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FULL PAPER

Synthesis and Characterization of Telmisartan-Derived Cell Death Modulators to Circumvent Imatinib Resistance in Chronic Myeloid Leukemia

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Abstract: New strategies to eradicate cancer stem cells in chronic myeloid leukemia (CML) include a combination of imatinib with peroxisome proliferator-activated receptor gamma (PPARγ) ligands. Recently, we identified the partial PPARγ agonist telmisartan as effective sensitizer of resistant K562 CML cells to imatinib treatment. Here, the importance of the heterocyclic core on the cell death modulating effects of the telmisartan-derived lead 4'-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid (**3b**) was investigated. Inspired by the pharmacodynamics of HYL-6d and the selective PPARγ ligand VSP-51, the benzimidazole was replaced by a carbazole or an indole core. The results indicate no correlation between PPARγ activation and sensitization of resistant CML cells to imatinib. The 2-COOH derivatives of the carbazoles or indoles achieved low activity at PPARγ, while the benzimidazoles showed 60-100% activation. Among the 2-CO_2CH_3 derivatives, only the ester of the lead (**2b**) slightly activated PPARγ. Sensitizing effects were further observed for this non-cytotoxic **2b** (80% cell death), and to a lesser extent for the lead **3b** or the 5-Br substituted ester of the benzimidazoles (**5b**).

Introduction

Chronic myeloid leukemia (CML) is characterized by a reciprocal chromosomal translocation causing the formation of the BCR-ABL fusion gene. This oncoprotein constitutes a deregulated tyrosine kinase with increased enzyme activity and promotes uncontrolled myelopoiesis.[1]

Patients in the chronic phase of CML are well treatable applying tyrosine kinase inhibitors (TKIs), e.g. the established drug imatinib. However, therapy has to be permanently continued, as otherwise a relapse occurs. Especially during the accelerated and blast crisis phase, TKI resistances complicate a successful treatment.[2] Furthermore, a reservoir of quiescent CML stem cells (SCs), insensitive to TKI therapy, persists in all stages of the disease and impedes a complete molecular response (CMR).[3-4]

Several approaches were pursued to develop a potential drug that eradicates CML SCs and overcomes resistance. One attempt aims the selective inhibition of the protein arginine methyltransferase 5 (PRMT5) with e.g. *N*-((9-ethyl-9*H*-carbazol-3-yl)methyl)-1,2,3,4-tetrahydronaphthalen-1-amine (PJ-68), which prevents growth of CML SCs in combination with imatinib.[5]

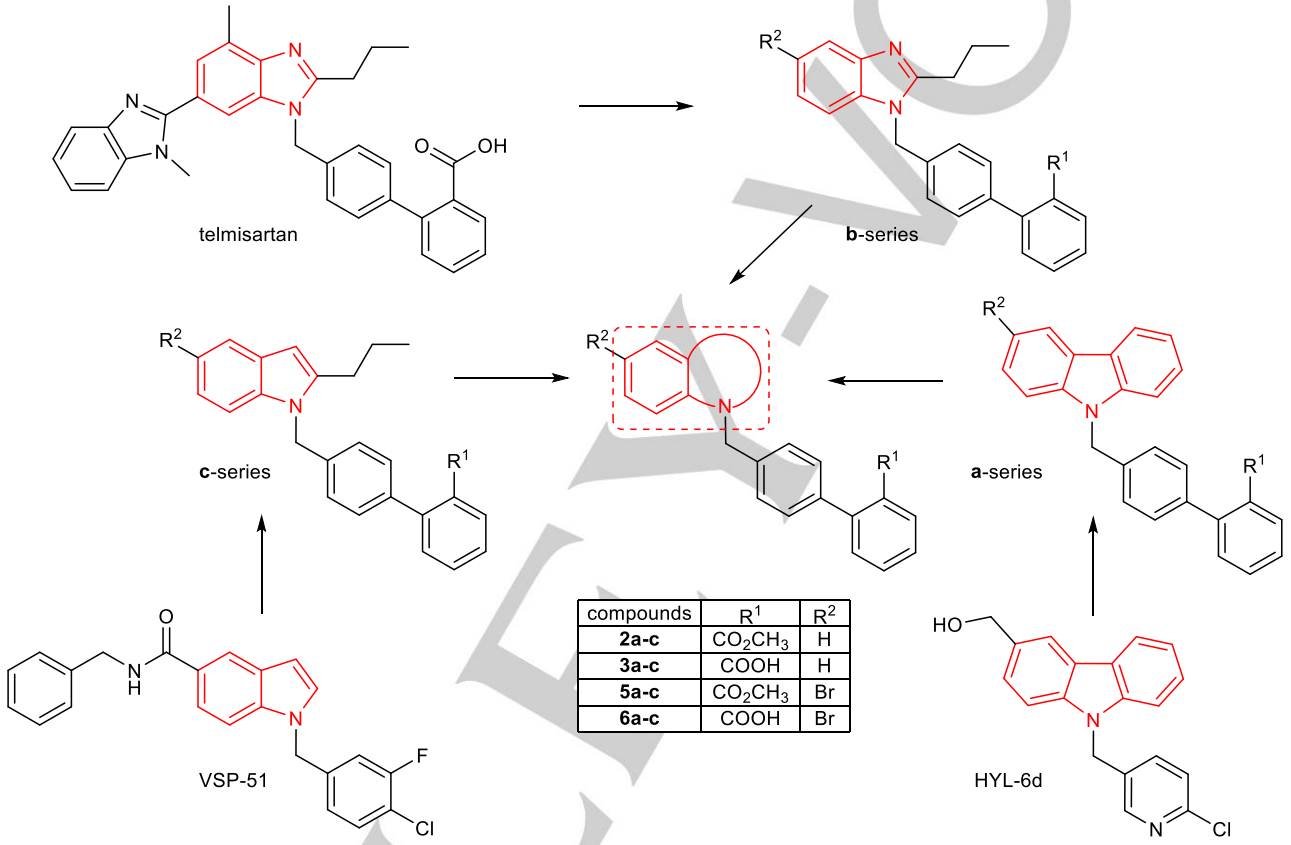
Another strategy to target leukemic SCs represents the combination therapy of imatinib with the peroxisome proliferator-activated receptor gamma (PPARγ) agonist pioglitazone, which yielded promising outcomes in the ACTIM phase II clinical trial.[6-8] The development of selective PPARγ modulators (SPPARγMs) has experienced a revival in the past few years, with the intention to improve the benefit-risk profile of PPARγ agonists in their clinical applications. Yet, as full agonists of PPARγ are associated with severe side effects[9], the investigations within our group focus on the well-tolerated partial agonist and SPPARγM telmisartan (Figure 1).[10-11]

The ability to circumvent imatinib resistance in K562-resistant CML cells has been experimentally verified for telmisartan and its 2-carboxamide derivative. The 4'-((2-propyl-1*H*-benzo[*d*]-imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid, identified as lead for the development of partial PPARγ agonists, was also included in this structure-activity relationship (SAR) studies. Compounds differing in substituents at position 4 of the heterocycle or in the derivatization of the 2-COOH have been evaluated concerning their influence on sensitization of imatinib-resistant CML cells.[12-13] In continuation, the relevance of the benzimidazole core on the cell death modulating effects was investigated by the exchange for related substructures.

Lamotte et al.[14] and Yi et al.[15] identified novel and promising selective PPARγ ligands, whereby VSP-51 (Figure 1) and others each comprise an indole core. Moreover, recent studies confirm a possible applicability of indoles for instance as anticancer agents in various types of leukemia. Herein, the interaction with different targets, e.g. with the apoptosis regulating proteins BCL2 and MCL1, but also with the BCR-ABL fusion gene was found to be involved in the mode of action.[16-18]

Carbazoles constitute other very interesting heterocyclic compounds with antitumor properties.[19-21] Representatives of this class were proven to possess potent activity against several human cancer cell lines or to be capable of sensitizing resistant cancer cells to chemotherapeutics. Despite their distinct mechanisms of action, these compounds have in common that they were optimized in SAR studies for tumor therapy by introduction of a carbazole moiety.

The same applies to HYL-6d (Figure 1), which caused high antiproliferative as well as antiangiogenic activity, and showed high structural analogy to SPPARγMs such as VSP-51. Moreover, carbazole derivatives succeeded to enter clinical trials and have even been approved for clinical use.[22]



compounds	R^1	R^2
2a-c	CO_2CH_3	H
3a-c	COOH	H
5a-c	CO_2CH_3	Br
6a-c	COOH	Br

Figure 1. Structural design of carbazole (a-series), benzimidazole (b-series), and indole (c-series) derivatives of telmisartan.

Accordingly, carbazole (a-series) and indole (c-series) derivatives of the lead 4'-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid were synthesized and transformed to the respective methyl esters (R^1 = COOH ↔ CO_2CH_3) (Figure 1). Additionally, the influence of a 5-Br substituent (R^2) was investigated (for the relevance of position 5 on the PPARγ activation of benzimidazole derivatives see[11, 23-24]).

