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Characterization of New PPARγ Agonists: Analysis of Telmisartan's Structural Components

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In addition to a proven efficacy in lowering blood pressure, the AT1 receptor blocker telmisartan has recently been shown to exert pleiotropic effects as a partial agonist of the nuclear peroxisome proliferator-activated receptor gamma (PPARγ). Based on these findings and an excellent side-effect profile, telmisartan may serve as a lead structure for the development of new PPARγ ligands. Therefore, we analyzed the structural components of telmisartan to identify those necessary for PPARγ activation. Synthesized compounds were tested in a differentiation assay using 3T3-L1 preadipocytes and a luciferase assay with COS-7 cells transiently transfected with pGal4-hPPARγDEF, pGal5-TK-pGL3 and pRL-CMV. The data obtained in this structure–activity relationship (SAR) study provide the basis for the development of new PPARγ ligands, which could lead to active compounds with a distinct, beneficial pharmacological profile compared with the existing full agonists. The basic 1-(biphenyl-4-ylmethyl)-1H-benzimidazole scaffold of telmisartan was identified as an essential moiety with either a carboxylic acid or tetrazole group at the C-2 position of the biphenyl. For maximum potency and activity, the alkyl chain in position 2 requires a minimum length of at least two C atoms (ethyl group), while the methyl group at position 4 of the benzimidazole core seems to contribute to partial activity. An additional benzimidazole at position 6 appears to be a further determinant of potency. Similar conclusions can be drawn for the methyl group in position 1.

Introduction

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily of ligand-activated transcription factors.[1–4] The most abundant isoform in adipose tissue, PPARγ, plays an important role in the regulation of insulin sensitivity.[5] Glitazones or thiazolidinediones, such as pioglitazone or rosiglitazone, are high-affinity ligands and full agonists for this receptor, and are currently used in the treatment of type 2 diabetes mellitus. Because of the side effects of glitazones, such as weight gain, edema and fluid retention, the characterization of new PPARγ ligands that retain metabolic efficacy without exerting adverse actions plays a central role in the development of new therapeutic strategies for insulin resistance and type 2 diabetes mellitus.[6] A promising new group of such ligands are selective PPARγ modulators (SPPARγMs), compounds that activate only a subset of the functions induced by cognate ligands or act in a cell-type-selective manner. A typical property observed in this new class of compounds is partial agonistic receptor activity.[7,8] It has been shown that a subgroup of AT_1 receptor blockers (ARBs) induced PPARγ activity, among which, telmisartan showed the highest efficacy.[9,10] Additionally, there is accumulating evidence that telmisartan and irbesartan are SPPARγMs.[11]

The aim of this study was to first identify essential structural components of telmisartan for PPARγ activation, and then to use this structure activity relationship (SAR) data for structural optimization to achieve a compound with high potency and a partial agonist activity profile. Telmisartan was divided into four parts, which were investigated for their PPARγ modulating activity (Figure 1). The structural modifications are listed in Figure 2, including some derivatives previously described by Narr and co-workers.[12]

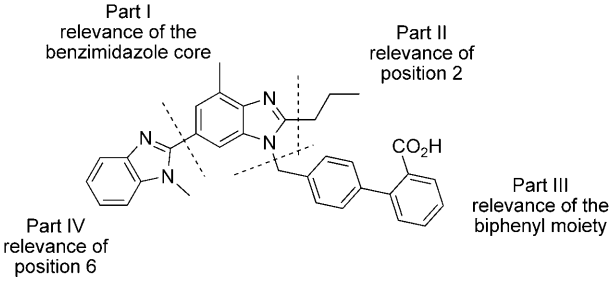

Figure 1. Telmisartan divided into four parts for investigating their relevance of PPARγ activation.

Part I included 2′-propyl-1*H*,3′*H*-2,5′-bibenzo[*d*]imidazole (**2**) and the biphenyl-2-carboxylic acid (BPA). The benzimidazole

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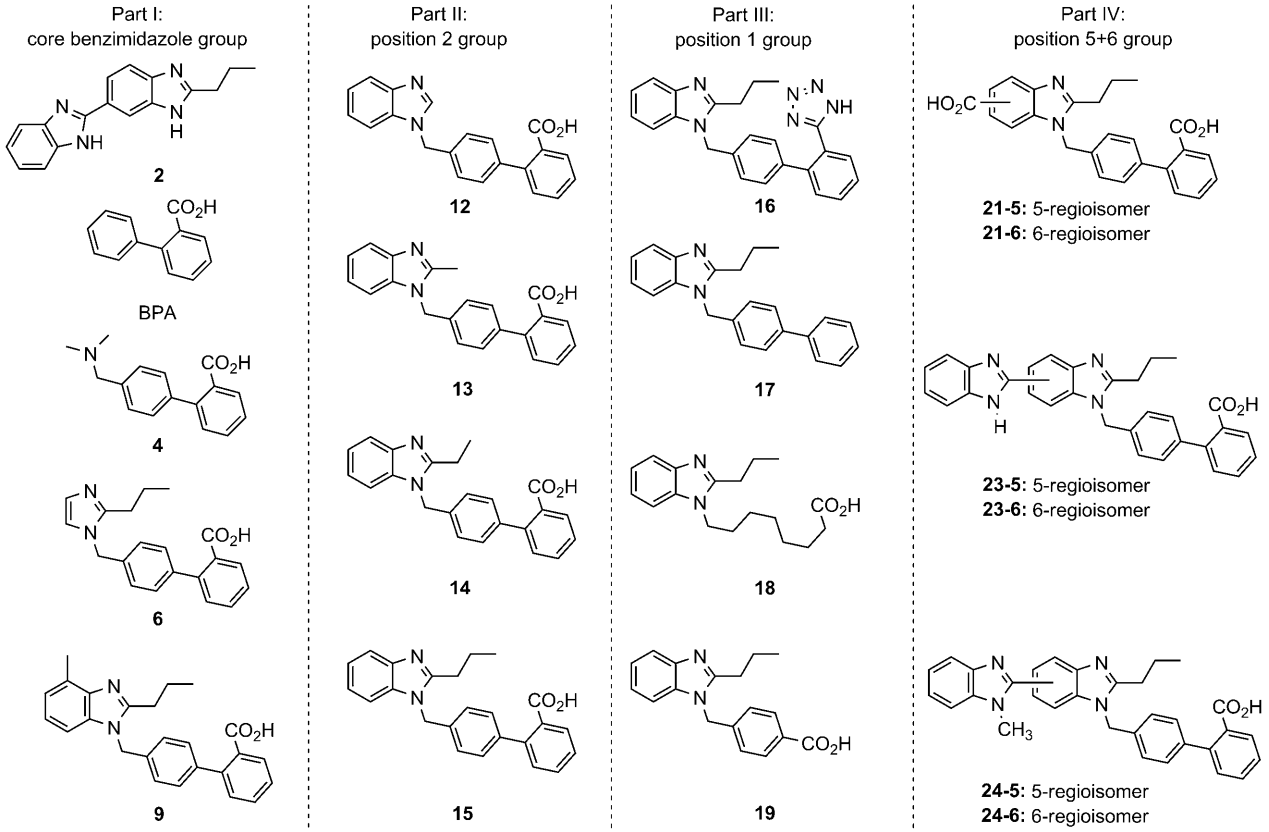
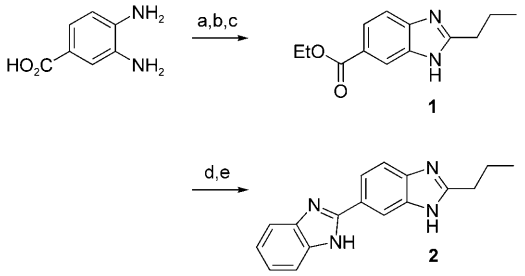


Figure 2. Overview and arrangement in groups of synthesized and tested compounds.

core was assembled stepwise in the synthesis of BPA derivatives **4**, **6** and **9** to show its relevance for PPARγ activation. In part II, we focused our attention on the influence of the alkyl chain at position 2 of the benzimidazole core. The chain length was varied from R = H to R = *n*Pr (compounds **12**–**15**). The compounds of part III provided information on the biphenyl moiety and the attached carboxyl group. The COOH group was replaced by a proton (compound **17**) or a bioisosteric tetrazole moiety (compound **16**), while a C_7 chain in compound **18** is able to mimic the biphenyl structure. Replacement of the biphenyl with a phenyl (compound **19**) provided data on the relevance of the orientation and distance of the carboxyl function to the benzimidazole. Part IV included compounds **21-5/6**, **23-5/6** and **24-5/6** probing the importance of the second benzimidazole in position 6 of telmisartan as well as the N-1 methyl group.

Chemistry

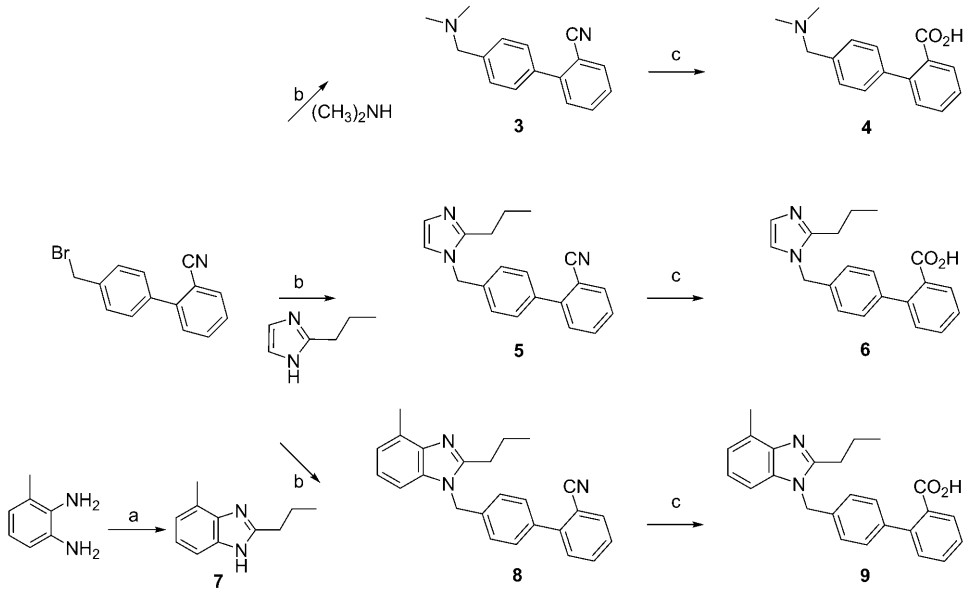
After esterification of 3,4-diaminobenzoic acid with ethanol/H_2SO_4, the free amino groups were acylated with butyryl chloride in anhydrous THF at room temperature, followed by cyclocondensation in toluene and *p*-toluenesulfonic acid to give compound **1** (Scheme 1).[13] Ester cleavage was carried out in aq NaOH (10%) and methanol (1:1). The cyclocondensation of the free carboxyl group with 1,2-benzenediamine in polyphosphoric acid at 150 °C yielded compound **2**.[14]



