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Research paper

Identification and development of non-cytotoxic cell death modulators: Impact of sartans and derivatives on PPARγ activation and on growth of imatinib-resistant chronic myelogenous leukemia cells

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ABSTRACT

4'-((2-Propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1′-biphenyl]-2-carboxylic acid derived from telmisartan was identified as lead for the design of cell death modulators. In this study, we evaluated the efficacy of telmisartan itself and other sartans in combination with imatinib against K562-resistant cells. The findings were directly used to further optimize the lead structure. Telmisartan and candesartan cilexetil represented the most effective sartans, thus the influence of carboxyl/methyl carboxylate groups at positions 7 (compounds **6**, **7**) or 4 (compounds **12**–**14**) at the benzimidazole core was studied. Additionally, according to the results of a former structure-activity study, telmisartan was transformed to the related amide (**1**). Telmisartan amide **1**, as well as the esters **6** and **12** markedly sensitized the resistant CML cells to imatinib treatment. Correlation with their potency to activate PPARγ is not given. Candesartan cilexetil, telmisartan and **1** showed the profile of partial agonists at PPARγ with EC_{50} values of 4.2, 4.3 and 9.1 μM, respectively, while **6** and **12** caused only marginal intrinsic activation at 10 μM (A_{max} = 22% and 13%). However, the repression of the STAT5 phosphorylation relates with the possibility to sensitize K562-resistant CML cells to imatinib treatment. It is worth mentioning that all compounds were per se non-cytotoxic at relevant concentrations.

1. Introduction

The treatment of cancer was revolutionized since tyrosine kinase inhibitors (TKIs) entered the market [1]. Chronic myeloid leukemia (CML), a hematopoietic malignancy, was the first neoplastic disease with rationally designed therapy. In 95% of all patients the BCR-ABL1 fusion protein is overexpressed, which is associated with increased tyrosine kinase activity. Imatinib, the most intensely studied TKI, acts as a competitive inhibitor at the ATP binding site of the BCR-ABL1 kinase. This leads to death of target cells along with mild adverse effects [1,2].

A major limitation of the therapy, however, is the failure of imatinib and other TKIs to completely eradicate leukemic cells [3,4]. Quiescent hematopoietic stem cells are highly insensitive to imatinib and remain viable in CML patients [5]. Besides primary resistance to imatinib, acquired resistance can be observed after prolonged treatment due to loss of response [2]. Per definition sustained deep molecular response (MR) is characterized by nearly undetectable BCR-ABL1 transcripts (PCR analyses). Only some 10% of patients, who were treated with imatinib and reached deep MR, are expected to remain in stable treatment-free remission, while the majority experiences a relapse [6]. Hence, the cure of CML cannot be achieved and research focuses on the overcome of imatinib resistance as well as the induction of sustained deep MR.

The angiotensin II type 1 receptor blocker (ARB) and partial peroxisome proliferator-activated receptor gamma (PPARγ) agonist telmisartan was already studied as anti-cancer agent. It revealed promising results towards TKI-resistant non-small cell lung cancer

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Abbreviations

TKI	tyrosine kinase inhibitor
CML	chronic myeloid leukemia
MR	molecular response
ARB	angiotensin II type 1 receptor blocker
PPARγ	peroxisome proliferator-activated receptor gamma
STAT5	signal transducer and activator of transcription 5
pSTAT5	phosphorylated signal transducer and activator of transcription 5
PI	propidium iodide
SAR	structure-activity relationship
MeOH	methanol
EG	ethylene glycol
PyBOP	benzotriazol-1-yl-oxytripyrrolidino-phosphonium hexafluorophosphate
HOBt	hydroxybenzotriazole
DIPEA	*N*,*N*-diisopropylethylamine
EA	ethyl acetate
EtOH	ethanol
PE	petroleum ether
THF	tetrahydrofuran
HRMS	high-resolution mass spectrometry
A_{max}	intrinsic activation
SD	standard deviation
SEM	standard error of the mean
ctr	control
pio.	pioglitazone
telmi.	telmisartan
cande. cil.	candesartan cilexetil
cande.	candesartan
irbe.	irbesartan
lo.	losartan
olme. med.	olmesartan medoxomil
olme.	olmesartan
DMEM	Dulbecco's Modified Eagle Medium
FCS	fetal calf serum
RPMI	Roswell Park Memorial Institute
PBS	phosphate-buffered saline