The significance of the heterocyclic core regarding the ability of the compounds to activate PPARγ on the one hand and to sensitize imatinib-resistant CML cells on the other was evaluated. For the latter, all compounds were tested in a cytotoxicity assay against K562-resistant CML cells with or without co-application of imatinib by propidium iodide (PI) FACS analyses. To exclude unselective cytotoxicity, a modified MTT assay with the non-malignant COS-7 cell line was performed. This cell line was also used in the transactivation assay (plasmids: pGal5-Tk-pGL3 and pGal4-hPPARγDEF) to estimate the PPARγ activation.

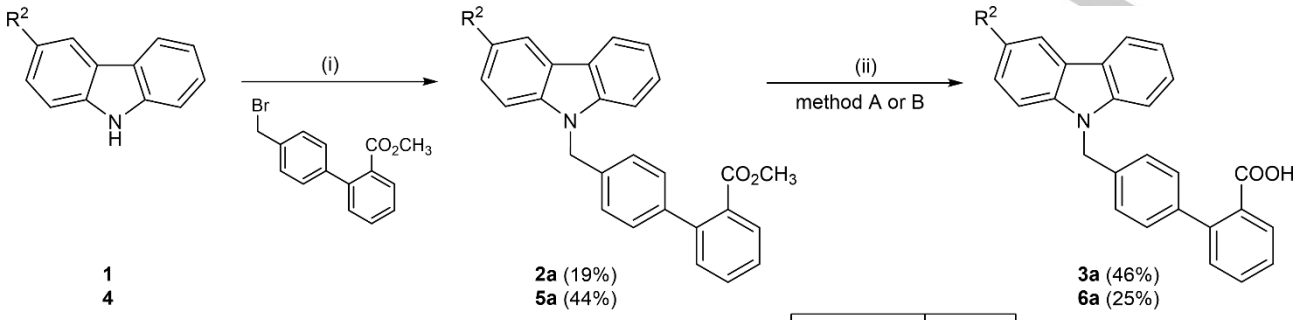
Results and Discussion

Synthesis

The syntheses of the compounds, based on already described procedures[11-12], are depicted in Schemes 1-3.

The carbazoles (a-series) were generated starting from the commercially available 9*H*-carbazole (**1**) and the 3-bromo-9*H*-carbazole (**4**) (Scheme 1). *N*-Alkylation with methyl 4'-

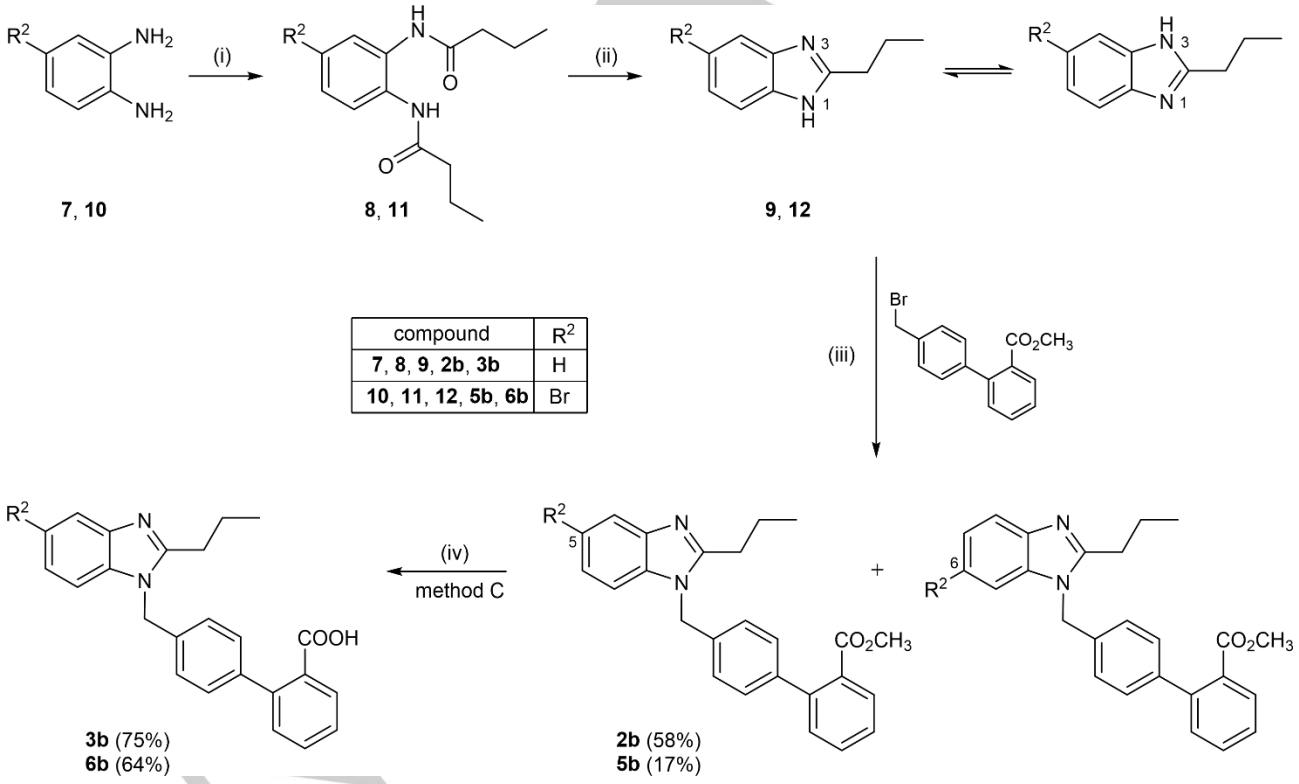
(bromomethyl)-[1,1'-biphenyl]-2-carboxylate was performed in the presence of the base NaH in anhydrous THF and yielded the respective methyl esters **2a** and **5a**. The carboxylic acids **3a** and **6a** were obtained from **2a**/**5a** by alkaline hydrolysis either with LiOH in THF at 60 °C (method A)[25] or with NaOH in MeOH at 65 °C (method B)[26].



compound	R^2
1, **2a**, **3a**	H
4, **5a**, **6a**	Br

Scheme 1. Synthesis of the carbazole (a-series) to yield the methyl esters **2a**/**5a** and the respective carboxylic acids **3a**/**6a**. Reagents and conditions: (i) NaH, anhydrous THF, rt; (ii) method A: 14% LiOH, THF, 60 °C; method B: 3 N NaOH, MeOH, 65 °C.

The synthesis of benzimidazoles (b-series) was realized with benzene-1,2-diamine (**7**) and 4-bromobenzene-1,2-diamine (**10**), which reacted in the first step with butyric anhydride and concentrated HCl to the bis-anilide derivatives **8** and **11** (Scheme 2).



compound	R^2
7, **8**, **9**, **2b**, **3b**	H
10, **11**, **12**, **5b**, **6b**	Br

Scheme 2. Synthesis of the benzimidazoles (b-series) to receive the methyl esters **2b**/**5b** and the respective carboxylic acids **3b**/**6b**. Reagents and conditions: (i) butyric anhydride, concentrated HCl, 120 °C; (ii) 4 N HCl, 100 °C; (iii) NaH, anhydrous DMF, rt; (iv) method C: KOH, EG, H_2O, 160 °C.

Heating with 4 N HCl led to ring closure yielding the respective benzimidazoles **9** and **12**. In this series, the *N*-alkylation, which was performed as described above but with anhydrous DMF instead of THF as solvent, resulted in an isomeric mixture due to

a possible alkylation of N1 or N3 (Scheme 2). The desired 5-substituted derivative **5b** was separated from the unwanted 6-substituted benzimidazole by column chromatography.

The distinction of the isomers was carried out by ^{1}H- and 2D-NMR spectroscopy.

Cross peaks between the NCH_2 protons and H7'' in the NOESY (nuclear overhauser enhancement spectroscopy) spectra allowed an assignment of the protons (Figure 2, blue). In the ^{1}H-NMR spectrum of **5b**, H7'' was split by an ortho coupling, which was superimposed by the signal of H6''. The 6-Br substituent of the other isomer, however, only enabled a meta splitting of H7'' (^{4}J = 1.9 Hz) and further caused a downshift from 7.40ppm (**5b**) to 7.65ppm.

