7.6 Hz, 1H), 7.55 (td, J = 7.6, 1.2 Hz, 1H), 7.44 (td, J = 7.5, 1.1 Hz, 1H), 7.36–7.23 (m, 5H), 7.23–7.19 (m, 2H), 5.63 (s, 2H), 3.92 (s, 3H), 2.93 (t, J = 7.5 Hz, 2H), 1.78 (quintet, J = 7.4 Hz, 2H), 1.41 (sextet, J = 7.4 Hz, 2H), 0.91 ppm (t, J = 7.4 Hz, 3H); MS (EI, 300 °C): *m/z* (%): 514 (8) $[M]^{+\bullet}$, 470 (100), 428 (59), 211 (48), 167 (56), 44 (68); Anal. calcd for $C_{33}H_{30}N_4O_2 \times 0.75\,H_2O$: C 75.05, H 6.01, N 10.61, found: C 75.17, H 6.31, N 10.82.

Compound **4-6** was isolated (CH_2Cl_2/MeOH, 9:1) as a colorless solid (38%): mp: 220 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.82 (s, 1H), 7.93 (d, J = 1.1 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.72–7.68 (m, 2H), 7.65 (dd, J = 8.3, 1.5 Hz, 1H), 7.60 (d, J = 7.4 Hz, 1H), 7.54 (td, J = 7.6, 1.4 Hz, 1H), 7.43 (td, J = 7.5, 1.2 Hz, 1H), 7.34 (dd, J = 7.6, 0.9 Hz, 1H), 7.32–7.22 (m, 4H), 7.19 (d, J = 8.2 Hz, 2H), 5.65 (s, 2H), 3.84 (s, 3H), 2.96 (t, J = 7.5 Hz, 2H), 1.79 (quintet, J = 7.4 Hz, 2H), 1.42 (sextet, J = 7.4 Hz, 2H), 0.92 ppm (t, J = 7.4 Hz, 3H); MS (EI, 220 °C): *m/z* (%) = 514 (6) $[M]^{+\bullet}$, 469 (100), 211 (32); Anal. calcd for $C_{33}H_{30}N_4O_2$: C 77.02, H 5.88, N 10.89, found: C 77.11, H 5.87, N 10.75.

4′-[(5-(1-Benzothiophen-2-yl)-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl]biphenyl-2-carboxylic acid (5-5): 1-Benzothiophen-2-ylboronic acid (0.413 g, 2.32 mmol), $Pd(PPh_3)_4$ (0.126 g, 0.12 mmol), and an aq Na_2CO_3 solution (5.8 mL, 0.4 M, 2.32 mmol) was added to a solution of 4′-[(5-bromo-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl]biphenyl-2-carbonitrile (0.5 g, 1.16 mmol) in MeCN (4.5 mL). The mixture was stirred at 90 °C under N_2 atmosphere for 5–6 h, cooled to RT and diluted with H_2O (25 mL). The reaction mixture was extracted with $CHCl_3$ (3×25 mL). The organic layers were combined and dried over Na_2SO_4. After filtration, the solvent was evaporated in vacuo, and the resulting crude product was purified by column chromatography (SiO_2) with stepwise gradient elution (CH_2Cl_2/MeOH, 98:2, 95:5, 9:1). The resulting 4′-[(5-(1-benzothiophen-2-yl)-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl]biphenyl-2-carbonitrile (74%, 0.415 g, 0.86 mmol) was treated with KOH (0.24 g, 4.3 mmol) as described in the general procedure for saponification to give a colorless solid (85%; 63% overall): mp: 272 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.73 (s, 1H), 7.99 (s, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.83–7.81 (m, 2H), 7.7 (dd, J = 7.5, 1 Hz, 1H), 7.65–7.60 (m, 2H), 7.54 (td, J = 7.26, 1.34 Hz, 1H), 7.45–7.29 (m, 6H), 7.15 (d, J = 8.2 Hz, 2H), 5.58 (s, 2H), 2.87 (t, J = 7.4 Hz, 2H), 1.80 (sextet, J = 7.3 Hz, 2H), 0.98 ppm (t, J = 7.3 Hz, 3H); MS (EI, 250 °C): *m/z* (%): 502 (36) $[M]^{+\bullet}$, 458 (61), 211 (41), 167 (100); Anal. calcd for $C_{32}H_{26}N_2O_2S$: C 76.47, H 5.21, N 5.57, found: C 76.61, H 5.37, N 5.76.