[7], adult T-cell leukemia/lymphoma [8], and cholangiocarcinoma [9]. Prost et al. [10] assessed the activation of PPARγ and the subsequent inhibition of STAT5 as main principle to overcome resistance. Therefore, we evaluated 4-substituted 4'-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1′-biphenyl]-2-carboxylic acids, initially designed as telmisartan-derived partial PPARγ agonists [11], against imatinib-resistant cells. After transformation to their 2-carboxamide derivatives, they represented strong sensitizers to imatinib therapy in the resistant K562 CML cell line. The respective carboxylic acids were ineffective [12]. These findings draw our attention to other ARBs as possible cell death modulators.

Oura et al. [13] already evaluated the sartans telmisartan, irbesartan, losartan, and valsartan regarding their inhibitory and antiproliferative impact on different cancer cell lines, such as hepatocellular carcinoma cells. As a result, growth inhibition, induction of cell cycle arrest, and apoptosis were observed. Additionally, Iñigo et al. [14] showed that losartan inhibits cell growth and induces apoptosis in K562 CML cells. These studies, however, did not provide an adequate treatment option for cancer therapy as the concentrations to achieve an efficacy were relatively high. Besides, the necessity to successfully circumvent the occurring drug resistance was not considered yet. Therefore, we evaluated selected sartans for PPARγ activation and eradication of imatinib-resistant cells. The sartans should cover a wide range of structural features. Telmisartan and candesartan (Fig. 1) comprise an *N*-([1,1′-biphenyl]-4-ylmethyl)-1*H*-benzo[*d*]imidazole core, while irbesartan, losartan, and olmesartan consist of an *N*-([1,1′-biphenyl]-4-ylmethyl)-1*H*-imidazole substructure (Fig. 1).

Compared to telmisartan, the 2-COOH group at the biphenyl moiety is bioisosterically replaced by a tetrazole ring in all other drugs. Furthermore, the heterocyclic cores of olmesartan and candesartan are substituted with a carboxylic group, respectively, which is either attached directly to the imidazole (olmesartan) or to the benzimidazole (candesartan). Both drugs are clinically applied as ester-prodrugs, which were also included in the present study. The ability of this broad spectrum of compounds to activate PPARγ in a transactivation assay and to induce cell death in K562-imatinib-resistant cells by PI (propidium iodide) FACS analyses was assessed according to previous findings [10,12].

The results of candesartan and its prodrug candesartan cilexetil revealed the high relevance of modified carboxyl groups for increased cell death modulating effects (see results). Therefore, telmisartan has been transformed to an amide (**1**) following our previous studies. Additionally, a carboxyl group was introduced at position 4 or 7 (analogously to candesartan) at the benzimidazole core of the 4'-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1′-biphenyl]-2-carboxylic acid, which represents our lead structure (Fig. 1). The compounds were investigated in relation to their respective methyl ester. STAT5 expression was determined to get an insight into the mode of action. Cytotoxicity was investigated in the COS-7 cell line by a modified MTT assay and further in the HS-5 cell line by PI FACS analyses.

2. Results and discussion

2.1. Synthesis

The synthesis of **1** (see Scheme 1) was performed analogously to Shan et al. [15], reacting telmisartan with the phosphonium salt based coupling reagent PyBOP, the base DIPEA and NH_4Cl.

The syntheses of compounds **6**, **7** and **12**–**14**, depicted in Schemes 2 and 3, are based on a previously described procedure [12].

2-Amino-3-nitrobenzoic acid (**2**) was esterified to **3** with H_2SO_4 in anhydrous MeOH [16]. The formation of the respective butyric anilide (**4**) was realized by heating **3** with butyric anhydride and concentrated HCl. *N*-Alkylation with methyl 4'-(bromomethyl)-[1,1′-biphenyl]-2-carboxylate in the presence of NaH gave compound **5**. In an one-pot reaction the reduction of the nitro group as well as ring closure were achieved by applying the reducing agent $SnCl_2$ and subsequent heating in EtOH. Ester cleavage of **6** with 2 N KOH in THF/EtOH finally yielded **7** [17]. This reaction pathway exclusively resulted in 7-substituted benzimidazole derivatives (Scheme 2).