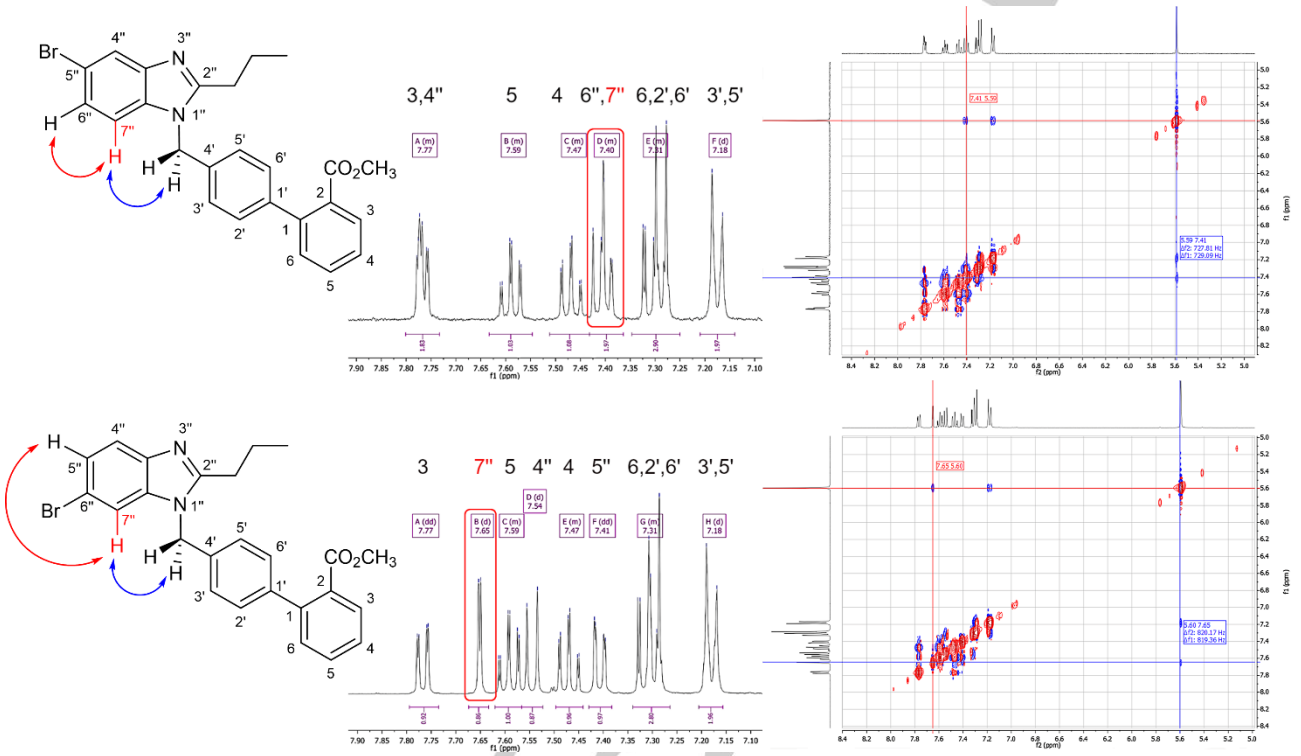


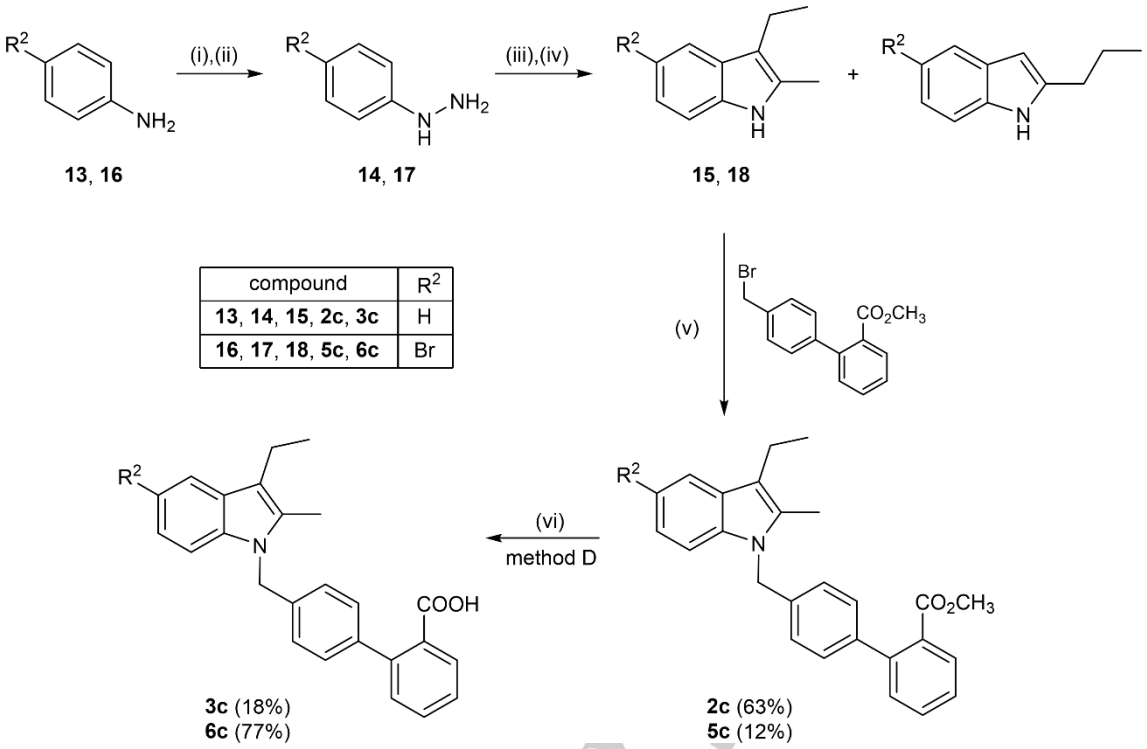
Figure 2. Extract of the ^{1}H-NMR spectra of **5b** (above) as well as its 6-Br isomer (below) and their respective NOESY spectra (solvent: acetone-d_6, 400 MHz). Saturation transfers indicated in blue, couplings in red.

In the final step, heating of **2b** or **5b** with KOH in ethylene glycol (EG) and H_2O at 160 °C (method C) hydrolyzed the ester and gave the carboxylic acids **3b** or **6b** in high yield.

In case of the indoles (c-series), the commercially available aniline (**13**) and 4-bromoaniline (**16**) were converted respectively to the phenylhydrazines **14** and **17** in a one-pot reaction (Scheme 3). After diazotization with $NaNO_2$ in 6 N HCl, the formed aryl diazonium salts were reduced with $SnCl_2$ in concentrated HCl[27]. The indole core was prepared *via* Fischer indole synthesis[28]. Thereby, **14** or **17** were refluxed with 2-pentanone in EtOH to give the aryl hydrazone intermediates *in situ*. The solvent was removed and after addition of $ZnCl_2$ in DMF, the mixture was heated at 120 °C.

Principally, two isomers can be formed during the ring closure reaction due to the use of an asymmetric substituted ketone (Scheme 3). Unfortunately, column chromatography of the crude product yielded only the 3-ethyl-2-methyl-substituted indoles in sufficient amount and purity. Most of the byproducts were intermediates, which did not convert to the expected indole core. Nevertheless, the obtained compounds **15** and **18** were used for *N*-alkylation with methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate and NaH in anhydrous DMF (→ **2c** and **5c**). Alkaline hydrolysis with 2 N KOH in THF/EtOH at 70 °C finally gave **3c** and **6c** (method D)[29].

Compounds **2a-c**, **3a-c**, **5a-c**, and **6a-c** showed characteristic ^{1}H- and ^{13}C-NMR as well as high-resolution mass spectra (HRMS) (see Supporting Information). High-performance liquid chromatography (HPLC) indicated sufficient purity. For synthesis protocols and the analytic data of intermediates as well as target compounds (**2**, **3**, **5**, and **6** of each series) see also Supporting Information.



Scheme 3. Synthesis of the indoles (c-series) to obtain the methyl esters **2c**/**5c** and the respective carboxylic acids **3c**/**6c**. Reagents and conditions: (i) $NaNO_2$, H_2O, 6 N HCl, –10 °C to 5 °C; (ii) $SnCl_2 \times 2\ H_2O$, concentrated HCl, –5 °C to rt; (iii) 2-pentanone, EtOH, 80 °C; (iv) $ZnCl_2$, DMF, 120 °C; (v) NaH, anhydrous DMF, rt; (vi) method D: 2 N KOH, THF, EtOH, 70 °C.

Biological activity

Transactivation assay

PPARγ interaction of the target compounds was evaluated *in vitro* in COS-7 cells, transiently transfected with the plasmids pGal5-Tk-pGL3 and pGal4-hPPARγDEF using a dual-luciferase reporter assay. The activity of the co-transfected pRenilla-CMV plasmid served for normalization and telmisartan (partial PPARγ agonist) as well as pioglitazone (full agonist) acted as positive controls. The maximum activation of pioglitazone at 10 µM was defined as A_{max} = 100% (EC_{50} = 1.05 µM). Figure 3 depicts the concentration-response curves of the transactivation assay, covering the concentrations 0.05 to 20 µM. The potency (EC_{50} values) and efficacy (A_{max}) of each compound at 10 µM are additionally summarized in Table 1.

A_{max} (61.3%) and the EC_{50} value (4.78 µM) of telmisartan are in agreement with previous results.[12] The same is true for the benzimidazole **3b**, representing the lead structure developed in a former study.[30] It reached the effect of telmisartan with A_{max} = 59.4% and EC_{50} = 6.98 µM.

Exchange of the heterocyclic core in **3b** by a carbazole or indole moiety reduced the activity at 10 µM to A_{max} = 25.6% (**3a**) and 39.2% (**3c**), respectively, while the potency remained nearly unchanged (EC_{50} = 10.4 µM and 6.86 µM, respectively).