4′-[(6-(1-Benzothiophen-2-yl)-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)-methyl]biphenyl-2-carboxylic acid (5-6): The synthesis was performed as described for compound **5-5**, however, starting with 4′-[(6-bromo-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl]biphenyl-2-carbonitrile (0.5 g, 1.16 mmol) to receive compound **5-6** as a colorless solid (58% overall): mp: 267 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.69 (s, 1H), 7.99 (s, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.81–7.79 (m, 2H), 7.69–7.66 (m, 2H), 7.58 (td, J = 8.3, 1.69 Hz, 1H), 7.51 (td, J = 7.56 Hz, J = 1.45 Hz, 1H), 7.41 (td, J = 7.57 Hz, J = 1.28 Hz, 1H), 7.37–7.28 (m, 5H), 7.17–7.15 (m, 2H), 5.63 (s, 2H), 2.83 (t, J = 7.4 Hz, 2H), 1.78 (sextet, J = 7.3 Hz, 2H), 0.95 ppm (t, J = 7.3 Hz, 3H); MS (EI, 250 °C): *m/z* (%): 502 (100) $[M]^{+\bullet}$, 211 (93); Anal. calcd for $C_{32}H_{26}N_2O_2S$: C 76.47, H 5.21, N 5.57, found: C 76.32, H 5.45, N 5.78.

4′-[(5-(1-Benzofuran-2-yl)-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)-methyl]biphenyl-2-carboxylic acid (6-5): The synthesis was performed as described for compound **5-5**, however, starting with 4′-[(5-bromo-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl]biphenyl-2-carbonitrile (0.5 g, 1.16 mmol) and 1-benzofuran-2-ylboronic acid (0.376 g, 2.32 mmol). After saponification, compound **6-5** was isolated as a colorless solid (70% overall): mp: 286 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.75 (s, 1H), 8.40 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.5 Hz, 1H), 7.73–7.68 (m, 2H), 7.64 (d, J = 8.3, 1H), 7.56–7.53 (m, 2H), 7.44 (td, J = 7.5 Hz, J = 1.2 Hz, 1H), 7.37–7.26 (m, 7H), 5.89 (s, 2H), 3.13 (t, J = 7.4 Hz, 2H), 1.80 (sextet, J = 7.3 Hz, 2H), 0.98 ppm (t, J = 7.3 Hz, 3H); MS (EI, 275 °C): *m/z* (%) = 486 (96) $[M]^{+\bullet}$, 247 (50), 211 (73), 167 (100); Anal. calcd for $C_{32}H_{26}N_2O_3 \times 0.5\,H_2O$: C 77.56, H 5.49, N 5.65, found: C 77.26, H 5.86, N 5.73.

4′-[(6-(1-Benzofuran-2-yl)-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)-methyl]biphenyl-2-carboxylic acid (6-6): The synthesis was performed as described for compound **5-5**, however, starting with 4′-[(6-bromo-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl]biphenyl-2-carbonitrile (0.5 g, 1.16 mmol) and 1-benzofuran-2-ylboronic acid (0.376 g, 2.32 mmol). After saponification, compound 6-6 was isolated as a colorless solid (59% overall): mp: 272 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.74 (s, 1H), 8.15 (d, J = 1.3 Hz, 1H), 7.79 (dd, J = 8.4 Hz, J = 1.6 Hz, 1H), 7.71 (dd, J = 7.7 Hz, J = 1.3 Hz, 1H), 7.66–7.61 (m, 3H), 7.55 (td, J = 7.6, J = 1.4 Hz, 1H), 7.44 (td, J = 7.6, J = 1.4 Hz, 1H), 7.38–7.22 (m, 6H), 7.18–7.14 (m, 2H), 5.59 (s, 2H), 2.87 (t, J = 7.4 Hz, 2H), 1.81 (sextet, J = 7.3 Hz, 2H), 0.98 ppm (t, J = 7.3 Hz, 3H); MS (EI, 260 °C): *m/z* (%): 486 (100) $[M]^{+\bullet}$, 247 (35), 211 (63); Anal. calcd for $C_{32}H_{26}N_2O_3$: C 78.99, H 5.39, N 5.76, found: C 78.91, H 5.37, N 5.84.