The 4-substituted compounds were synthesized as depicted in Scheme 3. Compound **2** was directly reduced with $SnCl_2$ to the 1,2-phenylenediamine derivative **8**. Then, **8** was transformed to the bis-butyric anilide **9** with butyric anhydride. Heating with 4 N HCl yielded the respective benzimidazole (**10**), which was esterified with H_2SO_4 in anhydrous MeOH. *N*-Alkylation of **11** with methyl 4'-(bromomethyl)-[1,1′-biphenyl]-2-carboxylate resulted in an isomeric mixture of 4- and 7-substituted benzimidazole

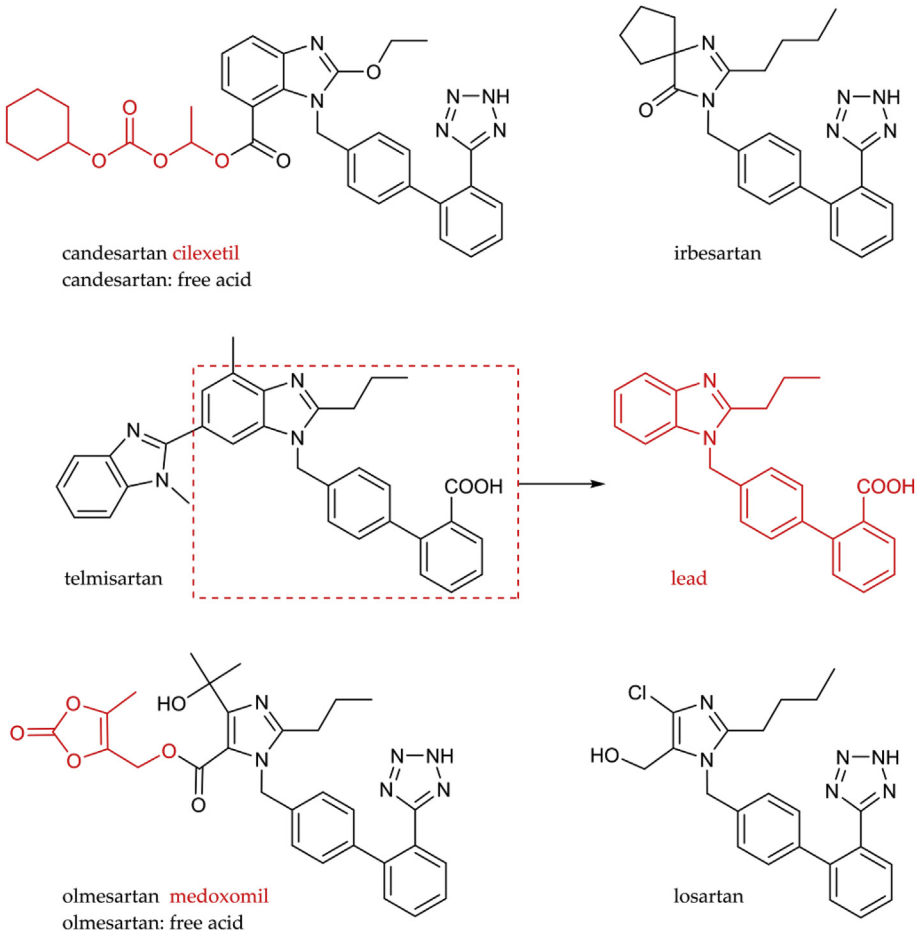


Fig. 1. Structures of the ARBs candesartan cilexetil (prodrug), candesartan, irbesartan, olmesartan medoxomil (prodrug), olmesartan, losartan, telmisartan and the derived lead.

derivatives, from which **12** was separated by column chromatography. The 7-substituted isomer was not obtained in sufficient amount and purity within this synthesis pathway, since the 4-substituted isomer was preferably formed (ratio of about 4:1). The subsequent hydrolysis of **12** with 2 N KOH in THF/EtOH yielded the mono-methyl ester derivative **13**. Only upon the use of stronger alkaline conditions and increased temperature, the bis-carboxylic acid **14** was formed.