Upon esterification, the resulting carbazole **2a** (A_{max} = 0.60%) and the indole **2c** (A_{max} = 3.16%) became inactive. Only the methyl ester **2b** achieved an activation of about 36% at a concentration of 20 µM (Figure 3).

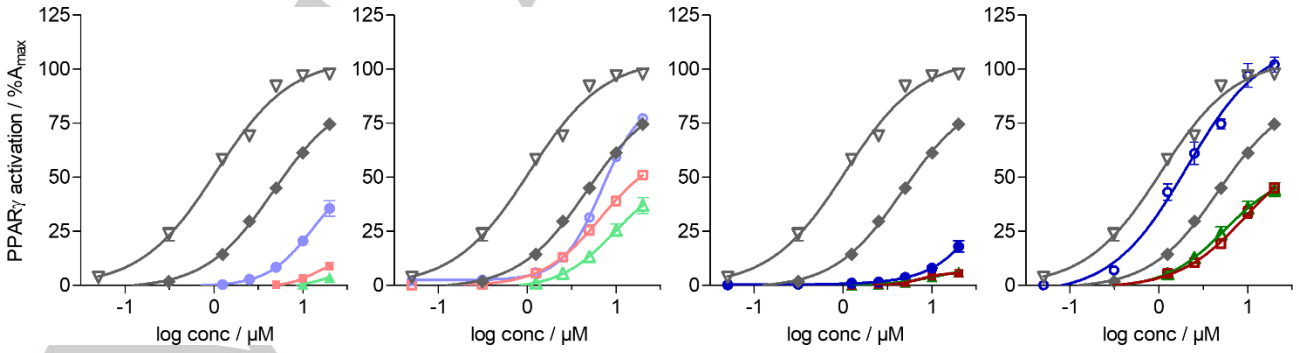
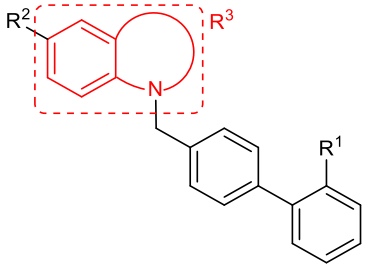


Figure 3. Concentration-response curves of the transactivation assay relating to PPARγ. COS-7 cells were transiently transfected with the plasmids pGal5-TK-pGL3 as well as pGal4-hPPARγDEF and subsequently stimulated with pioglitazone (▽), telmisartan (◆), or the compounds **2a** (▲), **2b** (●), **2c** (■), **3a** (△), **3b** (○), **3c** (□), **5a** (▲), **5b** (●), **5c** (■), **6a** (△), **6b** (○), **6c** (□), respectively. After 39 h of incubation, firefly-luciferase was measured and normalized with the activity of the co-transfected pRenilla-CMV. Data represent the mean ± SD of ≥ 3 independent experiments with three replicates each.

Table 1. PPARγ activation determined by the luciferase transactivation assay with COS-7 cells.



compound	R^1	R^2	R^3	A_{max}[a,c]	EC_{50}[b,c]
2a				0.60 ± 0.56	n.d.
2b	CO_2CH_3			20.5 ± 2.3	n.d.
2c		H		3.16 ± 0.49	n.d.
3a			a-series	25.6 ± 4.9	10.4 ± 3.9
3b	COOH			59.4 ± 3.7	6.98 ± 1.06
3c				39.2 ± 3.1	6.86 ± 1.58
5a				4.03 ± 0.40	n.d.
5b	CO_2CH_3		b-series	7.97 ± 0.50	n.d.
5c		Br		4.33 ± 0.55	n.d.
6a				35.8 ± 5.4	5.68 ± 1.07
6b	COOH			97.1 ± 9.51	2.05 ± 0.38
6c			c-series	33.8 ± 4.7	9.52 ± 0.74
pioglitazone				100	1.05 ± 0.21
telmisartan				61.3 ± 4.2	4.78 ± 0.79

[a] The activation (A_{max}) relates to the PPARγ activation caused by the compounds at 10 µM. The effect of pioglitazone at 10 µM was set to 100%.

[b] The potency (EC_{50} values) was calculated from the reflection point of the sigmoid concentration-response curves (Figure 3).

[c] Data represent the mean ± SD of ≥ 3 independent experiments (n.d.: not determinable).

Bromination of **3b** (→ **6b**) strongly increased the agonistic potency. The pharmacological profile changed to that of a full agonist with A_{max} = 97.1% and an EC_{50} = 2.05 µM, similar to the reference pioglitazone. The 5-Br substituent at **3a** (→ **6a**: A_{max} = 35.8%; EC_{50} = 5.68 µM) and **3c** (→ **6c**: A_{max} = 33.8%; EC_{50} = 9.52 µM) had just minor effects.

The brominated esters were completely inactive. Even **2b** (A_{max} = 20.5%) lost its low activity. **5b** as well as **5a** and **5c** showed an activation of only 4% to 8%, which is not different from the solvent control.

In conclusion, regarding the PPARγ activation, compounds with a benzimidazole core provided the best effects. The hydrophobic bromine substituent at position 5 changed the profile from a partial (**3b**) to a full agonist (**6b**). Esterification of the 2-COOH group led to nearly inactive compounds. The same is true, if a carbazole core is introduced. The indole derivatives, bearing a carboxylic group represented weak partial agonists.

Induction of cell death

In the next step, the compounds were evaluated to circumvent resistance. As mentioned above, imatinib represents an effective TKI in the treatment of CML. However, early relapse occurs due to therapy resistance.

Hui et al.[31] described the KD225 cell line as doxorubicin-resistant subclone of K562 CML cells. Our own results demonstrate that these cells are also imatinib-resistant (termed K562-resistant), since 1 µM only caused about 10-20% cell death as determined by PI FACS analyses (ctr. in Figure 4B).[12] Therefore, this cell line is suitable for studying the potency of the compounds to modulate the cell death induction of imatinib.

All compounds, with exception **2b**, were inactive even at a concentration of 10 µM. In each case, control-like effects (about 10%) were determined after an incubation time of 72 h. Merely **2b** slightly increased the cell death rate to 21% (Figure 4A).

Co-application of imatinib with the compounds showed that only those of the b-series (**2b**, **3b**, **5b**) modulated the potency of imatinib. The other compounds had no influence (Figure 4B) and caused control-like effects.

The benzimidazole 2-CO_2CH_3 derivative **2b** (10 µM) strongly sensitized the cells, so imatinib induced almost 80% cell death. Modifications at the heterocyclic core as well as alkaline hydrolysis to the 2-COOH group reduced the cell death modulating effects. Combination of imatinib with the brominated analog **5b** as well as the lead **3b** led to a cell death rate of 31%.

For comparison, the isomer of **5b** (6-Br) and the corresponding carboxylic acid were investigated as well. The 6-Br-COOH derivative, representing a full PPARγ agonist[11], failed to be a cell death modulator. Surprisingly, its methyl ester (6-Br-CO_2CH_3) sensitized the cells more effective (63% at 10 µM) than **5b** (31% at 10 µM) to imatinib treatment, but to a lesser extent than the unsubstituted ester **2b** (80% at 10 µM).

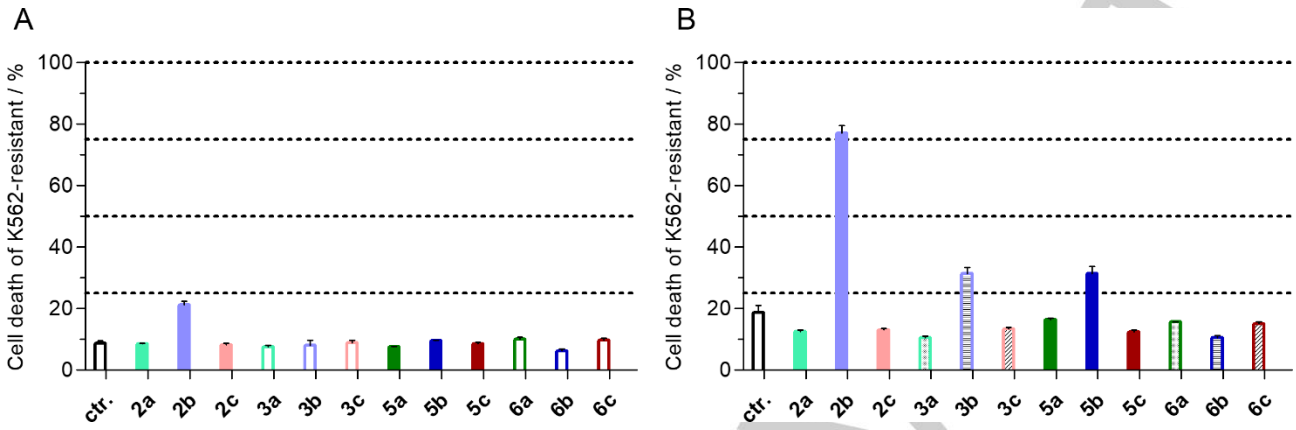


Figure 4. Induction of cell death in K562-resistant cells, treated with **2a-c**, **3a-c**, **5a-c**, or **6a-c** (each 10 µM) for 72 h, respectively, either without (A) or in combination with 1 µM of imatinib (B). A vehicle treated control without (ctr., A) or with imatinib (1 µM, ctr., B) was included. The detection of dead cells was conducted by PI FACS analyses. Data represent the mean + SEM of ≥ 4 independent experiments.