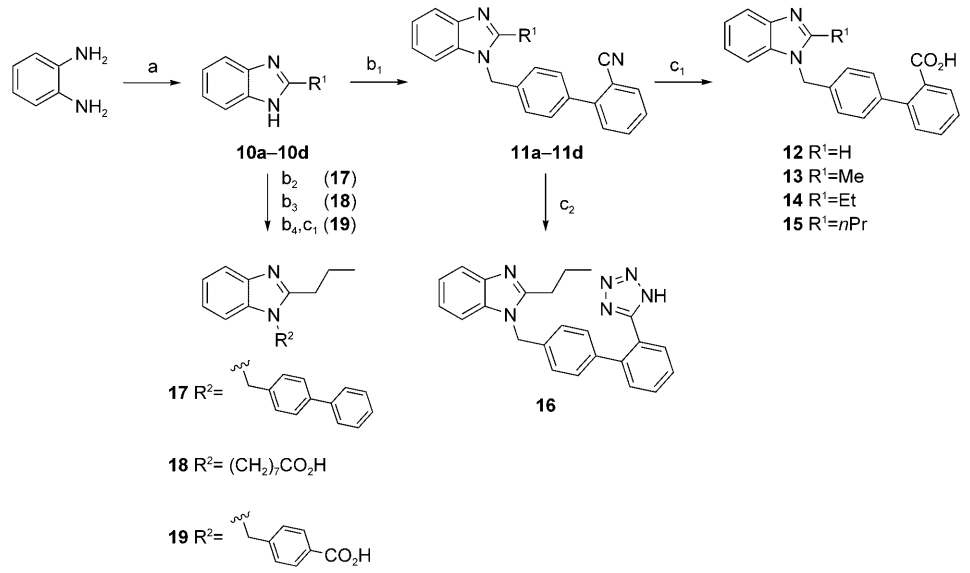
Scheme 1. *Reagents and Conditions*: a) EtOH, H_2SO_4, reflux; b) THF, butyryl chloride, RT; c) toluene, *p*TsOH·H_2O, reflux; d) MeOH, aq NaOH (10%), reflux; e) 1,2-benzenediamine, polyphosphoric acid, 150 °C.

The intermediates **3**, **5** and **8** were generated by reaction of 4′-(bromomethyl)-2-biphenylcarbonitrile with dimethylamine, 2-propylimidazole or compound **7** in anhydrous DMF and NaH. Compound **7** was prepared in advance by cyclocondensation of 3-methyl-1,2-benzenediamine with ethyl butanimidoate in ethanol. Hydrolysis of the nitrile group with KOH in ethylene glycol yielded **4**, **6**[15] and **9** (Scheme 2).

Scheme 3 shows the synthetic route for compounds **12**–**19**. The 2-substituted benzimidazoles **10a-d** were prepared by dissolving 1,2-benzenediamine in the appropriate *ortho*-ester and treating the solution dropwise with concentrated HCl to form the ring. Compounds **11a-d** and **17**–**19** were then obtained by N-alkylation with the respective alkyl halogenide using a protocol analogous to the method described for the preparation of



Scheme 2. *Reagents and Conditions*: a) ethyl butanimidoate hydrochloride, EtOH, reflux; b) NaH, DMF, 0 °C→RT; c) KOH, ethylene glycol, H_2O, 185 °C.



Scheme 3. *Reagents and Conditions*: a) $R^1C(OCH_3)_3$, HCl, RT; b_1) NaH, 4′-(bromomethyl)-2-biphenylcarbonitrile, DMF, 0 °C→RT; b_2) NaH, 4-(bromomethyl)biphenyl, DMF, 0 °C→RT; b_3) NaH, 8-bromooctanoic acid, DMF, 0 °C→RT; b_4) NaH, 4-(bromomethyl)benzonitrile, DMF, 0 °C→rt; c_1) ethylene glycol, KOH, 185 °C; c_2) NaN_3, NH_4Cl, DMF, 140 °C.

compounds **3**, **5** and **8**. Hydrolysis of nitriles **11 a-d** finally resulted in compounds **12**–**15**. Compound **11 d** was heated with NaN_3 and NH_4Cl in anhydrous DMF to 140 °C for 24 h to transform the nitrile into a tetrazole (**16**).

The isomers **20**-**5/6** were prepared by N-alkylation of compound **1** with 4′-(bromomethyl)-2-biphenylcarbonitrile and subsequent ester cleavage (Scheme 4). Treatment of the carbonitrile with KOH in ethylene glycol at 185 °C provided **21**-**5/6**. The synthesis of **22**-**5/6** started with the transformation of **20**-**5/6** into the acid chloride by using $SOCl_2$ in THF. The reaction mixture was then added directly to a solution of 1,2-benzenediamine in THF to form the acylated diamine allowing the formation of a benzimidazole by cyclocondensation. Saponification of **22**-**5/6** gave compounds **23**-**5/6**, which were reacted with MeI to give the *N*-methyl derivatives **24**-**5/6**.[14]

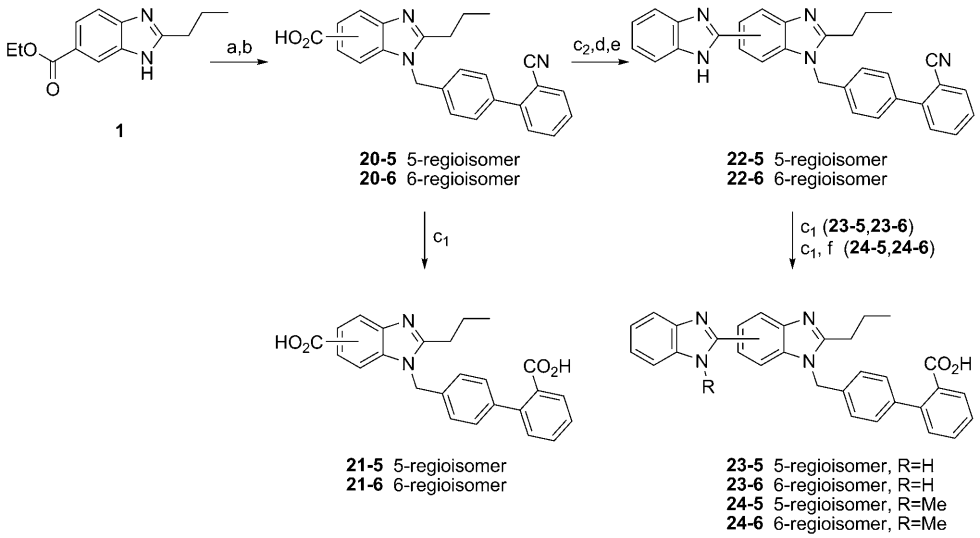
The structural assignment of **21**-**5/6**, **23**-**5/6** and **24**-**5/6** (to either the 5 or 6 regioisomer) and **9** (to the 4-methyl isomer) was performed by differential ^{1}H NMR Nuclear Overhauser Effect experiments (NOE-Diff) based on the saturation transfer from the benzylic methylene group to the C-7 proton (H-7) at the central benzimidazole. The observed NOE (CH_2, H-7; figure S2, Supporting Information) demonstrated unequivocally the positioning of the methyl group in compound **9** at C-4. Irradiation of the methylene protons in compounds **21**-**5/6**, **23**-**5/6** and **24**-**5/6** reduced the splitting of H-7 (5-isomer, doublet; 6-isomer, singlet; see figures S3–S8, Supporting Information).

Results and Discussion

In vitro SAR studies

Compounds were evaluated for PPARγ activation in vitro. PPARγ is known as the "master regulator" of adipocyte differentiation, and its activity closely correlates with the degree of differentiation analyzed by Oil Red O staining. Therefore, 3T3-L1 pre-/adipocyte differentiation was chosen as an established model for the assessment of cellular PPARγ activation screening (see Figure 3 and Figure 4, concentrations 1 and 10 μM of each compound were used; DMSO as vehicle (V); pioglitazone (P) and telmisartan (T) as positive controls).

No significant adipocyte differentiation was observed for BPA or compounds **2**, **4** and **6**. Compound **9** with the 4-methyl-1*H*-benzimidazole moiety induced adipocyte differentiation at 10 μM. Differentiation was also induced by compounds **12**–**15**, dependent on the C-2 alkyl chain. These findings demonstrated that a 1-(biphenyl-4-ylmethyl)-1*H*-benzimidazole structure is required for PPARγ activation. Comparison



Scheme 4. *Reagents and Conditions*: a) NaH, 4′-(bromomethyl)-2-biphenylcarbonitrile, DMF, 0 °C→RT; b) MeOH, aq NaOH (10%); c_1) KOH, ethylene glycol, H_2O, 185 °C; c_2) THF, $SOCl_2$, 60°; d) 1,2-benzenediamine, THF, 0 °C→RT; e) toluene, *p*TsOH·H_2O, 110°C; f) NaH, MeI, DMF, 0 °C→RT.

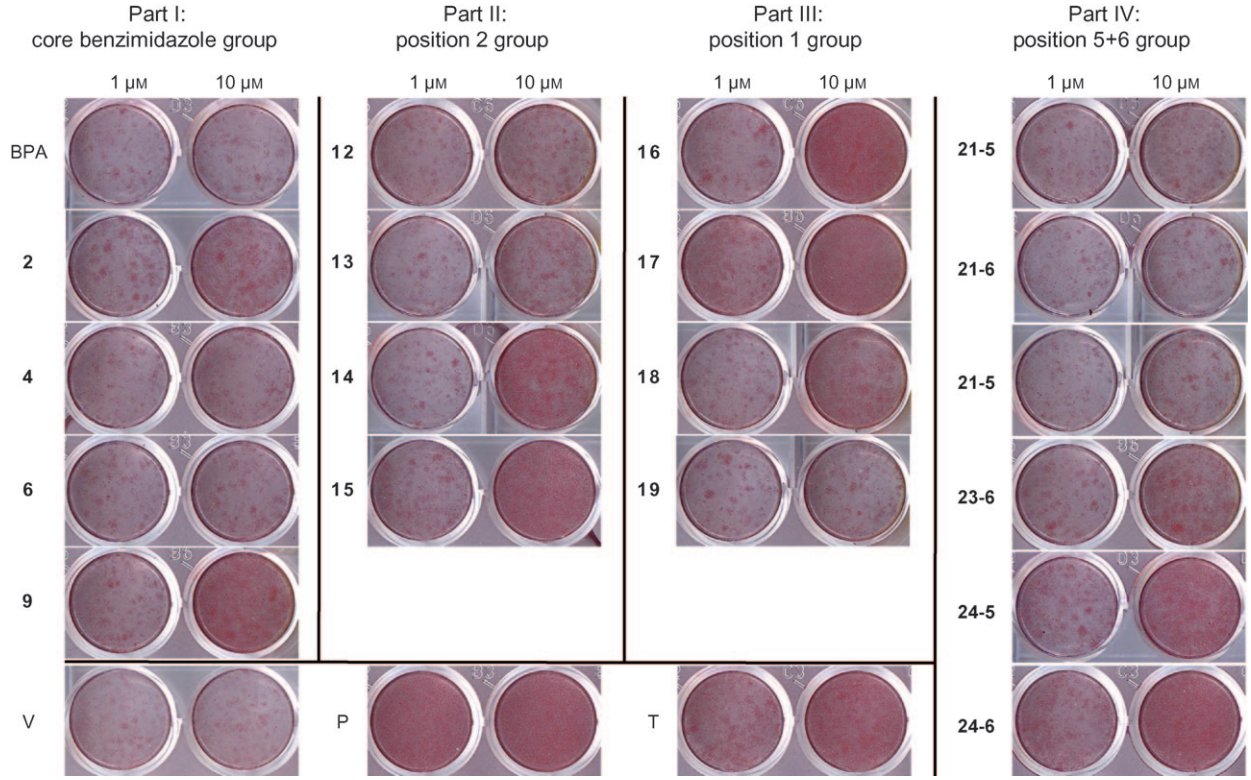


Figure 3. Adipocyte differentiation assay with 3T3-L1 cells in 24-well plates after 9 days differentiation ± the indicated compounds at 1 and 10 μM. DMSO as vehicle (V), pioglitazone (P) and telmisartan (T) as positive controls. Cells were stained with Oil Red O and one representative photograph out of three independent experiments is shown.