Compounds **6**, **7** and **12**–**14** were characterized applying ^{1}H and ^{13}C NMR spectroscopy as well as high-resolution mass spectrometry (HRMS). HPLC was performed to assess purity. The synthesis protocols and the analytic data of intermediates as well as target compounds are submitted as supplementary data.

2.2. Biological activity

Referring to previous findings [10,18–20], the ability of the compounds to activate PPARγ and their potency to circumvent imatinib resistance was studied.

2.2.1. Activation of PPARγ in a transactivation assay

Telmisartan, candesartan cilexetil, candesartan, irbesartan, losartan, olmesartan medoxomil, and olmesartan as well as the synthesized compounds **1**, **6**, **7**, and **12**–**14** were evaluated in a dual-luciferase reporter assay to assess their potency (EC_{50} values) and intrinsic activity (A_{max}) towards PPARγ. The results (A_{max}) listed in Table 1 refer to a concentration of 10 μM, according to the highest one used in the cytotoxicity assay with K562-resistant cells.

COS-7 cells were transiently transfected with the plasmids pGal5-TK-pGL3, pGal4-hPPARγDEF, pRenilla-CMV, and subsequently stimulated with the sartans or the synthesized compounds, respectively. Telmisartan and the full PPARγ agonist pioglitazone served as references. The maximum activation of pioglitazone was set to 100% and its half-maximal effective concentration (EC_{50} = 1.6 μM) was calculated from the concentration-activation curve (Fig. 2, concentrations 0.05–20 μM). The EC_{50} = 4.3 μM and A_{max} = 60.5% of telmisartan are in agreement with our previous findings [12].

Candesartan cilexetil showed the same profile as telmisartan (Fig. 2, A). It achieved an A_{max} of 59.8% at 10 μM and an EC_{50} value of 4.2 μM (Table 1). At the highest concentration used, the maximum effect was attained in both cases, which is lower than that of pioglitazone. Interestingly, candesartan itself did not cause receptor activation (Fig. 2, A) [21].

Irbesartan activated PPARγ to only 8.1% at 10 μM. Its potency cannot be calculated, as the concentration-activation curve did not reach the maximum (Fig. 2, B). Losartan, olmesartan medoxomil, and olmesartan (Fig. 2, C) were even completely inactive.

The structure-activity relationship (SAR) clearly indicated that a high PPARγ binding requires a benzimidazole core as realized in telmisartan. Regarding the benzimidazole derivative candesartan, only the ester-prodrug candesartan cilexetil yielded good receptor binding, while hydrolysis of the ester to candesartan strongly decreased the affinity. To further evaluate this effect, a COOH group was introduced in position 7 (**6**, **7**) or 4 (**12**–**14**) at the benzimidazole of the lead and the results were compared with that of the