These results are in accordance with findings of a recently published study.[13] A benzimidazole seems to be by far the most promising core to design derivatives with high potency to sensitize resistant CML cells to imatinib treatment. The investigations on the relevance of substituents at positions 5 and 6 of the benzimidazole will be part of a forthcoming study.

Modified MTT assay

The compounds were *per se* inactive, not only against K562-resistant cells, but also against the non-malignant COS-7 cell line used in the transactivation assay. All compounds were tested at defined concentrations in a modified MTT assay (Figure 5).

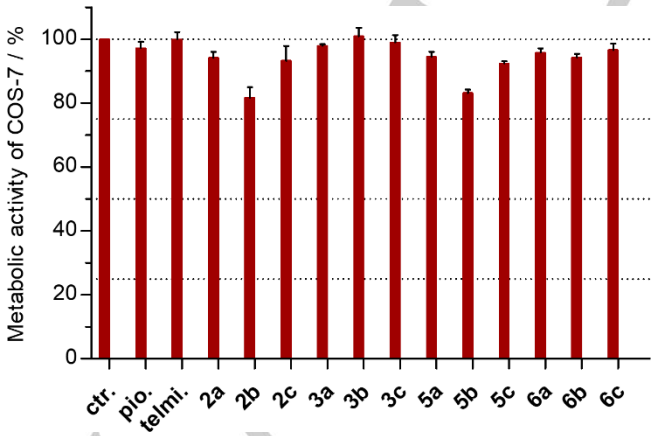


Figure 5. Metabolic activity of COS-7 cells treated with 20 µM of either vehicle (DMSO, ctr.) or **2a-c**, **3a-c**, **5a-c**, **6a-c**. The mean values + SD of ≥ 3 independent experiments are shown.

Metabolically competent cells reduce MTT dyes to purple-colored formazans. The formation of these products depends on the enzyme activity of mitochondrial dehydrogenases and allows the quantification of living cells.[32]

Cytotoxic compounds significantly reduce the metabolic activity of cells, measured after an incubation time of 72 h according to the manufacturer's protocol.

The derivatives **2a-c**, **3a-c**, **5a-c**, and **6a-c** did not have any impact on COS-7 cells. In all cases, the metabolic activity remained unchanged (higher than 80%, see Figure 5) at the highest concentration used (20 µM).

Conclusion

The results of this study extend our knowledge about PPARγ activation and cell death modulating effects of telmisartan derivatives. Rousselot et al.[6] investigated the synergistic effects of the full PPARγ agonist pioglitazone and imatinib in CML. They correlated the potency to activate PPARγ with the effectiveness to eradicate the CML SC pool in biological assays. Inspired by these investigations, we tested telmisartan in combination with imatinib in K562-resistant cells. Although it is only a partial agonist, telmisartan was even more effective to overcome imatinib resistance than pioglitazone. Extended testing of structurally modified compounds implied the independence of PPARγ activity and induction of cell death. These data further indicate that the heterocyclic core plays an essential role.

Therefore, we replaced the benzimidazole of the lead **3b** by an indole or carbazole in this SAR study and substituted position 5, which is relevant for PPARγ activation, with a bromine substituent. Compound **3b** is a partial PPARγ agonist that turned out to be a full agonist (**6b**) upon bromination. The respective methyl esters showed a reduced (**2b**) and missing (**5b**) activity.

Replacement of the benzimidazole (b-series) by a carbazole (a-series) or an indole (c-series) decreased both potency as well as

the efficacy. Again, the carboxylic acids (**3a**, **6a**, **3c**, **6c**) were moderately active and the methyl esters (**2a**, **5a**, **2c**, **5c**) remained inactive.

Although the methyl ester **2b** did not activate PPARγ at 10 µM, it sensitized the K562-resistant cells with high potency to imatinib treatment (1 µM) at the applied concentration. Imatinib in combination with **2b** induced a cell death rate of about 80%. Additionally, **2b** did not exert cytotoxicity *per se* as demonstrated in a modified MTT assay with non-malignant COS-7 cells.

Within the b-series, **3b** and **5b** can circumvent the resistance, if used as add-on to imatinib. Cell death rates of 30-40% were observed upon this co-application.

The modulating effect is limited to compounds of the b-series since none of the other compounds (carbazoles or indoles) sensitized the resistant cells to imatinib treatment.

Experimental Section

Chemistry

General materials and methods

The compounds **1**, **4**, **7**, **10**, **13**, and **16**, all reagents as well as other chemicals were purchased from Alfa Aesar, Sigma-Aldrich, or TCI Chemicals and used without further purification. Dichloromethane (DCM), ethyl acetate (EA), ethanol (EtOH), and methanol (MeOH) were distilled prior to usage, while petroleum ether (PE) was directly employed. Ethylene glycol (EG), *N*,*N*-dimethyl formamide (DMF), isopropanol, and tetrahydrofuran (THF) were purchased in appropriate quality. Column chromatography was conducted following both classic standard procedures and medium pressure liquid chromatography. For the latter, an Isolera One 3.0 Flash purification system (Biotage) was utilized. In either case, silica gel 60 (particle size 40-63 µm, 230-240 mesh) served as stationary phase. Thin-layer chromatography was carried out using Polygram SIL G/UV_{254} polyester foils covered with a 0.2 mm layer of silica gel as well as a fluorescence indicator (Macherey-Nagel) and were visualized with UV light (254 or 366 nm). Nuclear magnetic resonance spectra (NMR) were recorded using a 400 MHz Avance 4 Neo (Bruker) or 600 MHz Avance II (Bruker) spectrometer. Deuterated dimethyl sulfoxide (DMSO-d_6), acetone (acetone-d_6), methanol (CD_3OD), and water (D_2O) were used as solvents (all from Eurisotop or Alfa Aesar). Chemical shifts (δ) were referenced to the solvent peak or tetramethylsilane (TMS) as internal standard and are given in parts per million (ppm). Coupling constants (*J*) are reported in Hertz (Hz). Signals are described as follows: s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet. HRMS was conducted with an Orbitrap Elite system (Thermo Fisher Scientific). HPLC was used for the determination of purity. A Shimadzu Nexera-i LC 2040C 3D device equipped with the autosampler SIL 20A HT, the column oven CTO-10AS VP, the degasser DGU-20A, the detector SPD-M20A, and the pumps LC-20AD was applied. An RP-18 column (dimension 125×4 mm, 5 µm particle size, Knauer) was used and the chromatograms were analyzed with the program LabSolutions 5.86 (Shimadzu). The purity of ≥ 90% was assured for all compounds.

Syntheses of target compounds

General procedure for *N*-alkylation of the heterocycles with methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate: To a solution of the appropriate heterocycle (1 eq) in anhydrous DMF (1-3 ml/mmol) or anhydrous THF (2-3 ml/mmol), NaH (1.2 eq) was slowly added. After approximately 30 min of stirring at room temperature, methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate (1.1 eq) was added and stirring was continued for 10-16 h. The reaction mixture was diluted with water to double the volume and neutralized with 1 N HCl. Then, it was extracted with DCM (3×), the organic layers were combined, washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the resulting crude product was purified by flash chromatography with stepwise gradient elution (PE/EA, 9:1 to 3:7).

Methyl 4'-((9*H*-carbazol-9-yl)methyl)-[1,1'-biphenyl]-2-carboxylate (2a): From 9*H*-carbazole **1** (0.50 g, 3.0 mmol) in anhydrous THF (6 ml) with 60% NaH (1.79 g, 4.5 mmol) and methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate (1.0 g, 3.3 mmol). Colorless solid, yield: 19%. ^{1}H-NMR (400 MHz, acetone-d_6): δ 8.19 (d, 2H, ^3J = 7.8 Hz, H4'', H5''), 7.74 (dd, 1H, ^3J = 7.7 Hz, ^4J = 1.0 Hz, H3), 7.61 (d, 2H, ^3J = 8.3 Hz, H1'', H8''), 7.58-7.52 (m, 1H, H5), 7.49-7.40 (m, 3H, H4, H2'', H7''), 7.36 (dd, 1H, ^3J = 7.7 Hz, ^4J = 0.8 Hz, H6), 7.26-7.20 (m, 6H, H2', H3', H5', H6', H3'', H6''), 5.72 (s, 2H, NCH_2), 3.53 (s, 3H, CO_2CH_3). ^{13}C-NMR (101 MHz, acetone-d_6): δ 169.3, 142.5, 141.6, 141.2, 137.9, 132.3, 132.1, 131.4, 130.4, 129.5, 128.1, 127.4, 126.7, 123.9, 121.1, 120.0, 110.2, 52.0, 46.6. HRMS: *m/z* calculated for $C_{27}H_{21}NO_2$ [*M*+Na]$^+$: 414.1465, found: 414.1518.