of the effects of compounds **15** and **9** indicated a reduction in activity and partial PPARγ activity due to the presence of the 4-methyl group in this class of compounds. Shortening of the propyl chain at C-2 of compound **15** to ethyl (compound **14**), methyl (compound **13**) and proton (compound **12**) clearly underlines the relevance of the ethyl group as a minimum requirement for PPARγ activation.

Activation of PPARγ was diminished by replacing the biphenyl moiety of compound **15** by an octanoic acid (compound **18**), despite the possible orientation of the carboxyl group similar to compound **15**. Activation was completely abolished by replacement of the biphenyl with the phenyl ring (compound **19**). The importance of the biphenyl structure is consistent with previous data obtained by testing different

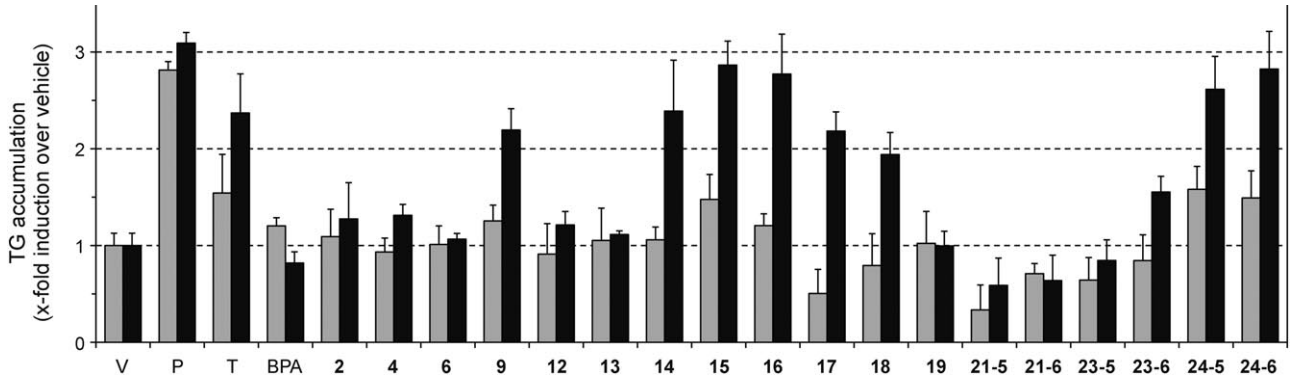


Figure 4. Compound screening in an adipocyte differentiation assay with 3T3-L1 cells in 24-well plates after 9 days differentiation ± the indicated compounds at 1 μM (■) and 10 μM (■). DMSO as vehicle (V), pioglitazone (P) and telmisartan (T) as positive controls. Cells were stained with Oil Red O and extracted with isopropanol (80% *v/v*). TG accumulation was measured by absorption of the dye at 515 nm. Values were compared to vehicle induction and are the means (±SD) of threefold determination in a single experiment.

ARBs for PPARγ activation.[9,10] Results from part III also provide information about the function of the carboxylic acid group. Bioisosteric replacement with the tetrazole moiety did not change PPARγ-dependent differentiation (compound **15** vs. **16**). Interestingly, decarboxylation (compound **17**) led to only a marginally reduced induction of cell differentiation. This means that the biphenyl structure plays a major role in PPARγ activation. Insertion of a carboxyl group at position 5 (regioisomer **21-5**) or 6 (regioisomer **21-6**) led to a loss of differentiation.

The insertion of a benzimidazole substituent at the benzimidazole core almost completely stopped differentiation (compound **15** vs. **23-5** and **23-6**). Low adipocyte differentiation was observed only for compound **23-6** at a concentration of 10 μM. Introduction of a 1-methyl group at the benzimidazole-2-yl residue increased the activity. Isomers **24-5** and **24-6** exhibited comparable activity compared with telmisartan at 1 μM and somewhat increased activity at a tenfold higher concentration. Again, the activity lowering effect of the 4-methyl group in telmisartan was documented. This important finding will be investigated in detail in subsequent SAR studies.

All test compounds at both concentrations (1 and 10 μM) were also investigated in a luciferase transactivation assay using COS-7 cells transiently transfected with pGal4-hPPARγ DEF and pGal5-Tk-pGL3 (Figure 5). This experiment was used as a screening assay to select potent compounds for further detailed analysis. The threshold for the selection was defined as >25% activation at 10 μM. Pioglitazone, as a full PPARγ agonist, was used as a positive control and its activation at 10 μM was defined as 100%.

The luciferase activation assay results correlated with those of the differentiation assay. Compounds **9**, **14**–**18**, **23-6, 24-5** and **24-6** showed activation comparable to telmisartan and were selected for further analysis. For full comparison of position 5 to 6, compound **23-5** was included also. The EC_{50} values and the maximum activation (A_{max}, %) were determined for these compounds (Table 1).

Telmisartan (EC_{50} = 5.1 μM, A_{max} = 56%), compound **15** (EC_{50} = 4.1 μM, A_{max} = 60%) and compound **16** (EC_{50} = 4.8 μM, A_{max} = 61%) were comparably active (Figure 6b). Shortening of the C-2-propyl group to ethyl (compound **14**) reduced the ac-

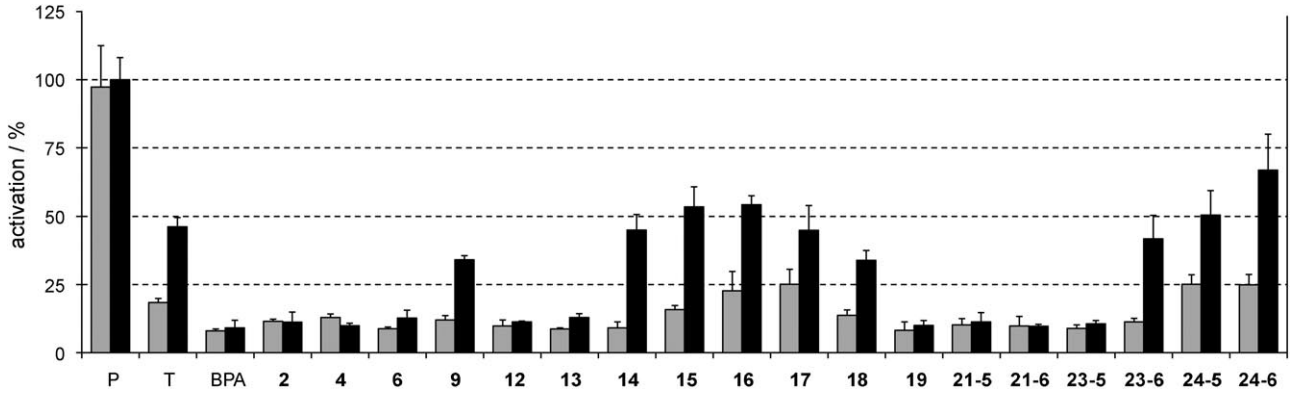


Figure 5. Compound screening in a luciferase transactivation assay. COS-7 cells were transiently transfected with the pGal4-hPPARγDEF and pGal5-Tk-pGL3 reporter followed by stimulation with the compounds as indicated. Firefly luciferase activity was measured after 36 h and normalized with activity of co-transfected renilla luciferase. Graph shows activation (%) of the luciferase gene by pioglitazone (P, 10 μM was defined as 100%), telmisartan (T), biphenyl-2-carboxylic acid (BPA) and the synthesized compounds **2**, **4**, **6**, **9**, **12**–**19**, **21-5/6**, **23-5/6** and **24-5/6** at 1 μM (■) and 10 μM (■). Values expressed are the means (±SD) of threefold determination in a single experiment.

Table 1. EC_{50} and maximum activation (A_{max}) values of pioglitazone, telmisartan and synthesized compounds **9**, **14**–**16**, **18**, **23-5/6** and **24-5/6**.

Cmpd	EC_{50} [μM][a]	A_{max} [%][a]
pioglitazone	0.3±0.1	100
telmisartan	5.1±0.2	56±7
9	7.5±2.0	56±4
14	8.3±2.2	48±3
15	4.1±0.6	60±3
16	4.8±1.3	61±6
18	8.2±2.0	46±4
23-5	21.3±7.6	24±6
23-6	10.1±2.5	54±9
24-5	4.8±3.7	58±10
24-6	3.3±1.0	71±4

[a] Data values represent the mean ±SD of three independent experiments.

tivity (EC_{50} = 8.3 μM, A_{max} = 48%) as did a methyl group at C-4 (compound **9**) (Figure 6a). The present data were in contrast to our initial hypothesis that enhanced hydrophobicity would increase potency (compound **9** vs. **15**).[9]

The interpretation of the results regarding the PPARγ binding is difficult. The aim of this SAR study was the identification of essential parts of telmisartan for receptor activation. Determination of possible antagonistic properties or competition experiments with pioglitazone will be part of a forthcoming study.

In accordance with the results of the differentiation assay, the carboxyl or tetrazole group at the biphenyl led to identical results. Substitution for a proton (compound **17**) reduced the maximum activation to 20%. However, results from the concentration–activation curve have to be interpreted cautiously for compound **17** due to high variations in measurements of this activation quantity. Therefore, no EC_{50} value was calculated or further interpretation made.

The relevance of the biphenyl moiety between carboxyl group and the benzimidazole core demonstrated again the reduced potency of compound **18** (EC_{50} = 8.2 μM, A_{max} = 46%).