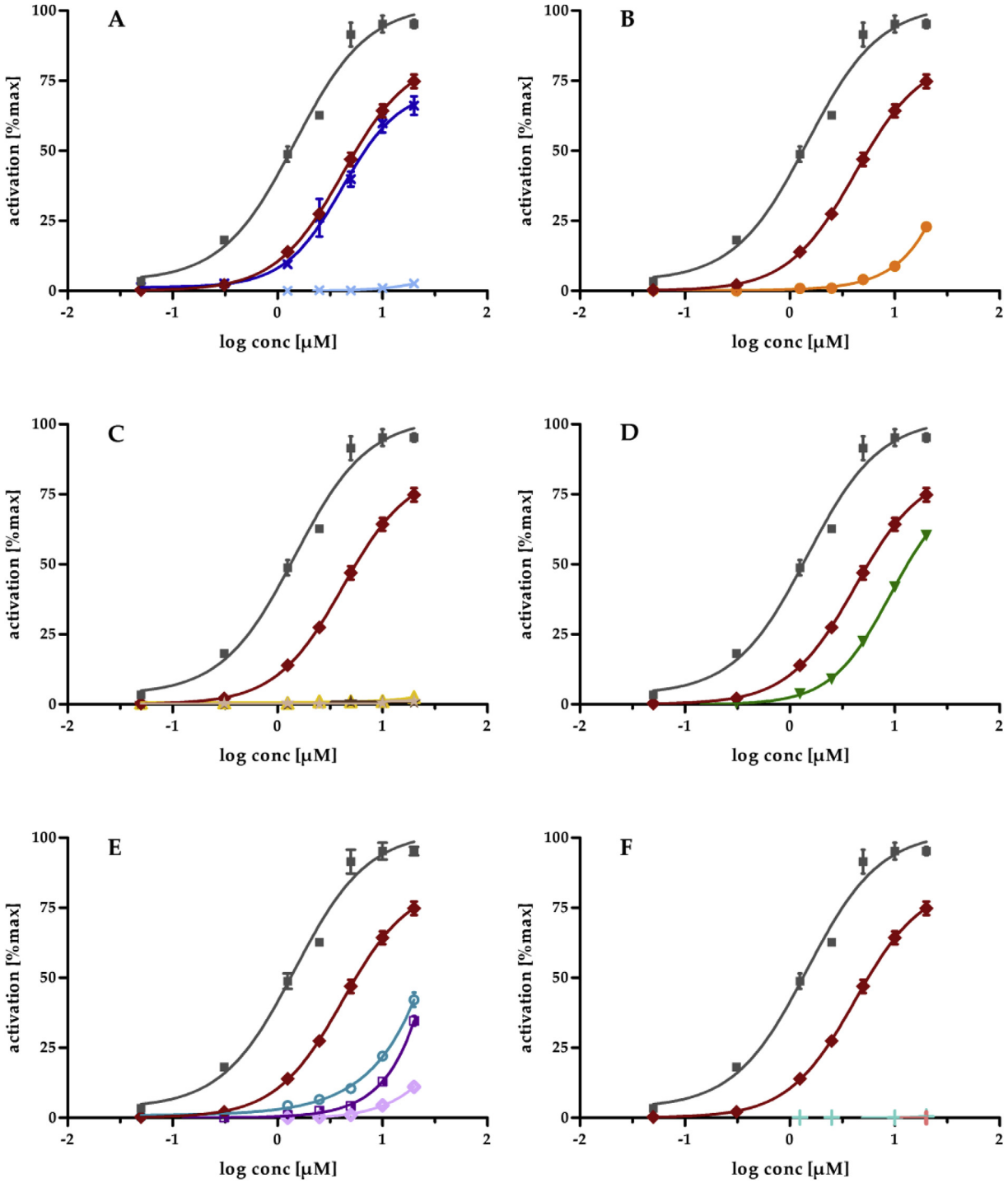
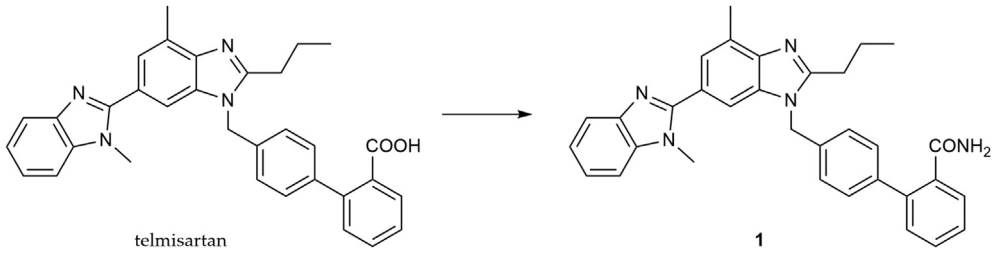
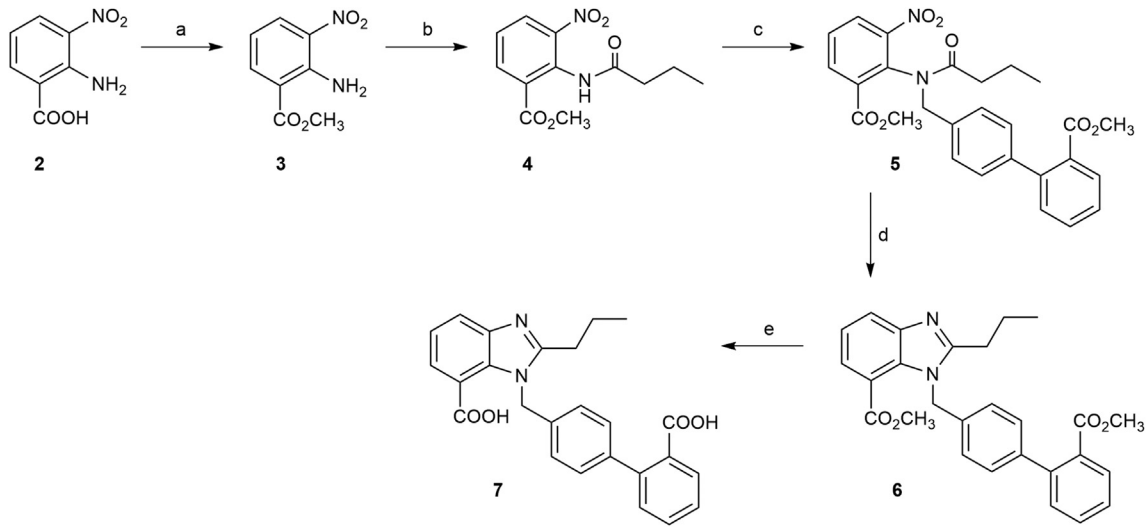