Methyl 4'-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylate (2b): From compound **9** (0.50 g, 3.1 mmol) in anhydrous DMF (6 ml), with NaH (0.15 g, 3.7 mmol) and methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate (1.05 g, 3.4 mmol). Colorless solid, yield: 58%. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.72 (dd, 1H, ^3J = 7.7 Hz, ^4J = 1.4 Hz, H3), 7.64-7.55 (m, 2H, H5, H4''), 7.53-7.42 (m, 2H, H4, H7''), 7.38 (dd, 1H, ^3J = 7.7 Hz, ^4J = 1.3 Hz, H6), 7.24 (d, 2H, ^3J = 8.3 Hz, H2', H6'), 7.21-7.10 (m, 4H, H3', H5', H5'', H6''), 5.54 (s, 2H, NCH_2), 3.54 (s, 3H, CO_2CH_3), 2.84 (t, 2H, ^3J = 7.5 Hz, $CH_2CH_2CH_3$), 1.84-1.71 (m, 2H, $CH_2CH_2CH_3$), 0.96 (t, 3H, ^3J = 7.4 Hz, $CH_2CH_2CH_3$). ^{13}C-NMR (101 MHz, DMSO-d_6): δ 168.37, 155.06, 142.43, 139.66, 136.24, 135.37, 131.48, 130.74, 130.46, 129.31, 128.52, 127.50, 126.39, 121.67, 121.33, 118.50, 110.14, 51.81, 45.72, 28.56, 20.33, 13.79. HRMS: *m/z* calculated for $C_{25}H_{24}N_2O_2$ [*M*+H]$^+$: 385.1911, found: 385.1937.

Methyl 4'-((3-ethyl-2-methyl-1*H*-indol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylate (2c): From **15** (0.08 g, 0.5 mmol) in anhydrous DMF (1.4 ml), with NaH (0.03 g, 1.5 mmol) and methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate (0.17 g, 0.6 mmol). Light brown oil, yield: 63%. ^{1}H-NMR (400 MHz, acetone-d_6): δ 7.75 (dd, 1H, ^3J = 7.7 Hz, ^4J = 1.5 Hz, H3), 7.62-7.48 (m, 2H, H5, H4''), 7.48-7.41 (m, 1H, H4), 7.40-7.31 (m, 2H, H6, H7''), 7.22 (d, 2H, ^3J = 8.2 Hz, H2', H6'), 7.08-6.99 (m, 4H, H3', H5', H5'', H6''), 5.44 (s, 2H, NCH_2), 3.56 (s, 3H, CO_2CH_3), 2.78 (q, 2H, ^3J = 7.5 Hz, CH_2CH_3), 2.36 (s, 3H, CH_3), 1.22 (t, 3H, ^3J = 7.5 Hz, CH_2CH_3). ^{13}C-NMR (101 MHz, acetone-d_6): δ 140.9, 138.8, 132.1, 131.4, 130.4, 129.4, 128.1, 126.9, 121.4, 119.5,

118.7, 110.0, 52.0, 46.7, 18.2, 16.1, 10.2. HRMS: *m/z* calculated for $C_{26}H_{25}NO_2$ [*M*+Na]$^+$: 406.1778, found: 406.1774.

Methyl 4'-((3-bromo-9*H*-carbazol-9-yl)methyl)-[1,1'-biphenyl]-2-carboxylate (5a): From 3-bromo-9*H*-carbazole **4** (0.50 g, 2.0 mmol) in anhydrous THF (6 ml) with 60% NaH (1.2 g, 3.0 mmol) and methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate (0.68 g, 2.2 mmol). Colorless solid, yield: 44%. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.45 (d, 1H, ^4J = 2.0 Hz, H4''), 8.25 (d, 1H, ^3J = 7.7 Hz, H5''), 7.73-7.64 (m, 3H, H3, H1'', H8''), 7.61-7.53 (m, 2H, H5, H2''), 7.53-7.40 (m, 2H, H4, H7''), 7.35 (dd, 1H, ^3J = 7.8 Hz, ^4J = 1.3 Hz, H6), 7.29-7.21 (m, 1H, H6''), 7.21-7.17 (m, 4H, H2', H3', H5', H6'), 5.73 (s, 2H, NCH_2), 3.53 (s, 3H, CO_2CH_3). ^{13}C-NMR (101 MHz, DMSO-d_6): δ 168.3, 140.8, 140.6, 139.5, 138.9, 136.6, 131.5, 130.7, 130.5, 129.3, 128.4, 128.2, 127.4, 126.7, 126.6, 124.2, 123.0, 121.3, 121.0, 119.5, 111.7, 111.2, 109.9, 51.8, 45.4. HRMS: *m/z* calculated for $C_{27}H_{20}BrNO_2$ [*M*+Na]$^+$: 492.0570, found: 492.0624.

Methyl 4'-((5-bromo-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylate (5b) and methyl 4'-((6-bromo-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylate (6-Br-CO_2CH_3): From **12** (0.50 g, 2.1 mmol) in anhydrous DMF (2 ml) with NaH (0.06 g, 2.5 mmol) and methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate (0.70 g, 2.3 mmol).

5b: Colorless solid, yield: 17%. ^{1}H-NMR (400 MHz, acetone-d_6): δ 7.83-7.72 (m, 2H, H3, H4''), 7.62-7.54 (m, 1H, H5), 7.50-7.43 (m, 1H, H4), 7.43-7.35 (m, 2H, H6'', H7''), 7.35-7.24 (m, 3H, H6, H2', H6'), 7.17 (d, 2H, ^3J = 8.5 Hz, H3', H5'), 5.57 (s, 2H, NCH_2), 3.57 (s, 3H, CO_2CH_3), 2.89 (t, 2H, ^3J = 7.4 Hz, $CH_2CH_2CH_3$), 1.93-1.79 (m, 2H, $CH_2CH_2CH_3$), 1.00 (t, 3H, ^3J = 7.4 Hz, $CH_2CH_2CH_3$). ^{13}C-NMR (101 MHz, acetone-d_6): δ 157.73, 142.31, 141.60, 136.71, 132.21, 132.15, 131.39, 130.43, 129.68, 128.25, 127.17, 125.36, 122.37, 114.83, 112.44, 52.07, 47.20, 21.36, 14.19. HRMS: *m/z* calculated for $C_{25}H_{23}BrN_2O_2$ [*M*+H]$^+$: 463.1016, found: 463.1054.

6-Br-CO_2CH_3: Colorless solid, yield: 13%. ^{1}H-NMR (400 MHz, acetone-d_6): δ 7.77 (dd, 1H, ^3J = 7.7 Hz, ^4J = 1.4 Hz, H3), 7.65 (dd, 1H, ^4J = 1.9 Hz, H7''), 7.62-7.56 (m, 1H, H5), 7.54 (d, 1H, ^3J = 8.5 Hz, H4''), 7.50-7.44 (m, 1H, H4), 7.41 (dd, 1H, ^3J = 7.6 Hz, ^4J = 1.3 Hz, H5''), 7.35-7.26 (m, 3H, H6, H2', H6'), 7.18 (d, 2H, ^3J = 8.5 Hz, H3', H5'), 5.59 (s, 2H, NCH_2), 3.57 (s, 3H, CO_2CH_3), 2.89 (t, 2H, ^3J = 7.6 Hz, $CH_2CH_2CH_3$), 1.94-1.80 (m, 2H, $CH_2CH_2CH_3$), 1.01 (t, 3H, ^3J = 7.4 Hz, $CH_2CH_2CH_3$). ^{13}C-NMR (101 MHz, acetone-d_6): δ 169.4, 157.3, 143.2, 142.4, 141.6, 138.0, 136.8, 132.3, 132.2, 131.4, 130.4, 129.7, 128.3, 127.2, 125.4, 121.3, 115.3, 113.8, 52.1, 47.1, 21.4, 14.2.