The concentration–activation curves of **23-5**, **23-6**, **24-5** and **24-6** are shown in Figure 6c. Compared with telmisartan, compounds **23-5** and **23-6** are less active. For the 6-isomer **23-6**, an EC_{50} value of 10.1 μM and an A_{max} value of 54% was calculated. Regioisomerization of the benzimidazol-2-yl moiety to the 5 position drastically reduced the activity (EC_{50} = 21.3 μM; A_{max} = 24%). This effect was not as pronounced for compounds **24-5** and **24-6**. Compound **24-5** showed the same curve as telmisartan while the corresponding 6-isomer was more active (EC_{50} = 3.3 μM) and also caused a higher maximum activation (A_{max} = 71%). These findings further confirmed the importance of the methyl groups at the benzimidazole core and the 5/6-substituent for full agonistic activity.

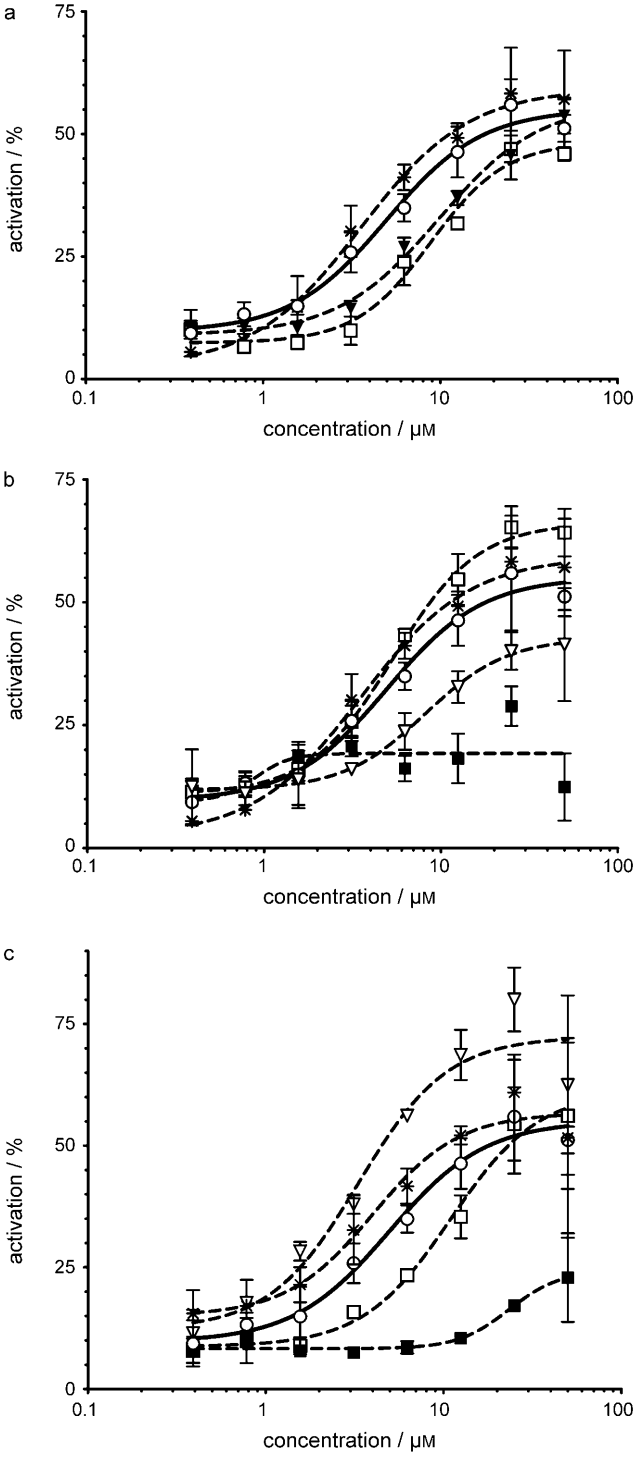


Figure 6. Compounds tested in luciferase activation assay using COS-7 cells transiently transfected with pGal4-hPPARγDEF and pGal5-Tk-pGL3. Data points represent the mean (±SD) of threefold determination in a single representative experiment. Activation (%) of the luciferase gene in COS-7 cells by pioglitazone (not shown, 10 μM, defined as 100%), telmisartan (○), and the synthesized compounds. a) compounds **9** (▼), **14** (□) and **15** (*); b) compounds **15** (*), **16** (□), **17** (■) and **18** (▽); c) compounds **23-5** (■), **23-6** (□), **24-5** (*) and **24-6** (▽).

Conclusions

The results described in this paper can be summarized as followed, the 1-(biphenyl-4-ylmethyl)-1*H*-benzimidazole is the essential core of telmisartan. An alkyl chains at C-2 enhanced the activity when ≥ ethyl. This might be the consequence of enhanced lipophilic contacts in the ligand-binding domain (LBD).

The 2-COOH located on the biphenyl system can be exchanged by a bioisosteric tetrazole without change in receptor activation. Interestingly, decarboxylation did not cause the expected loss of activity and so we assume that H bonds between the COOH and amino acids in the binding cavity do not play an essential role.

It is postulated that telmisartan binds in an angular conformation in the LBD to render a specific attachment of the 6-benzimidazole moiety. This would be in accordance with the different effects observed with compounds **23**-**5** and **23**-**6**. However, introduction of an *N*-methyl group (compound **24**-**5**) drastically increased the activation of compound **23**-**5** and to some extent that of compound **23**-**6** also (see **24**-**6**). Furthermore, the results from compounds **9**, **15** and **24**-**6** indicated that a C-4 methyl group, as present in telmisartan, reduced the hormonal profile to that of a partial agonist. However, this finding has to be confirmed in a further SAR study.

The telmisartan moieties important for PPARγ activation activity are depicted in Figure 7. These initial SAR results allow an assessment of the PPARγ activating properties of different ARBs shown in Figure 8. However, to get a complete overview of PPAR activation it will be necessary to evaluate the importance of PPAR subtype (α and δ) activation, too.

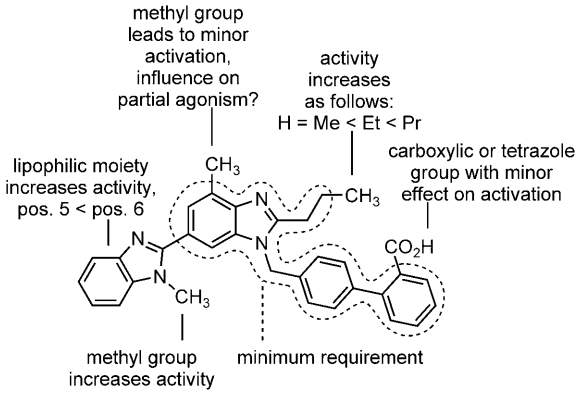


Figure 7. Overview of important moieties of telmisartan for PPARγ activation. The minimum core structure required for activation is highlighted (-----).

From our point of view, these findings provide the basis for future research regarding the further elucidation of key structural features of telmisartan and their impact on PPARγ activity. They could also be the origin for the development of new PPARγ ligands with a distinct pharmacological profile compared to existing full agonists, useful in treating metabolic diseases such as insulin resistance and diabetes.

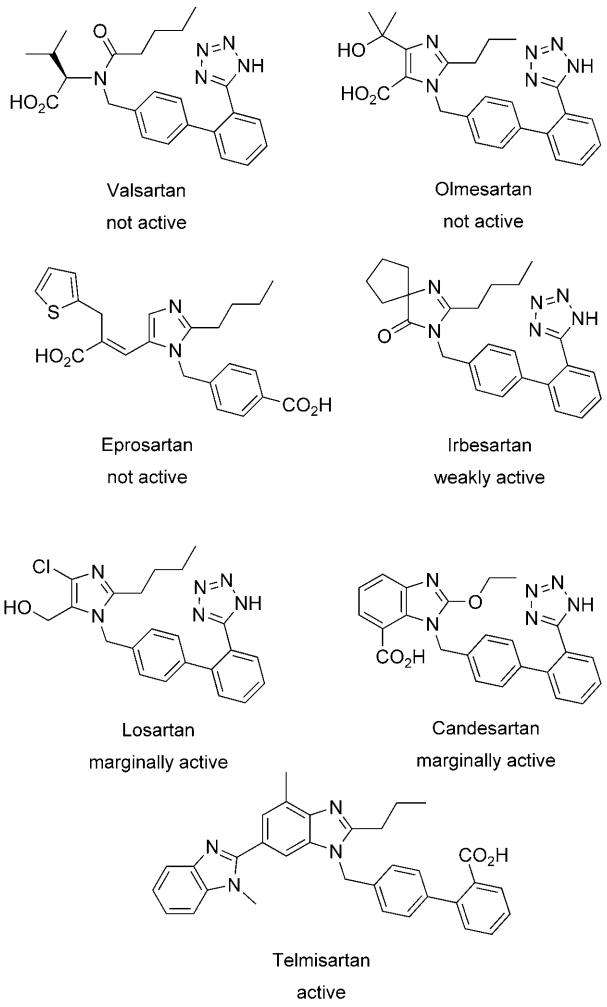


Figure 8. Structural comparisons of clinically used ARBs regarding their PPARγ activating properties.[10,16,17]

Experimental Section

Chemistry

All reagents and solvents were purchased from Acros Organics, Sigma–Aldrich, Alfa Aesar or Merck. All reactions were monitored by TLC, performed on silica gel plates 60 F_{254} (Merck, Darmstadt, Germany). Visualization on TLC was achieved by UV light. Column chromatography was performed with Merck silica gel 60H, grain size <0.063 mm, 230 mesh ASTM (Darmstadt, Germany). Melting points were measure using a B 545 Büchi (Flawil/Schweiz) capillary melting point apparatus. ^{1}H NMR experiments were carried out on an Avance DPX-400 spectrometer (Bruker, Karlsruhe, Germany) at 400 MHz using TMS as an internal standard. Elemental analyses were conducted by the micro laboratory of the Freie Universität of Berlin. EI-MS spectra were recorded on a CH-7A-Varian MAT, 70 eV (Melbourne, Australia). Microplate Reader FLASHScan S12 (Analytik Jena AG, Jena/Germany) Microlumat: Victor2 1420 Multilabel Counter (Wallac, Perkin–Elmer, Life sciences, Turku/Finnland).

General procedure for N-alkylation with 4′-(bromomethyl)-2-biphenylcarbonitrile: A stirred solution of the appropriate secondary amine (1 mmol) in anhyd DMF (3 mL) was cooled to 0 °C and treated with NaH (2 mmol). After ~30 min (or after no more visible evolution of hydrogen) 4′-(bromomethyl)-2-biphenylcarbonitrile (1.1 mmol) was added slowly and stirred at 0 °C for 1 h before warming to RT and stirring for a further 2–5 h. The reaction mixture was poured into aq HCl (1 mL, 6 N) with crushed ice (25 g) and extracted with $CHCl_3$ (3×15 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The crude product was purified by column chromatography with stepwise gradient elution (DCM/MeOH, 99:1, 98:2, 95:5).