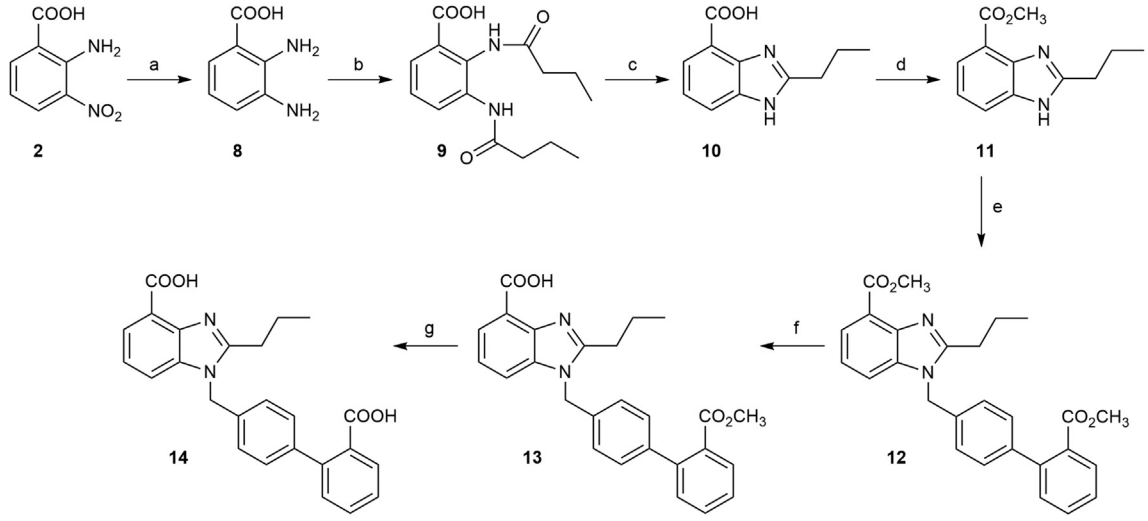
Fig. 2. Concentration-dependent activation of PPARγ. COS-7 cells were transiently transfected with the plasmids pGal5-TK-pGL3, pGal4-hPPARγDEF, pRenilla-CMV, and subsequently stimulated with the two references pioglitazone (■) and telmisartan (◆), as well as the ARBs, candesartan cilexetil (✕), candesartan (✕), irbesartan (●), losartan (△), olmesartan medoxomil (★), olmesartan (★), and the compounds **1** (▼), **6** (○), **12** (◨), **13** (◇), **7** (+), **14** (▮), respectively. The data represent the mean ± SD of ≥3 independent experiments with threefold determination.



Scheme 1. Derivatization of telmisartan to the amide **1**. Reagents and conditions: PyBOP, NH_4Cl, DIPEA, anhydrous DMF, 0 °C to rt.



Scheme 2. Synthesis of compounds **6** and **7**. Reagents and conditions: (a) H_2SO_4, anhydrous MeOH, 65 °C; (b) butyric anhydride, concentrated HCl, 120 °C; (c) methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate, NaH, anhydrous DMF, rt; (d) $SnCl_2 \times 2\ H_2O$, EtOH, 80 °C; (e) 2 N KOH, THF, EtOH, 80 °C.