Biology

General: Pioglitazone was obtained from Enzo Life Sciences (Germany) and telmisartan was extracted as previously described.[10a]

PPARγ transactivation assay: Transient transfection (Invitrogen) and dual-luciferase reporter assay (Promega) was performed according the manufacturer's protocol. COS-7 cells were seeded in 96-well plates at a density of 1×10^5 cells/well in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS/1% penicillin/streptomycin and incubated at 37 °C for 20 h prior to transfection. After changing to serum-free medium, cells were transfected with Lipofectamine 2000 (0.25 μL; Invitrogen), pGal4-hPPARγDEF (4.5 ng), pGal5-TK-pGL3 (45 ng) and p*Renilla*-CMV (3 ng) in Opti-MEM (25 μL; Gibco).[20] After 4 h, the synthesized compounds, pioglitazone, telmisartan or vehicle (DMSO) were added in indicated concentrations, and luciferase activity was measured after 36 h.

Differentiation assay: Murine 3T3-L1 preadipocytes (CL-173; ATCC, USA) were maintained in DMEM supplemented with FBS (10%) and differentiated by a modified protocol previously described.[21] Briefly, postconfluent preadipocytes were treated with complete medium containing dexamethasone (1 μM) and insulin (0.17 μM) for 3 days. After medium change, cells were incubated with complete medium containing only insulin (0.17 μM) for further 3 days and with solely complete medium for another 3 days. During the whole stimulation period, the medium contained either one of the synthesized compounds, pioglitazone, telmisartan or vehicle (DMSO) at three different concentrations (0.1 μM, 1 μM and 10 μM). At day 9 of differentiation, cells were washed with phosphate buffered saline and stained with Oil-Red O for 1 h.

Time-resolved fluorescence resonance energy transfer in vitro: Cell-free LanthaScreen TR-FRET PPARγ coactivator assay (Invitrogen)[22] was used according to the manufacturer's protocol. Briefly, the recombinant ligand binding domain of PPARγ (PPARγ-LBD) is tagged with glutathione S-transferase (GST), and can be recognized by an anti-GST antibody. Dilution series of the synthesized compounds, pioglitazone and telmisartan were produced in triplicates and combined with PPARγ-LBD and anti-GST antibody in black 384-well plates (Corning). Maximum concentration of pioglitazone represented the positive assay control and DMSO served as a negative control. Binding of the agonist induced a conformational change of the PPARγ-LBD, resulting in an increased affinity for coactivator peptides. In this assay, cofactor peptides are labeled with fluorescein (coactivators: SRC1-GPQTPQAQQKSLLQQLLTE, PGC-1α-EAEEPSLLKKLLLAPANTQ, TRAP220-NTKNHPMLMNLLKDNPAQD and corepressor: NCoR1-DPASNLGLEDIIRKALMGSFDDK). Close proximity of terbium and fluorescein increased the FRET signal. Fluorescence intensities were measured with a filter-based EnVision plate reader (PerkinElmer) and FRET signal was calculated by the ratio of the emission signal at 520 nm (fluorescein) and 495 nm (terbium). Instrument settings were consistent with the specifications of the manufacturer for LanthaScreen assays and were tested with LanthaScreen Tb instrument control kit (Invitrogen).

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Keywords: cofactors · PPARγ · SPPARMs · structure–activity relationships · telmisartan