General procedure for saponification of carbonitriles: A stirred solution of the respective carbonitrile (1 mmol), KOH (5 mmol) in H_2O (1 mmol) and ethylene glycol (4 mL) was heated at 185 °C for 5–6 h, with additional H_2O (1 mmol) carefully added hourly. After 5–6 h the reaction mixture was cooled to 100 °C, and diluted further with H_2O (8 mL). The solution was acidified to pH 5–6 with aq HCl (6 N) and stirred for 15 min to complete the precipitation. The crude solid was purified by column chromatography with stepwise gradient elution (DCM/MeOH, 95:5, 9:1, 8:2) and recrystallization from MeOH.

Ethyl(2-propyl-1*H*-benzo[*d*]imidazole)-6-carboxylate (1): A solution of 3,4-diaminobenzoic acid (4 g, 26.3 mmol) in anhyd EtOH (80 mL) and concd H_2SO_4 (1.41 mL, 26.3 mmol) was heated at reflux for 5–10 h. The reaction was cooled, poured into aq $NaHCO_3$ (160 mL, 5%) and extracted with $CHCl_3$ (3×50 mL). The aqueous phase was maintained at pH 8 to extract the desired ethyl-3,4-diaminobenzoate only. The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The crude product (3.55 g, 19.7 mmol, 75%) was dissolved in THF (30 mL) and treated with butyryl chloride (4.1 mL, 39.4 mmol) dropwise. The reaction mixture was stirred for 1 h at RT. The reaction was poured into aq $NaHCO_3$ (50 mL, 5%) and extracted with $CHCl_3$ (3×35 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. The crude product (ethyl-3,4-dibutanamidobenzoate, 6.12 g, 19.1 mmol, 97%) was dissolved in a suspension of toluene (190 mL) and *p*TsOH·H_2O (7.27 g, 38.2 mmol) and refluxed for 3 h. The reaction was worked up as in the previous step and the crude product was purified by column chromatography with stepwise gradient elution (DCM/MeOH, 98:2, 95:5, 9:1) to give the title compound as a colorless solid, 96% (67%, 2 steps); ^{1}H NMR ([D$_6$]DMSO): δ = 12.53 (s, 1H), 8.08 (s, 1H), 7.78 (dd, J = 8.4, 1.2 Hz, 1H), 7.54 (s, 1H), 4.31 (q, J = 7.1 Hz, 2H), 2.82 (t, J = 7.5 Hz, 2H), 1.80 (sextet, J = 7.4 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H); MS (EI, 100 °C): m/z (%) = 232 $[M]^{+\cdot}$ (39), 217 (19), 204 (100), 187 (15).

2′-Propyl-1*H*,3′*H*-2,5′-bibenzo[*d*]imidazole (2): A solution of compound **1** (0.5 g, 2.15 mmol) in aq NaOH (10%) and MeOH (1:1, 9 mL) was refluxed for 2 h. The resulting free acid (0.4 g, 2 mmol, 93%) was dissolved in polyphosphoric acid (9 g) at 150 °C and treated with 1,2-benzenediamine in small portions. After stirring at 150 °C for 24 h the mixture was allowed to cool and water was added in small portions. The pH was adjusted to pH 9 by the addition of concd NH_3 to the cooled reaction flask (ice bath). The precipitate was collected and recrystallized from DCM/MeOH to give the title compound as colorless crystals (31%); mp: 338–341 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.78 (s, 1H), 12.44 (s, 1H), 8.27 (s, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.59 (s, 3H), 7.20–7.14 (m, 2H), 2.83 (t, J = 7.5 Hz, 2H), 1.82 (sextet, J = 7.4 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H); MS (EI, 250 °C): m/z (%) = 276 $[M]^{+\cdot}$ (89), 261 (20), 248 (100). Anal. calcd for $C_{17}H_{16}N_4$: C 73.89, H 5.84, N 20.27, found: C 73.59, H 5.63, N 20.07.

4′-[(Dimethylamino)methyl]biphenyl-2-carbonitrile (3): Compound **3** was prepared from dimethylamine hydrochloride (0.15 g, 1.84 mmol), 4′-(bromomethyl)-2-biphenylcarbonitrile (0.54 g, 2 mmol), NaH (88 mg, 3.7 mmol) and anhyd DMF (5 mL) following the N-alkylation general procedure. The product was purified by column chromatography with stepwise gradient elution (DCM/MeOH, 95:5, 9:1) to give compound **3** as a colorless solid (72%); ^{1}H NMR ([D$_6$]DMSO): δ = 7.95 (d, J = 7.6 Hz, 1H), 7.79 (t, J = 7.7 Hz, 1H), 7.63 (d, J = 7.7 Hz, 1H), 7.60–7.52 (m, 3H), 7.44 (d, J = 8.0 Hz, 2H), 3.45 (s, 2H), 2.15 (s, 6H).

4′-[(Dimethylamino)methyl]biphenyl-2-carboxylic acid (4): The compound was prepared from compound **3** (0.5 g, 2.1 mmol), KOH (0.59 g, 10.5 mmol) in H_2O (0.038 mL, 1 mmol) and ethylene glycol (8 mL) following the general procedure for saponification of carbonitriles to give compound **4** as a colorless solid (20%); mp: 136–140 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 7.61 (d, J = 7.4 Hz, 1H), 7.47 (t, J = 7.3 Hz, 1H), 7.38 (t, J = 7.5 Hz, 1H), 7.35–7.31 (m, 5H), 3.59 (s, 2H), 2.25 (s, 6H); MS (EI, 210 °C): m/z (%) = 255 $[M]^{+\cdot}$ (58), 211 (28), 58 (100). Anal. calcd for $C_{16}H_{17}NO_2 \times 2H_2O$: C 65.96, H 6.71, N 5.49, found: C 65.73, H 6.42, N 5.35.

4′-[(2-Propyl-1*H*-imidazole-1-yl)methyl]biphenyl-2-carbonitrile (5): Compound **5** was prepared from 2-propyl-1*H*-imidazole (0.5 g, 4.5 mmol), 4′-(bromomethyl)-2-biphenylcarbonitrile (1.35 g, 5 mmol), NaH (0.22 g, 9 mmol) and anhyd DMF (7 mL) following the general procedure for N-alkylation to give compound **5** as a colorless solid (56%); ^{1}H NMR ([D$_6$]DMSO): δ = 7.94 (dd, J = 7.6, 1.2 Hz, 1H), 7.79 (td, J = 7.7, 1.3 Hz, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.57 (d, J = 8.4 Hz, 3H), 7.25 (d, J = 8.2 Hz, 2H), 7.17 (d, J = 1.2 Hz, 1H), 6.83 (d, J = 1.2 Hz, 1H), 5.26 (s, 2H), 2.56 (t, J = 7.5 Hz, 2H), 1.60 (sextet, J = 7.5 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H).

4′-[(2-Propyl-1*H*-imidazole-1-yl)methyl]biphenyl-2-carboxylic acid (6): The compound was prepared from compound **5** (0.5 g, 1.66 mmol), KOH (0.466 g, 8.3 mmol) in H_2O (0.03 mL, 1.66 mmol) and ethylene glycol (6 mL) following the general procedure for saponification of carbonitriles to give compound **6** as a colorless solid (11%); mp: 214–215 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.77 (s, 1H), 7.71 (dd, J = 7.7, 1.3 Hz, 1H), 7.56 (td, J = 7.5, 1.4 Hz, 1H), 7.45 (td, J = 7.6, 1.3 Hz, 1H), 7.36 (dd, J = 7.7, 1.1 Hz, 1H), 7.31 (d, J = 8.2 Hz, 2H), 7.14 (m, 3H), 6.82 (d, J = 1.2 Hz, 1H), 5.20 (s, 2H), 2.56 (t, J = 7.6 Hz, 2H), 1.60 (sextet, J = 7.5 Hz, 2H), 0.88 (t, J = 7.4 Hz, 3H); MS (EI, 70 °C): m/z (%) = 320 $[M]^{+\cdot}$ (36), 292 (32), 211 (100). Anal. calcd for $C_{20}H_{20}N_2O_2$: C 74.98, H 6.29, N 8.74, found: C 74.72, H 6.51, N 8.73.

4-Methyl-2-propyl-1*H*-benzo[*d*]imidazole (7): A stirred solution of 3-methyl-1,2-benzenediamine (0.25 g, 2 mmol) in anhyd EtOH (10 mL) was treated with ethyl butanimidoate hydrochloride (0.38 g, 2.5 mmol) and refluxed for 3 h. The reaction was cooled, poured into aq $NaHCO_3$ (50 mL, 5%) and extracted with $CHCl_3$ (3× 50 mL). The product was purified by column chromatography with stepwise gradient elution (DCM/MeOH 95:5, 9:1) to give compound **7** as a colorless solid (97%); ^{1}H NMR ([D$_6$]DMSO): δ = 12.04 (s, 1H), 7.27 (d, J = 7.9 Hz, 1H), 7.02 (t, J = 7.6 Hz, 1H), 6.93 (d, J = 7.2 Hz, 1H), 2.83 (t, J = 7.6 Hz, 2H), 2.54 (s, 3H), 1.77 (sextet, J = 7.4 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H).

4′-[(2-Propyl-4-methyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carbonitrile (8): Compound **8** was prepared from **7** (0.33 g, 1.9 mmol), 4′-(bromomethyl)-2-biphenylcarbonitrile (0.57 g, 2.1 mmol), NaH (0.09 g, 3.8 mmol) and anhyd DMF (6 mL) following the general procedure for N-alkylation to give the title compound as a colorless solid (86%); ^{1}H NMR ([D$_6$]DMSO): δ = 7.93 (d, J = 7.7 Hz, 1H), 7.76 (td, J = 7.7, 1.3 Hz, 1H), 7.59–7.53 (m, 4H), 7.29 (d, J = 7.9 Hz, 1H), 7.21 (d, J = 8.2 Hz, 2H), 7.05 (t, J = 7.6 Hz, 1H), 6.97 (d, J = 7.2 Hz, 1H), 5.57 (s, 2H), 2.84 (t, J = 7.6 Hz, 2H), 2.53 (s, 3H), 1.76 (sextet, J = 7.4 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H).