Scheme 3. Synthesis of compounds **12**, **13**, and **14**. Reagents and conditions: (a) $SnCl_2 \times 2\ H_2O$, HCl, EtOH, rt; (b) butyric anhydride, concentrated HCl, 120 °C; (c) 4 N HCl, 100 °C; (d) H_2SO_4, anhydrous MeOH, 65 °C; (e) methyl 4'-(bromomethyl)-[1,1'-biphenyl]-2-carboxylate, NaH, anhydrous DMF, rt; (f) 2 N KOH, THF, EtOH, 80 °C; (g) 6 N KOH, EG, 160 °C.

respective methyl esters.

In relation to the lead, a strong decrease of intrinsic activity was observed for **6**, **7** and **12**–**14** (Fig. 2, E-F). The 7-CO_2CH_3 group at the benzimidazole together with the 2-CO_2CH_3 group reduced A_{max} at 10 μM from 58.5% (lead) to 22% (**6**) and even to 0% after hydrolysis of the esters (**7**). Compound **14**, which bears a 4-COOH at the benzimidazole, was inactive too. Esterification of the 2-COOH as well marginally increased the efficiency (2-CO_2CH_3 (**13**) 4.5% < 2/4-CO_2CH_3 (**12**) 13%).

Derivatization of telmisartan to the amide **1** also lowers the receptor activation and potency (Fig. 2, D). From the curve in Fig. 2, D an A_{max} of 41.9% and an EC_{50} value of 9.1 μM were calculated.

Table 1
Intrinsic activity (A_{max}) and potency (EC_{50}) determined in a transactivation assay with PPARγ.

compound	A_{max} at 10 μM [%][a,c]	EC_{50} [μM][b,c]
candesartan cilexetil	59.8 ± 6.5	4.2 ± 0.7
candesartan	0.9 ± 0.1	n.d.
irbesartan	8.1 ± 0.5	n.d.
losartan	1.6 ± 0.3	n.d.
olmesartan medoxomil	0.7 ± 0.1	n.d.
olmesartan	0.5 ± 0.1	n.d.
1	41.9 ± 2.3	9.1 ± 0.8
6	22.0 ± 2.2	n.d.
7	0	n.d.
12	13.0 ± 0.3	n.d.
13	4.5 ± 1.2	n.d.
14	0	n.d.
pioglitazone	100	1.6 ± 0.3
telmisartan	60.5 ± 4.1	4.3 ± 0.5

[a] A_{max} indicates the response of the compounds related to the maximum activation of pioglitazone (100%, 10 μM).
[b] EC_{50} values were calculated by means of the concentration-activation curves (Fig. 2).
[c] Data represent the mean ± SD of ≥3 independent experiments (n.d., not determinable).

2.2.2. Modified MTT assay

Pioglitazone, the selected sartans, and all synthesized target compounds were evaluated in a modified MTT assay regarding their cytotoxic potential in COS-7 cells. The assay is based on the formation of purple colored formazan derivatives indicating cell viability. This process is driven by NAD(P)H-dependent cellular dehydrogenases. Their enzyme activity correlates with functional mitochondria, which reflects the number of viable cells [22]. The metabolic activity of actively growing cells markedly decreases upon exposure to cytotoxic molecules.

Neither pioglitazone and the sartans, nor the compounds **1**, **6**, **7**, **12**–**14** affected the metabolic activity of COS-7 cells at a concentration of 10 μM (used concentration in the cell death assays). At a concentration of 20 μM, which was applied within the transactivation assay, only **12** caused a slightly stronger decrease in metabolic activity of about 20% (Fig. 3). Nevertheless, all compounds can be stated as non-cytotoxic.

2.2.3. Induction of cell death

Prost et al. [10] described to overcome imatinib resistance by the combination therapy of imatinib with the full PPARγ agonist pioglitazone. Following this approach, we investigated the cell death modulating effects of the partial PPARγ agonists telmisartan, telmisartan amide **1**, and candesartan cilexetil as well as the less/non-agonistic ARBs candesartan, irbesartan, losartan, olmesartan medoxomil, olmesartan and the carboxyl/methyl carboxylate bearing 4'-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1'-biphenyl]-2-carboxylic acid derivatives **6**, **7**, **12**–**14** in K562-resistant CML cells.