[1] M. Lehrke, M. A. Lazar, *Cell* **2005**, *123*, 993 – 999.
[2] a) G. Boden, M. Zhang, *Expert Opin. Invest. Drugs* **2006**, *15*, 243 – 250; b) R. W. Nesto, D. Bell, R. O. Bonow, V. Fonseca, S. M. Grundy, E. S. Horton, M. Le Winter, D. Porte, C. F. Semenkovich, S. Smith, L. H. Young, R. Kahn, *Diabetes Care* **2004**, *27*, 256 – 263; c) W. Wei, Y. Wan, *PPAR Res.* **2011**, *2011*, 867180.
[3] a) J. H. Choi, A. S. Banks, J. L. Estall, S. Kajimura, P. Bostrom, D. Laznik, J. L. Ruas, M. J. Chalmers, T. M. Kamenecka, M. Bluher, P. R. Griffin, B. M. Spiegelman, *Nature* **2010**, *466*, 451 – 456; b) J. H. Choi, A. S. Banks, T. M. Kamenecka, S. A. Busby, M. J. Chalmers, N. Kumar, D. S. Kuruvilla, Y. Shin, Y. He, J. B. Bruning, D. P. Marciano, M. D. Cameron, D. Laznik, M. J. Jurczak, S. C. Schurer, D. Vidovic, G. I. Shulman, B. M. Spiegelman, P. R. Griffin, *Nature* **2011**, *477*, 477 – 481.
[4] a) E. J. Murphy, T. J. Davern, A. O. Shakil, L. Shick, U. Masharani, H. Chow, C. Freise, W. M. Lee, N. M. Bass, *Dig. Dis. Sci.* **2000**, *45*, 549 – 553; b) J. D. Lewis, A. Ferrara, T. Peng, M. Hedderson, W. B. Bilker, C. P. Quesenberry, Jr., D. J. Vaughn, L. Nessel, J. Selby, B. L. Strom, *Diabetes Care* **2011**, *34*, 916 – 922.