4′-[(2-Propyl-4-methyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carboxylic acid (9): The compound was prepared from **8** (0.6 g, 1.64 mmol), KOH (0.46 g, 8.2 mmol) in H_2O (0.03 mL, 1.64 mmol) and ethylene glycol (6 mL) following the general procedure for saponification of carbonitriles to give the title compound

as a colorless solid (63%); mp: 279–280 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.73 (s, 1H), 7.70 (dd, J = 7.7, 1.2 Hz, 1H), 7.54 (td, J = 7.6, 1.4 Hz, 1H), 7.43 (td, J = 7.5, 1.3 Hz, 1H), 7.33 (dd, J = 7.7, 0.9 Hz, 1H), 7.30–7.26 (m, 3H), 7.10 (d, J = 8.3 Hz, 2H), 7.05 (t, J = 7.6 Hz, 1H), 6.97 (d, J = 7.2 Hz, 1H), 5.51 (s, 2H), 2.84 (t, J = 7.6 Hz, 2H), 2.53 (s, 3H), 1.76 (sextet, J = 7.5 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H); MS (EI, 150 °C): m/z (%) = 384 [M]$^{+\cdot}$ (83), 356 (100), 355 (70), 211 (97). Anal. calcd for $C_{25}H_{24}N_2O_2 \times 0.3H_2O$: C 77.02, H 6.36, N 7.19, found: C 77.09, H 6.18, N 7.28.

Procedure for the preparation of 10a-d: A stirred suspension of 1,2-benzenediamine (0.5 g, 4.6 mmol) in the respective trimethyl orthoester (18.4 mmol) was treated dropwise with concd HCl at RT until the suspension turned clear and an exothermic reaction started. When the reaction mixture reached pH 8, aq $NaHCO_3$ (50 mL, 5%) was added and the reaction mixture was extracted with $CHCl_3$ (3×25 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. The crude product was purified by column chromatography with DCM/MeOH (95:5).

1*H*-Benzo[*d*]imidazole (10a): From 1,2-benzenediamine (0.5 g, 4.6 mmol) and trimethyl orthoformate (2 mL, 18.4 mmol) to give the title compound as a light-brown solid (95%); ^{1}H NMR ([D$_6$]DMSO): δ = 12.42 (s, 1H), 8.2 (s, 1H), 7.64 (d, J = 6.75, 1H), 7.52 (d, J = 6.71, 1H), 7.25–7.07 (m, 2H); MS (EI, 30 °C): m/z (%) = 118 [M]$^{+\cdot}$ (100), 91 (26), 64 (13).

2-Methyl-1*H*-benzo[*d*]imidazole (10b): From 1,2-benzenediamine (0.5 g, 4.6 mmol) and trimethyl orthoacetate (2.3 mL, 18.4 mmol) to give the title compound as a colorless solid (94%); ^{1}H NMR ([D$_6$]DMSO): δ = 12.15 (s, 1H), 7.58–7.28 (m, 2H), 7.18–6.91 (m, 2H), 2,47 (s, 3H); MS (EI, 30 °C): m/z (%) = 132 [M]$^{+\cdot}$ (100), 131 (64).

2-Ethyl-1*H*-benzo[*d*]imidazole (10c): From 1,2-benzenediamine (0.5 g, 4.6 mmol) and trimethyl orthopropionate (2.6 mL, 18.4 mmol) to give the title compound as a colorless solid (98%); ^{1}H NMR ([D$_6$]DMSO): δ = 12.14 (s, 1H), 7.60–7.24 (m, 2H), 7.23–6.93 (m, 2H), 2.82 (q, J = 7.6, 2H), 1.31 (t, J = 7.6, 3H); MS (EI, 30 °C): m/z (%) = 146 [M]$^{+\cdot}$ (70), 145 (100).

2-Propyl-1*H*-benzo[*d*]imidazole (10d): From 1,2-benzenediamine (2 g, 18.5 mmol) and trimethyl orthobutyrate (11.8 mL, 74 mmol) to give the title compound as a colorless solid (95%); ^{1}H NMR ([D$_6$]DMSO): δ = 12.05 (s, 1H), 7.48–7.35 (m, 2H), 7.13–6.93 (m, 2H), 2.75 (t, J = 7.5, 2H), 1.77 (sextet, J = 7.4, 2H), 0.91 (t, J = 7.4, 3H); MS (EI, 50 °C): m/z (%) = 160 [M]$^{+\cdot}$ (29), 145 (20), 132 (100).

Procedure for the preparation of 11a-d: The compounds were prepared from compounds **10a-d** following the general procedure for N-alkylation.

4′-[(1*H*-Benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carbonitrile (11a): From **10a** (0.51 g, 4.3 mmol) and 4′-(bromomethyl)-2-biphenylcarbonitrile (1.28 g, 4.7 mmol) to give the title compound as a colorless solid (89%); ^{1}H NMR ([D$_6$]DMSO): δ = 8.48 (s, 1H), 7.94 (d, J = 7.7 Hz, 1H), 7.77 (td, J = 7.7, 1.2 Hz, 1H), 7.68 (dd, J = 7.1, 1.9 Hz, 1H), 7.62–7.55 (m, 5H), 7.45 (d, J = 8.2 Hz, 2H), 7.23 (m, 2H), 5.61 (s, 2H); MS (EI, 125 °C): m/z (%) = 309 [M]$^{+\cdot}$ (46), 192 (100).

4′-[(2-Methyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carbonitrile (11b): From **10b** (0.57 g, 4.3 mmol) and 4′-(bromomethyl)-2-biphenylcarbonitrile (1.28 g, 4.7 mmol) to give the title compound as a colorless solid (93%); ^{1}H NMR ([D$_6$]DMSO): δ = 7.95 (d, J = 7.7 Hz, 1H), 7.75 (t, J = 7.7, 1H), 7.63–7.49 (m, 6H), 7.26 (d, J = 8.2 Hz, 2H), 7.16 (m, 2H), 5.57 (s, 2H), 2.56 (s, 3H); MS (EI, 150 °C): m/z (%) = 323 [M]$^{+\cdot}$ (53), 192 (100).

4′-[(2-Ethyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carbonitrile (11c): From **10c** (0.66 g, 4.5 mmol) and 4′-(bromomethyl)-2-biphenylcarbonitrile (1.36 g, 5 mmol) to give the title compound as a colorless solid (87%); ^{1}H NMR ([D$_6$]DMSO): δ = 7.95 (d, J = 7.7 Hz, 1H), 7.76 (t, J = 7.7, 1H), 7.64–7.47 (m, 6H), 7.24 (d, J = 8.0 Hz, 2H), 7.17 (m, 2H), 5.59 (s, 2H), 2.89 (q, J = 5.6 Hz, 2H), 1.31 (t, J = 5.0, 3H); MS (EI, 125 °C): m/z (%) = 337 [M]$^{+\cdot}$ (41), 192 (100).

4′-[(2-Propyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carbonitrile (11d): From **10d** (1.4 g, 8.74 mmol) and 4′-(bromomethyl)-2-biphenylcarbonitrile (2.61 g, 9.6 mmol) to give the title compound as a colorless solid (77%); ^{1}H NMR ([D$_6$]DMSO): δ = 8.00 (d, J = 8.1 Hz, 1H), 7.78 (dd, J = 8.1, 1.4 Hz, 1H), 7.66 (td, J = 7.7, 1.6 Hz, 1H), 7.56 (d, J = 8.4 Hz, 2H), 7.50–7.39 (m, 5H), 7.18 (d, J = 8.5 Hz, 2H), 5.53 (s, 2H), 3.14 (t, J = 7.4 Hz, 2H), 2.00 (sextet, J = 7.5 Hz, 2H), 1.08 (t, J = 7.4 Hz, 3H); MS (EI, 300 °C): m/z (%) = 351 [M]$^{+\cdot}$ (73), 323 (100), 322 (79), 192 (71).

Procedure for the preparation of 12–15: The compounds were prepared from compounds **11a-d** following the general procedure for saponification of carbonitriles.

4′-[(1*H*-Benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carboxylic acid (12): From **11a** (1.18 g, 3.8 mmol) and KOH (1.07 g, 19 mmol) to give the title compound as a colorless solid (32%); mp: 253–255 °C, ^{1}H NMR ([D$_6$]DMSO): δ = 12.73 (s, 1H), 8.45 (s, 1H), 7.72–7.67 (m, 2H), 7.60 (d, J = 7.4 Hz, 1H), 7.55 (td, J = 7.6, 1.4 Hz, 1H), 7.44 (td, J = 7.5, 1.1 Hz, 1H), 7.36–7.29 (m, 5H), 7.26–7.19 (m, 2H), 5.56 (s, 2H); MS (EI, 225 °C): m/z (%) = 328 [M]$^{+\cdot}$ (31), 284 (17), 211 (100). Anal. calcd for $C_{21}H_{16}N_2O_2 \times 0.25H_2O$: C 75.77, H 5.00, N 8.42, found: C 75.79, H 5.14, N 8.39.

4′-[(2-Methyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carboxylic acid (13): From **11b** (1.29 g, 3.99 mmol) and KOH (0.466 g, 8.3 mmol) to give the title compound as a colorless solid (53%); mp: 251–252 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.72 (s, 1H), 7.70 (dd, J = 7.6, 1.2 Hz, 1H), 7.58–7.51 (m, 3H), 7.43 (td, J = 7.5, 1.2 Hz, 1H), 7.34 (d, J = 7.5 Hz, 1H), 7.29 (d, J = 8.2 Hz, 2H), 7.20–7.13 (m, 4H), 5.51 (s, 2H); MS (EI, 150 °C): m/z (%) = 342 [M]$^{+\cdot}$ (51), 211 (100). Anal. calcd for $C_{22}H_{18}N_2O_2 \times 0.5H_2O$: C 75.20, H 5.45, N 7.97, found: C 75.02, H 5.58, N 8.34.

4′-[(2-Ethyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carboxylic acid (14): From **11c** (1.32 g, 3.9 mmol) and KOH (1.09 g, 19.5 mmol) to give the title compound as a colorless solid (73%); mp: 249–251 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.73 (s, 1H), 7.70 (dd, J = 7.7, 1.2 Hz, 1H), 7.61–7.59 (m, 1H), 7.56–7.51 (m, 2H), 7.43 (td, J = 7.6, 1.2 Hz, 1H), 7.33 (dd, J = 7.7, 1.0 Hz, 1H), 7.28 (d, J = 8.2 Hz, 2H), 7.19–7.14 (m, 2H), 7.13 (d, J = 8.2 Hz, 2H), 5.53 (s, 2H), 2.87 (q, J = 7.5 Hz, 2H), 1.30 (t, J = 7.5 Hz, 3H); MS (EI, 200 °C): m/z (%) = 356 [M]$^{+\cdot}$ (58), 211 (100). Anal. calcd for $C_{23}H_{20}N_2O_2$: C 77.51, H 5.66, N 7.86, found: C 77.60, H 5.70, N 7.82.

4′-[(2-Propyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carboxylic acid (15): From **11d** (1 g, 2.85 mmol) and KOH (0.8 g, 14.3 mmol) to give the title compound as a colorless solid (43%); mp: 254–255 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 12.74 (s, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.60–7.58 (m, 1H), 7.54–7.49 (m, 2H), 7.43 (t, J = 7.5 Hz, 1H), 7.33 (d, J = 7.7 Hz, 1H), 7.29 (d, J = 8.2 Hz, 2H), 7.18–7.16 (m, 2H), 7.12 (d, J = 8.1 Hz, 2H), 5.54 (s, 2H), 2.84 (t, J = 7.5 Hz, 2H),

1.79 (sextet, $J=7.4$ Hz, 2H), 0.96 (t, $J=7.4$ Hz, 3H); MS (EI, 225 °C): m/z (%) = 370 $[M]^{+\cdot}$ (62), 342 (79), 211 (100). Anal. calcd for $C_{24}H_{22}N_2O_2$: C 77.81, H 5.99, N 7.56, found: C 77.86, H 6.17, N 7.59.