Methyl 4'-((5-bromo-3-ethyl-2-methyl-1*H*-indol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylate (5c): From **18** (0.09 g, 0.4 mmol) in anhydrous DMF (1 ml) with 60% NaH (0.02 g, 0.8 mmol) and methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate (0.13 g, 0.4 mmol). Light brown solid, yield 12%. ^{1}H-NMR (400 MHz, acetone-d_6): δ 7.75 (dd, 1H, ^3J = 8.1 Hz, ^4J = 1.3 Hz, H3), 7.67 (d, 1H, ^4J = 1.9 Hz, H4''), 7.62-7.53 (m, 1H, H5), 7.50-7.42 (m, 1H, H4), 7.39 (dd, 1H, ^3J = 8.0 Hz, ^4J = 1.3 Hz, H6), 7.33 (d, 1H, ^3J = 8.6 Hz, H7''), 7.24 (d, 2H, ^3J = 8.3 Hz, H2', H6'), 7.16 (dd, 1H, ^3J = 8.6 Hz, ^4J = 2.0 Hz, H6''), 7.04 (d, 2H, ^3J = 8.3 Hz, H3', H5'), 5.47 (s, 2H, NCH_2), 3.57 (s, 3H, CO_2CH_3), 2.76 (q, 2H, ^3J = 7.5 Hz, CH_2CH_3), 2.37 (s, 3H, CH_3), 1.20 (t, 3H, ^3J = 7.5 Hz, CH_2CH_3). ^{13}C-NMR (101 MHz, acetone-d_6): δ 169.4, 142.5, 141.1, 138.3, 136.3, 134.9, 132.3, 132.1, 131.4, 130.5, 130.4, 129.5, 128.2, 126.8, 123.8, 121.2, 114.3, 112.5, 111.9, 52.1, 46.9, 18.0, 16.0, 10.3. HRMS: *m/z* calculated for $C_{26}H_{24}BrNO_2$ [*M*+H]$^+$: 462.1063, found: 462.1152.

General procedures for saponification of the methyl esters:

Method A: THF (50 ml/mmol) was applied to dissolve the respective methyl ester. 14% LiOH (5 ml/mmol) was added and it was heated at 60 °C for 140 h. The reaction mixture was diluted with water to double the volume and acidified to a pH of 5 with 1 N HCl. Then, it was extracted with EA (3×), the organic layers were combined, washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the resulting crude product was purified by flash column chromatography with stepwise gradient elution (PE/EA, 9:1 to 7:3).

Method B: The respective methyl ester was dissolved in MeOH (50 ml/mmol), 3 N NaOH (1 ml/mmol) was added and the solution was heated at 65 °C for 72 h. After adding water, 6 N HCl was used to reach a pH of 1. It was extracted with DCM (3×), the organic layers were combined, washed with brine, and dried over anhydrous Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the resulting crude product was purified by flash column chromatography with stepwise gradient elution (PE/EA, 9:1 to 7:3).

Method C: KOH (2 eq) was dissolved in EG (0.5-1.5 ml/mmol) and added to the respective methyl ester (1 eq). After adding a few drops of water, the mixture was heated at 160 °C for 14 h. Water was added to double the volume and the reaction mixture was acidified to a pH of 3 with 6 N HCl. It was extracted with EA (3×), the organic layers were combined, washed with brine, and dried over anhydrous Na_2SO_4. Then it was filtered, the solvent was removed under reduced pressure and the resulting crude product was purified by chromatography with stepwise gradient elution (DCM/MeOH, 95:5 to 9:1).

Method D: The respective methyl ester was dissolved in EtOH and THF (each 1.9 ml/mmol). 2 N KOH (4 ml/mmol) was added and the mixture was heated at 70 °C for 18 h. After adding water, 6 N HCl was used to reach a pH of 2. The formed precipitate was sucked off, washed with cold water, and dried *in vacuo*. The crude product was purified by recrystallization from MeOH.

4'-((9*H*-Carbazol-9-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid (3a): Method A: from **2a** (0.10 g, 0.3 mmol) in THF (15 ml) with 14% LiOH (1.3 ml). Colorless oil, yield: 46%. ^{1}H-NMR (400 MHz, acetone-d_6): δ 8.19 (d, 2H, ^3J = 7.8 Hz, H4'', H5''), 7.81 (dd, 1H, ^3J = 7.7 Hz, ^4J = 1.5 Hz, H3), 7.62 (d, 2H, ^3J = 8.2 Hz, H1'', H8''), 7.57-7.50 (m, 1H, H5), 7.50-7.40 (m, 3H, H4, H2'', H7''), 7.33 (dd, 1H, ^3J = 7.6 Hz, ^4J = 1.3 Hz, H6), 7.30-7.20 (m, 6H, H2', H3', H5', H6', H3'', H6''), 5.71 (s, 2H, NCH_2). ^{13}C-NMR (151 MHz, CD_3OD): δ 142.4, 142.1, 141.9, 138.0, 131.6, 131.5, 130.1, 129.9, 128.1, 127.4, 126.9, 124.3, 121.1, 120.2, 110.2, 46.9. HRMS: *m/z* calculated for $C_{26}H_{19}NO_2$ [*M*+Na]$^+$: 400.1308, found: 400.1300.

4'-((2-Propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid (3b): Method C: from **2b** (0.50 g, 1.3 mmol) with KOH (0.15 g, 2.6 mmol) in EG (2 ml). Colorless solid, yield: 75%. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.80 (br s, 1H, COOH), 7.67 (dd, 1H, ^3J = 7.6 Hz, ^4J = 1.5 Hz, H3), 7.62-7.56 (m, 1H, H4''), 7.54-7.47 (m, 2H, H5, H7''), 7.44-7.38 (m, 1H, H4), 7.34-7.27 (m, 3H, H6, H2', H6'), 7.20-7.14 (m, 2H, H5'', H6''), 7.11 (d, 2H, ^3J = 8.2 Hz, H3', H5'), 5.53 (s, 2H, NCH_2), 2.84 (t, 2H, ^3J = 7.5 Hz, $CH_2CH_2CH_3$), 1.87-1.71 (m, 2H, $CH_2CH_2CH_3$), 0.96 (t, 3H, ^3J = 7.4 Hz, $CH_2CH_2CH_3$). ^{13}C-NMR (101 MHz, DMSO-d_6): δ 169.9, 155.4, 140.9, 140.8, 135.8, 134.9, 132.7, 131.4, 130.9, 129.6, 129.2, 127.9, 126.9, 123.3, 123.2, 117.8, 111.5, 46.6, 28.5, 20.7, 14.2. HRMS: *m/z* calculated for $C_{24}H_{22}N_2O_2$ [*M*+H]$^+$: 371.1754, found: 371.1773.

4'-((3-Ethyl-2-methyl-1*H*-indol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid (3c): Method D: from **2c** (0.04 g, 0.1 mmol) in EtOH and THF (each 0.2 ml) with 2 N KOH (0.4 mmol). Colorless solid, yield: 18%. ^{1}H-NMR (400 MHz, acetone-d_6): δ 7.83 (dd, 1H, ^3J = 7.9 Hz, ^4J = 1.5 Hz, H3), 7.61-7.53 (m, 2H, H5, H4''), 7.49-7.44 (m, 1H, H4), 7.41-7.34 (m, 2H, H6, H7''), 7.30 (d, 2H, ^3J = 8.2 Hz, H2', H6'), 7.10-7.00 (m, 4H, H3', H5', H5'', H6''), 5.47 (s, 2H, NCH_2), 2.79 (q, 2H, ^3J = 7.5 Hz, CH_2CH_3), 2.38 (s, 3H, CH_3), 1.23 (t, 3H, ^3J = 7.5 Hz, CH_2CH_3). ^{13}C-NMR (101 MHz, acetone-d_6): δ 169.6, 142.6, 141.2, 138.8, 137.6, 132.8, 132.6, 131.9, 131.6, 130.5, 129.6, 129.5, 128.8, 128.0, 126.8, 121.4, 119.5, 118.7, 114.4, 110.0, 46.6, 18.2, 16.1, 10.2. HRMS: *m/z* calculated for $C_{25}H_{23}NO_2$ [*M*+Na]$^+$: 392.1621, found: 392.1626.

4'-((3-Bromo-9*H*-carbazol-9-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid (6a): Method B: from **5a** (0.34 g, 0.7 mmol) in MeOH (34 ml) with 3 N NaOH (0.6 ml). Off-white solid, yield 25%. ^{1}H-NMR (400 MHz, acetone-d_6): δ 8.37 (d, 1H, ^3J = 1.9 Hz, H4''), 8.24 (d, 1H, ^3J = 7.8 Hz, H5''), 7.81 (dd, 1H, ^3J = 7.7 Hz, ^4J = 1.5 Hz, H3), 7.71-7.37 (m, 6H, H4, H5, H1'', H2'', H7'', H8''), 7.36-7.20 (m, 6H, H6, H2', H3', H5', H6', H6), 5.73 (s, 2H, NCH_2). ^{13}C-NMR (101 MHz, acetone-d_6): δ 169.6, 142.5, 142.0, 141.6, 140.3, 137.3, 132.6, 131.9, 131.6, 130.5, 129.7, 129.2, 128.1, 127.6, 127.3, 125.7, 123.9, 122.8, 121.6, 120.6, 112.4, 112.2, 110.6, 46.7, 18.9. HRMS: *m/z* calculated for $C_{26}H_{18}BrNO_2$ [*M*+Na]$^+$: 478.0413, found: 478.0469.