[5] J. P. Berger, A. E. Petro, K. L. Macnaul, L. J. Kelly, B. B. Zhang, K. Richards, A. Elbrecht, B. A. Johnson, G. Zhou, T. W. Doebber, C. Biswas, M. Parikh, N. Sharma, M. R. Tanen, G. M. Thompson, J. Ventre, A. D. Adams, R. Mosley, R. S. Surwit, D. E. Moller, *Mol. Endocrinol.* **2003**, *17*, 662 – 676.
[6] R. T. Nolte, G. B. Wisely, S. Westin, J. E. Cobb, M. H. Lambert, R. Kurokawa, M. G. Rosenfeld, T. M. Willson, C. K. Glass, M. V. Milburn, *Nature* **1998**, *395*, 137 – 143.
[7] J. B. Bruning, M. J. Chalmers, S. Prasad, S. A. Busby, T. M. Kamenecka, Y. He, K. W. Nettles, P. R. Griffin, *Structure* **2007**, *15*, 1258 – 1271.
[8] T. S. Hughes, M. J. Chalmers, S. Novick, D. S. Kuruvilla, M. R. Chang, T. M. Kamenecka, M. Rance, B. A. Johnson, T. P. Burris, P. R. Griffin, D. J. Kojetin, *Structure* **2012**, *20*, 139 – 150.
[9] a) S. C. Benson, H. A. Pershadsingh, C. I. Ho, A. Chittiboyina, P. Desai, M. Pravenec, N. Qi, J. Wang, M. A. Avery, T. W. Kurtz, *Hypertension* **2004**, *43*, 993 – 1002; b) M. Schupp, M. Clemenz, R. Gineste, H. Witt, J. Janke, S. Helleboid, N. Hennuyer, P. Ruiz, T. Unger, B. Staels, U. Kintscher, *Diabetes* **2005**, *54*, 3442 – 3452.
[10] a) M. Goebel, M. Clemenz, B. Staels, T. Unger, U. Kintscher, R. Gust, *ChemMedChem* **2009**, *4*, 445 – 456; b) M. Goebel, B. Staels, T. Unger, U. Kintscher, R. Gust, *ChemMedChem* **2009**, *4*, 1136 – 1142; c) M. Goebel, G. Wolber, P. Markt, B. Staels, T. Unger, U. Kintscher, R. Gust, *Bioorg. Med. Chem.* **2010**, *18*, 5885 – 5895.
[11] J. Charton, S. Girault-Mizzi, C. Sergheraert, *Chem. Pharm. Bull.* **2005**, *53*, 492 – 497.
[12] U. J. Ries, G. Mihm, B. Narr, K. M. Hasselbach, H. Wittneben, M. Entzeroth, J. C. van Meel, W. Wienen, N. H. Hauel, *J. Med. Chem.* **1993**, *36*, 4040 – 4051.
[13] A. Casimiro-Garcia, G. F. Filzen, D. Flynn, C. F. Bigge, J. Chen, J. A. Davis, D. A. Dudley, J. J. Edmunds, N. Esmaeil, A. Geyer, R. J. Heemstra, M. Jalaie, J. F. Ohren, R. Ostroski, T. Ellis, R. P. Schaum, C. Stoner, *J. Med. Chem.* **2011**, *54*, 4219 – 4233.
[14] Y. Lamotte, P. Martres, N. Faucher, A. Laroze, D. Grillot, N. Ancellin, Y. Saintillan, V. Beneton, R. T. Gampe, Jr., *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1399 – 1404.
[15] E. D. Rosen, P. Sarraf, A. E. Troy, G. Bradwin, K. Moore, D. S. Milstone, B. M. Spiegelman, R. M. Mortensen, *Mol. Cell* **1999**, *4*, 611 – 617.
[16] a) K. Henriksen, I. Byrjalsen, P. Qvist, H. Beck-Nielsen, G. Hansen, B. J. Riis, H. Perrild, O. L. Svendsen, J. Gram, M. A. Karsdal, C. Christiansen, *Diabetes Metab. Res. Rev.* **2011**, *27*, 392 – 401; b) F. L. Dunn, L. S. Higgins, J. Fredrickson, A. M. DePaoli, *J. Diabetes Complications* **2011**, *25*, 151 – 158.
[17] Y. Guan, C. Hao, D. R. Cha, R. Rao, W. Lu, D. E. Kohan, M. A. Magnuson, R. Redha, Y. Zhang, M. D. Breyer, *Nat. Med.* **2005**, *11*, 861 – 866.
[18] T. S. Pavlov, J. D. Imig, A. Staruschenko, *PPAR Res.* **2010**, *2010*, 703735.
[19] S. Rocchi, F. Picard, J. Vamecq, L. Gelman, N. Potier, D. Zeyer, L. Dubuquoy, P. Bac, M. F. Champy, K. D. Plunket, L. M. Leesnitzer, S. G. Blanchard, P. Desreumaux, D. Moras, J. P. Renaud, J. Auwerx, *Mol. Cell* **2001**, *8*, 737 – 747.
[20] E. Raspé, L. Madsen, A. M. Lefebvre, I. Leitersdorf, L. Gelman, J. Peinado-Onsurbe, J. Dallongeville, J. C. Fruchart, R. Berge, B. Staels, *J. Lipid Res.* **1999**, *40*, 2099 – 2110.
[21] M. Schupp, J. Janke, R. Clasen, T. Unger, U. Kintscher, *Circulation* **2004**, *109*, 2054 – 2057.
[22] F. J. Schopfer, M. P. Cole, A. L. Groeger, C. S. Chen, N. K. Khoo, S. R. Woodcock, F. Golin-Bisello, U. N. Motanya, Y. Li, J. Zhang, M. T. Garcia-Barrio, T. K. Rudolph, V. Rudolph, G. Bonacci, P. R. Baker, H. E. Xu, C. I. Batthyany, Y. E. Chen, T. M. Hallis, B. A. Freeman, *J. Biol. Chem.* **2010**, *285*, 12321 – 12333.

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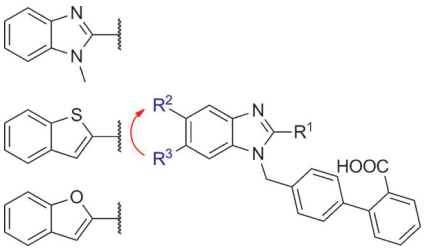
Changes to the core: The AT_1-receptor blocker telmisartan is a partial PPARγ agonist with promising in vitro and in vivo properties for the treatment of insulin resistance. However, potency and efficacy need to be optimized to accomplish sufficient plasma levels for PPARγ activation. Therefore, we performed modifications at position 2, 5 and 6 in order to increase potency, but also to analyze the structure–activity relationship and its influence on cofactor recruitment.



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Characterization of Telmisartan-Derived PPARγ Agonists: Importance of Moiety Shift from Position 6 to 5 on Potency, Efficacy and Cofactor Recruitment