4′-[(2-Propyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-tetrazole (16): Compound **11d** (1 g, 2.85 mmol) was dissolved in a solution of NH_4Cl (1.98 g, 37 mmol) and NaN_3 (2.4 g, 37 mmol) in DMF (30 mL). The reaction mixture was heated to 140 °C for 21 h and then cooled and acidified with diluted HCl (0.1 N). The first precipitation was collected by filtration, but not used. After one week, further precipitate was collected and recrystallized from MeOH to give the title compound as colorless crystals (11%); mp: 236–237 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=16.25$ (s, 1H), 7.68–7.61 (m, 2H), 7.61–7.52 (m, 2H), 7.52–7.46 (m, 2H), 7.20–7.14 (m, 2H), 7.07–7.02 (m, 4H), 5.49 (s, 2H), 2.79 (t, $J=7.5$ Hz, 2H), 1.74 (sextet, $J=7.4$ Hz, 2H), 0.94 (t, $J=7.4$ Hz, 3H); MS (EI, 275 °C): m/z (%) = 394 $[M]^{+\cdot}$ (16), 366 (14), 28 (100). Anal. calcd for $C_{24}H_{22}N_6$: C 73.07, H 5.62, N 21.30, found: C 73.42, H 5.74 , N 20.98.

1-(Biphenyl-4-ylmethyl)-2-propyl-1*H*-benzo[*d*]imidazole (17): From **10d** (0.25 g, 1.56 mmol) and 4-(bromomethyl)biphenyl (0.468 g, 1.72 mmol) following the general procedure for N-alkylation to give the title compound as a colorless solid (66%); mp: 105–109 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=7.65$–7.58 (m, 5H), 7.50–7.41 (m, 3H), 7.35 (t, $J=7.2$ Hz, 1H), 7.19–7.14 (m, 4H), 5.54 (s, 2H), 2.85 (t, $J=7.5$ Hz, 2H), 1.78 (q, $J=7.4$ Hz, 2H), 0.96 (t, $J=7.4$ Hz, 3H); MS (EI, 150 °C): m/z (%) = 326 $[M]^{+\cdot}$ (52), 298 (51), 297 (27), 167 (100). Anal. calcd for $C_{23}H_{22}N_2$: C 84.63, H 6.79, N 8.58, found: C 84.58, H 6.45, N 8.58.

8-(2-Propyl-1*H*-benzo[*d*]imidazole-1-yl)octanoic acid (18): A stirred solution of compound **10d** (0.5 g, 3.12 mmol) in anhyd DMF (5 mL) was cooled to 0 °C and treated with NaH (0.25 g, 60% dispersion in mineral oil, 6.24 mmol). Separately, a stirred solution of 8-bromooctanoic acid (0.765 g, 3.43 mmol) in anhyd DMF (3 mL) was treated with NaH (0.137 g, 60% dispersion in mineral oil, 3.43 mmol) at 0 °C. After ~30 min (or after no more visible emergence of H_2) the solution of 8-bromooctanoic acid was added slowly to the solution of **10d** following the general procedure for N-alkylation to give the title compound as colorless crystals (38%); mp: 100–101 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=11.98$ (s, 1H), 7.53 (dd, $J=6.2$, 2.2 Hz, 1H), 7.48 (d, $J=7.6$ Hz, 1H), 7.14 (t, $J=1.9$ Hz, 1H), 4.16 (t, $J=7.4$ Hz, 2H), 2.81 (t, $J=7.5$ Hz, 2H), 2.17 (t, $J=7.4$ Hz, 2H), 1.82 (sextet, $J=7.4$ Hz, 2H), 1.68 (t, $J=6.8$ Hz, 2H), 1.46 (quintet, $J=7.2$ Hz, 2H), 1.31–1.22 (m, 6H), 1.00 (t, $J=7.4$ Hz, 3H); MS (EI, 150 °C): m/z (%) = 302 $[M]^{+\cdot}$ (40), 259 (62), 187 (60), 173 (51), 146 (92), 132 (100). Anal. calcd for $C_{18}H_{26}N_2O_2$: C 71.49, H 8.67, N 9.26, found: C 71.40, H 8.52, N 9.20.

4-[(2-Propyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]benzoic acid (19): 4-[(2-Propyl-1*H*-benzo[*d*]imidazole-1-yl)methyl]benzonitrile was prepared from **10d** (0.25 g, 1.56 mmol) and 4-(bromomethyl)benzonitrile (0.337 g, 1.72 mmol) following the general procedure for N-alkylation to give a colorless solid (94%); ^{1}H NMR ([D$_6$]DMSO): $\delta=$ 7.74 (d, $J=8.4$ Hz, 2H), 7.65–7.61 (m, 1H), 7.45–7.43 (m, 1H), 7.21–7.15 (m, 4H), 5.53 (s, 2H), 2.85 (t, $J=7.5$ Hz, 2H), 1.69 (sextet, $J=$ 7.4 Hz, 2H), 0.92 (t, $J=7.4$ Hz, 3H); MS (EI, 125 °C): m/z (%) = 275 $[M]^{+\cdot}$ (68), 246 (100), 116 (40). Compound **19** was prepared from the benzonitrile intermediate (0.4 g, 1.45 mmol) and KOH (0.407 g, 7.25 mmol) following the general procedure for saponification of carbonitriles to give the title compound as a colorless solid (49%); mp: 219–220 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=12.94$ (s, 1H), 7.89 (d, $J=$ 8.4 Hz, 2H), 7.61–7.58 (m, 1H), 7.43–7.41 (m, 1H), 7.18–7.13 (m, 4H), 5.58 (s, 2H), 2.79 (t, $J=7.5$ Hz, 2H), 1.74 (sextet, $J=7.4$ Hz, 2H), 0.93 (t, $J=7.4$ Hz, 3H); MS (EI, 100 °C): m/z (%) = 294 $[M]^{+\cdot}$ (72), 266 (100), 265 (85), 159 (29), 135 (38), 131 (57). Anal. calcd for $C_{18}H_{18}N_2O_2$: C 77.95, H 5.12, N 7.90, found: C 77.74, H 5.16, N 7.91.

1-[(2′-Cyanobiphenyl-4-yl)methyl]-2-propyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid (20-5) and 1-[(2′-cyanobiphenyl-4-yl)methyl]-2-propyl-1*H*-benzo[*d*]imidazole-6-carboxylic acid (20-6): Compound **1** (3.5 g, 15.1 mmol) was treated as described for the procedure of N-alkylation with 4′-(bromomethyl)-2-biphenylcarbonitrile (4.5 g, 16.6 mmol) to give an isomeric mixture of ethyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-2-propyl-1*H*-benzo[*d*]imidazole-5-carboxylate and ethyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-2-propyl-1*H*-benzo[*d*]imidazole-6-carboxylate as a colorless solid (5.66 g, 14.3 mmol, 95%). After cleavage of the ester by refluxing in aq NaOH (10%) and MeOH (60 mL, 1:1) for 2 h, it was possible to separate the regioisomers by column chromatography with a DCM/MeOH (9:1). The 5-regioisomer was also isolated by recrystallization from MeOH or DCM/MeOH to give compound **20-5** as a colorless solid (37% in total); ^{1}H NMR ([D$_6$]DMSO): $\delta=12.68$ (s, 1H), 8.19 (d, $J=1.5$ Hz, 1H), 7.93 (dd, $J=7.7$, 0.8 Hz, 1H), 7.83 (dd, $J=8.5$, 1.6 Hz, 1H), 7.77 (td, $J=7.7$, 1.3 Hz, 1H), 7.62–7.54 (m, 5H), 7.24 (d, $J=8.3$ Hz, 2H), 5.65 (s, 2H), 2.88 (t, $J=7.5$ Hz, 2H), 1.79 (sextet, $J=7.4$ Hz, 2H), 0.96 (t, $J=7.4$ Hz, 3H); MS (EI, 200 °C): m/z (%) = 395 $[M]^{+\cdot}$ (41), 367 (70), 192 (100). **20-6**: colorless solid (18% in total); ^{1}H NMR ([D$_6$]DMSO): $\delta=12.76$ (s, 1H), 8.12 (d, $J=1.5$ Hz, 1H), 7.93 (dd, $J=$ 7.8, 1.5 Hz, 1H), 7.81 (dd, $J=8.4$, 1.6 Hz, 1H), 7.76 (td, $J=7.7$, 1.4 Hz, 1H), 7.67 (d, $J=8.4$ Hz, 1H), 7.62–7.54 (m, 4H), 7.21 (d, $J=$ 8.3 Hz, 2H), 5.70 (s, 2H), 2.89 (t, $J=7.5$ Hz, 2H), 1.80 (sextet, $J=$ 7.4 Hz, 2H), 0.96 (t, $J=7.4$ Hz, 3H); MS (EI, 200 °C): m/z (%) = 395 $[M]^{+\cdot}$ (50), 367 (69), 192 (100).

1-[(2′-Carboxybiphenyl-4-yl)methyl]-2-propyl-1*H*-benzo[*d*]imidazole-5-carboxylic acid (21-5) and 1-[(2′-carboxybiphenyl-4-yl)-methyl]-2-propyl-1*H*-benzo[*d*]imidazole-6-carboxylic acid (21-6): The compounds were prepared from **20-5** (1 g, 2.5 mmol) and **20-6** (1 g, 2.5 mmol) following the general procedure for saponification of carbonitriles. **21-5**: colorless solid (65%); mp: 295–297 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=12.69$ (s, 2H), 8.17 (d, $J=1.5$ Hz, 1H), 7.82 (dd, $J=8.5$, 1.6 Hz, 1H), 7.70 (dd, $J=7.7$, 1.4 Hz, 1H), 7.60 (d, $J=$ 8.5 Hz, 1H), 7.54 (td, $J=7.5$, 1.4 Hz, 1H), 7.43 (td, $J=7.6$, 1.3 Hz, 1H), 7.33 (dd, $J=7.7$, 1.2 Hz, 1H), 7.29 (d, $J=8.3$ Hz, 2H), 7.13 (d, $J=8.3$ Hz, 2H), 5.59 (s, 2H), 2.86 (t, $J=7.5$ Hz, 2H), 1.79 (sextet, $J=$ 7.4 Hz, 2H), 0.96 (t, $J=7.4$ Hz, 3H); MS (EI, 200 °C): m/z (%) = 414 $[M]^{+\cdot}$ (51), 386 (48), 211 (100). Anal. calcd for $C_{25}H_{22}N_2O_4\times0.5H_2O$: C 70.91, H 5.47, N 6.62, found: C 70.76, H 5.55, N 6.51. **21-6**: colorless solid (68%); mp: 256–259 °C; ^{1}H NMR ([D$_6$]DMSO): $\delta=12.72$ (s, 2H), 8.12 (d, $J=1.5$ Hz, 1H), 7.81 (dd, $J=8.5$, 1.6 Hz, 1H), 7.66 (dd, $J=$ 7.7, 1.4 Hz, 1H), 7.60 (d, $J=8.5$ Hz, 1H), 7.54 (td, $J=7.5$, 1.4 Hz, 1H), 7.43 (td, $J=7.6$, 1.3 Hz, 1H), 7.33 (dd, $J=7.7$, 1.2 Hz, 1H), 7.28 (d, $J=8.3$ Hz, 2H), 7.10 (d, $J=8.3$ Hz, 2H), 5.64 (s, 2H), 2.88 (t, $J=$ 7.5 Hz, 2H), 1.78 (sextet, $J=7.4$ Hz, 2H), 0.95 (t, $J=7.4$ Hz, 3H); MS (EI, 200 °C): m/z (%) = 414 $[M]^{+\cdot}$ (44), 386 (45), 211 (100). Anal. calcd for $C_{25}H_{22}N_2O_4\times0.25H_2O$: C 71.67, H 5.41, N 6.69, found: C 71.27, H 5.71, N 6.64.