4'-((5-Bromo-2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid (6b): Method C: from **5b** (0.08 g, 0.2 mmol) with KOH (0.05 g, 0.9 mmol) in EG (1 ml). Colorless solid, yield: 64%. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.73 (br s, 1H, COOH), 7.79 (d, 1H, ^4J = 1.9 Hz, H4''), 7.70 (dd, 1H, ^3J = 7.8 Hz, ^4J = 1.4 Hz, H3), 7.58-7.47 (m, 2H, H5, H7''), 7.47-7.39 (m, 1H, H4), 7.36-7.25 (m, 4H, H6, H2', H6', H6''), 7.11 (d, 2H, ^3J = 8.2 Hz, H3', H5'), 5.55 (s, 2H, NCH_2), 2.84 (t, 2H, ^3J = 7.5 Hz, $CH_2CH_2CH_3$), 1.84-1.70 (m, 2H, $CH_2CH_2CH_3$), 0.95 (t, 3H, ^3J = 7.4 Hz, $CH_2CH_2CH_3$). ^{13}C-NMR (101 MHz, DMSO-d_6): δ 169.49, 156.71, 143.86, 140.40, 140.15, 135.67, 134.51, 132.26, 130.84, 130.42, 129.11, 128.73, 127.33, 126.25, 124.37, 120.95, 113.69, 112.09, 45.88, 28.53, 20.20, 13.75. HRMS: *m/z* calculated for $C_{24}H_{21}BrN_2O_2$ [*M*+H]$^+$: 449.0859, found: 449.0900.

4'-((5-Bromo-3-ethyl-2-methyl-1*H*-indol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid (6c): Method D: from **5c** (0.07 g, 0.2 mmol) in EtOH (0.3 ml) and THF (0.3 ml) with 2 N KOH (0.7 ml). Off-white solid, yield 77%. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.71 (br s, 1H, COOH), 7.72-7.62 (m, 2H, H3, H4''), 7.57-7.49 (m, 1H, H5), 7.48-7.36 (m, 2H, H4, H7''), 7.32 (dd, 1H, ^3J = 7.7 Hz, ^4J = 1.3 Hz, H6), 7.25 (d, 2H, ^3J = 8.1 Hz, H2', H6'), 7.15 (dd, 1H, ^3J = 8.6 Hz, ^4J = 2.0 Hz, H6''), 6.98 (d, 2H, ^3J = 8.0 Hz, H3, H5), 5.43 (s, 2H, NCH_2), 2.69 (q, 2H, ^3J = 7.5 Hz, CH_2CH_3), 2.32 (s, 3H, CH_3), 1.15 (t, 3H, ^3J = 7.5 Hz, CH_2CH_3). ^{13}C-NMR (101 MHz, DMSO-d_6): δ 169.56, 140.45, 139.67, 137.17, 134.82, 134.06, 132.31, 130.78, 130.39, 129.01, 128.60, 127.23, 125.89, 122.65, 119.90, 112.84, 111.46, 111.28, 45.62, 16.91, 15.65, 9.92. HRMS: *m/z* calculated for $C_{25}H_{22}BrNO_2$ [*M*-H]$^-$: 446.0750, found: 446.0772.

Biology

General cell culture methods

The monkey kidney-derived cell line COS-7 (ATCC) was cultured as a monolayer culture in Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/l glucose and 584 mg/l L-glutamine (GE Healthcare), without sodium pyruvate or phenol red, supplemented with fetal calf serum (FCS 10%, Sigma-Aldrich). The chronic myelogenous leukemia cell lines K562 (ATCC) and K562-resistant were cultivated in Roswell Park Memorial Institute (RPMI) 1640 medium (Lonza) supplemented with 10% FCS, 100 U/ml penicillin (Lonza), 100 µg/ml streptomycin (Lonza), and 2 mM L-glutamine. The K562-resistant cell line was received from Ernesto Yague and was originally described as subclone of K562 cells that shows a doxorubicin resistance (termed KD225 by Hui et al.[31]).

All cell lines were incubated in a humidified atmosphere (5% CO_2/95% air) at 37 °C and passaged twice a week. The final concentration of DMSO in cell-based assays never exceeded 0.1% and vehicle-treated controls were always included.

PPARγ transactivation assay

The PPARγ transactivation assay was performed according to our previous studies.[12-13] Transient transfection (TransIT-LT1, MoBiTec) and the dual-luciferase reporter assay (Promega) were applied according to the manufacturer's protocol. After seeding of COS-7 cells in 96 well plates (10^4 cells per well) in triplicates, they were incubated at 37 °C under a humidified atmosphere (5% CO_2/95% air) for 24 h. TransIT-LT1 served as reagent for transient transfection with the plasmids pGal5-TK-pGL3 (90 ng), pGal4-hPPARγDEF (9 ng), and pRenilla-CMV (3 ng) in phosphate-buffered saline (PBS) prior to further incubation for 7 h. Then, the respective compounds, telmisartan, pioglitazone, or vehicle (DMSO) were added at indicated concentrations. The samples were incubated for 39 h. After washing with PBS, lysis was induced by freezing (–80 °C) of the cells. The appropriate buffers were added to complete lysis and to determine luciferase activity with the EnSpire multimode plate reader (PerkinElmer). Thereby, renilla luciferase activity served as internal control and for normalization.[33] The results of the compounds **2a-c**, **3a-c**, **5a-c**, and **6a-c** as well as the references pioglitazone and telmisartan are represented by the mean ± SD of ≥ 3 independent experiments with three replicates each.

Determination of cell death by flow cytometry

In accordance with our former work, cell death was measured by PI FACS analyses.[12-13, 34] Herein, K562-resistant cells were seeded in 24-well plates (2×10^5 cells per well) and the compounds were added in the selected concentrations. After incubation for 72 h at 37 °C under a humidified atmosphere, the cells were harvested and stained with PI/Triton-X100 for 2 h at 4 °C. Subsequently the cells were subjected to forward/sideward scatter analyses using a CytomicsFC-500 Beckman Coulter. Dead cells were detected as stained nuclei in the sub-G1 marker window. The results of ctr. (DMSO), **2a-c**, **3a-c**, **5a-c**, and **6a-c** are represented by the mean + SEM of ≥ 3 independent experiments. All compounds were proven to be soluble in the applied concentrations by microscopy.

Determination of metabolic activity

COS-7 cells were seeded in 96-well plates (2×10^3 cells per well) in triplicates before incubating under a humidified atmosphere for 24 h. Each compound was added at the respective concentration and it was incubated for 72 h. The metabolic activity was determined with a modified MTT assay (EZ4U kit, Biomedica) according to the manufacturer's instructions. The metabolic activity in the absence of the compounds (ctr., DMSO) was set to 100%. The results of pioglitazone, telmisartan, and the compounds **2a-c**, **3a-c**, **5a-c**, and **6a-c** are represented by the mean + SD of ≥ 3 independent experiments with three replicates each.[35]

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Conflict of interest

The authors declare no conflict of interest.

Keywords: chronic myeloid leukemia, imatinib resistance, peroxisome proliferator-activated receptor gamma, sensitizers, structure-activity relationship

Supporting Information: The Supporting Information is available free of charge at the Wiley Online Library https://onlinelibrary.wiley.com/journal/18607187. Syntheses of intermediates. Analytical data including NMR spectroscopy, HRMS and HPLC of all compounds.

Abbreviations: CML, chronic myeloid leukemia; TKI, tyrosine kinase inhibitor; PPARγ, peroxisome proliferator-activated receptor gamma; SPPARγM, selective peroxisome proliferator-activated receptor gamma modulator; SCs, stem cells; CMR, complete molecular response; PRMT5, protein arginine methyltransferase 5; SAR, structure-activity relationship; PI, propidium iodide; MTT, 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; THF, tetrahydrofuran; MeOH, methanol; EG, ethylene glycol; NOESY, nuclear overhauser enhancement spectroscopy; EtOH, ethanol; eq, equivalents; HRMS, high-resolution mass spectrometry; HPLC, high-performance liquid chromatography; A_{max}, intrinsic activation; SD, standard deviation; SEM, standard error of the mean; ctr., control; pio., pioglitazone; telmi., telmisartan; EA, ethyl acetate; PE, petroleum ether; DMEM, Dulbecco's Modified Eagle Medium; FCS, fetal calf serum; RPMI, Roswell Park Memorial Institute; PBS, phosphate-buffered saline

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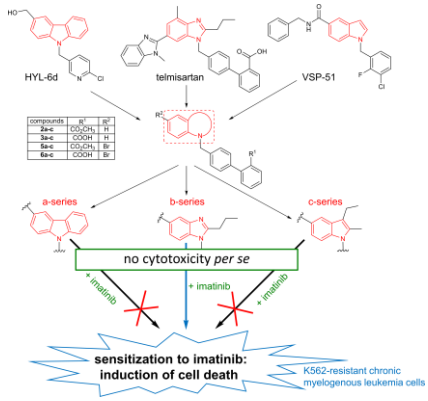

Heterocyclic compounds with carbazole, benzimidazole, and indole core were synthesized as sensitizers to imatinib treatment in resistant chronic myelogenous leukemia cells. Regarding their potency as cell death modulators, the benzimidazole derivatives were clearly favored over the respective carbazoles or indoles. These results are of importance for further development of effective sensitizers to circumvent tyrosine kinase inhibitor resistance.