4′-[(2-Propyl-2′,5-bi-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carbonitrile (22-5) and 4′-[(2-propyl-2′,6-bi-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carbonitrile (22-6): A solution of **20-5/6** (1 g, 2.5 mmol) in THF (5 mL) was treated with $SOCl_2$ (0.091 mL, 1.25 mmol) and heated to 60 °C for 1 h. After cooling, the reaction mixture was added to a stirred solution of 1,2-benzenediamine (541 mg, 5 mmol) in THF (5 mL) at 0 °C and stirred for 2 h at RT. After the standard work up previously described for compound **1**, the product (mono-acylated 1,2-benzenediamine, 580 mg,

1.2 mmol, 48%) was treated with toluene (12 mL) and *p*TsOH·H_2O (456 mg, 2.4 mmol) as described for the preparation of compound **1** to give compound **22-5** as a colorless solid (92%, 39% total); ^{1}H NMR ([D_6]DMSO): δ = 13.06 (s, 1H), 8.43 (d, J = 1.4 Hz, 1H), 8.09 (dd, J = 8.5, 1.6 Hz, 1H), 7.94 (dd, J = 7.8, 1.1 Hz, 1H), 7.77 (td, J = 7.7, 1.3 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.61–7.56 (m, 6H), 7.28 (d, J = 8.3 Hz, 2H), 7.23–7.17 (m, 2H), 5.67 (s, 2H), 2.90 (t, J = 7.5 Hz, 2H), 1.81 (sextet, J = 7.5 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H); MS (EI, 225 °C): *m/z* (%) = 467 [M]$^{+\cdot}$ (100), 438 (26), 275 (18), 192 (68). **22-6**: colorless solid (98%, 47% total); ^{1}H NMR ([D_6]DMSO): δ = 12.82 (s, 1H), 8.35 (d, J = 1.1 Hz, 1H), 8.04 (dd, J = 8.4, 1.6 Hz, 1H), 7.93 (dd, J = 7.7, 1.0 Hz, 1H), 7.78–7.72 (m, 2H), 7.63–7.49 (m, 6H), 7.27 (d, J = 8.3 Hz, 2H), 7.20–7.13 (m, 2H), 5.70 (s, 2H), 2.89 (t, J = 7.5 Hz, 2H), 1.81 (sextet, J = 7.4 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H); MS (EI, 225 °C): *m/z* (%) = 467 [M]$^{+\cdot}$ (100), 438 (28), 275 (18), 192 (65).

4′-[(2-Propyl-2′,5-bi-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carboxylic acid (23-5): The compound was prepared from **22-5** (0.45 g, 0.96 mmol) following the general procedure for saponification of carbonitriles to give the title compound as a colorless solid (62%); mp: 275–276 °C; ^{1}H NMR ([D_6]DMSO): δ = 12.81 (s, 2H), 8.39 (d, J = 1.1 Hz, 1H), 8.07 (dd, J = 8.5, 1.6 Hz, 1H), 7.70–7.62 (m, 3H), 7.52 (t, J = 7.1 Hz, 2H), 7.42 (t, J = 7.3 Hz, 1H), 7.33–7.31 (m, 3H), 7.18–7.15 (m, 4H), 5.60 (s, 2H), 2.88 (t, J = 7.6 Hz, 2H), 1.81 (sextet, J = 7.4 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H); MS (EI, 200 °C): *m/z* (%) = 486 [M]$^{+\cdot}$ (22), 442 (78), 211 (27), 167 (100), 44 (61). Anal. calcd for $C_{31}H_{26}N_4O_2 \times 0.25H_2O$: C 75.82, H 5.44, N 11.41, found: C 75.90, H 5.73, N 11.73.

4′-[(2-Propyl-2′,6-bi-1*H*-benzo[*d*]imidazole-1-yl)methyl]biphenyl-2-carboxylic acid (23-6): The compound was prepared from **22-6** (0.55 g, 1.17 mmol) following the general procedure for saponification of carbonitriles to give the title compound as a colorless solid (58%); mp: 263–265 °C; ^{1}H NMR ([D_6]DMSO): δ = 12.82 (s, 2H), 8.31 (d, J = 0.9 Hz, 1H), 8.02 (dd, J = 8.4, 1.6 Hz, 1H), 7.76–7.66 (m, 4H), 7.53–7.49 (m, 2H), 7.41 (td, J = 7.5, 1.2 Hz, 1H), 7.34–7.28 (m, 3H), 7.19–7.14 (m, 3H), 7.07 (d, J = 8.3 Hz, 1H), 5.64 (s, 2H), 2.88 (t, J = 7.4 Hz, 2H), 1.82 (sextet, J = 7.5 Hz, 2H), 0.98 (t, J = 7.3 Hz, 3H); MS (EI, 200 °C): *m/z* (%) = 486 [M]$^{+\cdot}$ (17), 442 (77), 211 (24), 167 (100), 44 (64). Anal. calcd for $C_{31}H_{26}N_4O_2 \times 0.25H_2O$: C 75.82, H 5.44, N 11.41, found: C 75.72, H 5.87, N 11.39.

4′-[(1′-Methyl-2-propyl-2′,5-bi-1*H*-benzo[*d*]imidazole]-1-yl)methyl]biphenyl-2-carboxylic acid (24-5): The compound was prepared from **23-5** (0.29 g, 0.6 mmol) following the same procedure as described for the preparation of **24-6** (below) to give the title compound as a colorless solid (29%); mp: 185–189 °C; ^{1}H NMR ([D_6]DMSO): δ = 12.77 (s, 1H), 8.07 (d, J = 1.2 Hz, 1H), 7.72–7.66 (m, 4H), 7.61 (d, J = 7.6 Hz, 1H), 7.54 (td, J = 7.6, 1.2 Hz, 1H), 7.43 (td, J = 7.5, 1.1 Hz, 1H), 7.35–7.31 (m, 3H), 7.29 (td, J = 7.5, 1.3 Hz, 1H), 7.24 (td, J = 7.4, 1.3 Hz, 1H), 7.21–7.17 (m, 2H), 5.63 (s, 2H), 3.92 (s, 3H), 2.91 (t, J = 7.5 Hz, 2H), 1.83 (sextet, J = 7.4 Hz, 2H), 1.00 (t, J = 7.4 Hz, 3H); MS (EI, 225 °C): *m/z* (%) = 500 [M]$^{+\cdot}$ (2), 455 (100), 211 (11). Anal. calcd for $C_{32}H_{28}N_4O_2 \times 0.25H_2O$: C 76.09, H 5.69, N 11.09, found: C 76.22, H 6.03, N 10.81.

4′-[(1′-Methyl-2-propyl-2′,6-bi-1*H*-benzo[*d*]imidazole]-1-yl)methyl]biphenyl-2-carboxylic acid (24-6): A solution of compound **23-6** (0.33 g, 0.68 mmol) in DMF (3 mL) was treated with NaH (2 mmol) and then subsequently by dropwise addition of MeI (0.047 mL, 0.75 mmol in 3 mL DMF). The reaction was worked up and purified as described for the general procedure of N-alkylation to give compound **24-6** as a colorless solid (21%); mp: 253–254 °C; ^{1}H NMR ([D_6]DMSO): δ = 12.87 (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), 7.30–7.22 (m, 4H), 7.19 (d, J = 8.2 Hz, 2H), 5.65 (s, 2H), 3.83 (s, 3H), 2.94 (t, J = 7.5 Hz, 2H), 1.84 (sextet, J = 7.4 Hz, 2H), 1.00 (t, J = 7.4 Hz, 3H); MS (EI, 250 °C): *m/z* (%) = 500 [M]$^{+\cdot}$ (3), 455 (100), 211 (13). Anal. calcd for $C_{32}H_{28}N_4O_2$: C 76.78, H 5.64, N 11.19, found: C 76.84, H 5.85, N 11.39.

Biology

Telmisartan (tablets, 80 mg) and pioglitazone (tablets, 45 mg) were obtained from the pharmacy, the active compound was extracted with $CHCl_3$, purified by chromatography and recrystallized from MeOH.

Differentiation Assay: Murine 3T3-L1 preadipocytes were cultured in DMEM (+10% FCS) and differentiated by a modified previously described protocol[9] and incubated for 2 d. Postconfluent preadipocytes were treated for 3 d with complete medium (dexamethasone, 1 μM; insulin, 0.17 μM). The medium was replaced and the cells were incubated for a further 3 d with insulin (0.17 μM), and for the last 3 days with only complete medium. Cells were treated the whole time period with either vehicle (DMSO) as negative control, pioglitazone and telmisartan as positive controls or the synthesized compounds until day 9 of differentiation, after which the cells were washed with phosphate-buffered saline and stained with Oil Red O.[9] For quantification, the dye was extracted with isopropanol (80% *v/v*) and the absorption was measured at 515 nm.

PPARγ Transactivation Assay: Transient transfection (Invitrogen) and luciferase assays (Promega) were performed as described in the manufacturer's protocol. COS-7 cells (8×10^5/well in a 96-well plate) were seeded the day before and transfected for each well using 0.25 μL Lipofectamine 2000 (Invitrogen) with 4.5 ng pGal4-hPPARγDEF, 45 ng pGal5-TK-pGL3 and 3 ng pRL-CMV in 25 μL Opti-MEM (Gibco).[18] After 4 h, a sixth part of the transfection medium volume of DMEM (+10% FCS) plus the requisite compounds or vehicle (DMSO) was added, and luciferase activity was measured after 36 h.

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