To prove imatinib resistance, the K562 and K562-resistant cell lines were comparatively examined upon treatment with increasing concentrations of imatinib [12]. In the K562 cell line 1 μM of imatinib induced 46% cell death, whereas in the K562-resistant cells only 17% dead cells were detected by PI FACS analyses. Thus, imatinib resistance in the latter cell line was confirmed (for detailed information see SI, Fig. S19).

All tested compounds alone (10 μM) revealed effects comparable to the vehicle (DMSO) treated control (Fig. 4, A: ctr.; cell death rate: 7%) or the imatinib control (1 μM; Fig. 4, B: ctr.; cell death rate: 17%) in K562-resistant cells. The same is true, if imatinib, candesartan, olmesartan medoxomil, olmesartan, losartan, **7**, or **14** were combined with imatinib. The compounds could not sensitize the cells to imatinib treatment. In contrast, imatinib administered together with irbesartan and candesartan cilexetil caused 31% and 42% cell death, together with telmisartan or its amide **1** even 63% and 89%, respectively (Fig. 4, B). Also **6** and **12** were highly active sensitizers. This effect is nearly independent on the position of the carboxylic ester at the benzimidazole core (cell death rate: 83% each). However, ester cleavage reduced the effects. Compounds **7** and **14** were completely inactive (cell death rate: 12%). The mono ester **13** was of medium activity (cell death rate: 56%).

The potency of telmisartan, **1**, **6**, **12**, and **13** as cell death modulators for imatinib was further concentration-dependently proven in K562-resistant CML cells. The cells were incubated for 72 h with 1 μM of imatinib in combination with increasing concentrations (1–10 μM) of the selected compounds (Fig. 5).

1, **6**, and **12** showed nearly equal concentration-activity curves. At 2.5 μM they mediated a cell death rate of about 50–60% for imatinib. Compound **13** showed the same activity profile at reduced concentrations. Only at 5 and 10 μM, the effects were somewhat lower (54% and 56% dead cells, respectively). In contrast, telmisartan was less active up to a concentration of 2.5 μM and reached

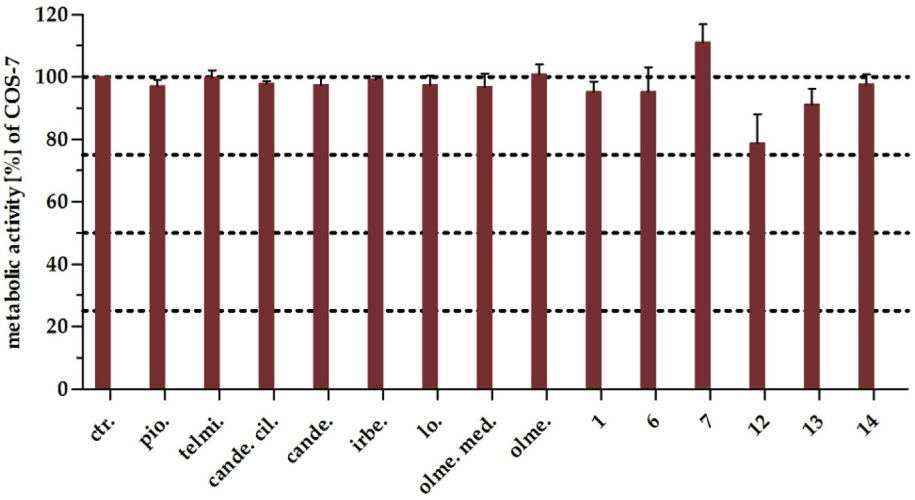


Fig. 3. Metabolic activity of COS-7 cells treated with 20 μM of either vehicle (ctr., DMSO), pioglitazone (pio.), telmisartan (telmi.), candesartan cilexetil (cande. cil.), candesartan (cande.), irbesartan (irbe.), losartan (lo.), olmesartan medoxomil (olme. med.), olmesartan (olme.), **1**, **6**, **7**, or **12**–**14**. The mean values +SD of ≥3 independent experiments are shown.

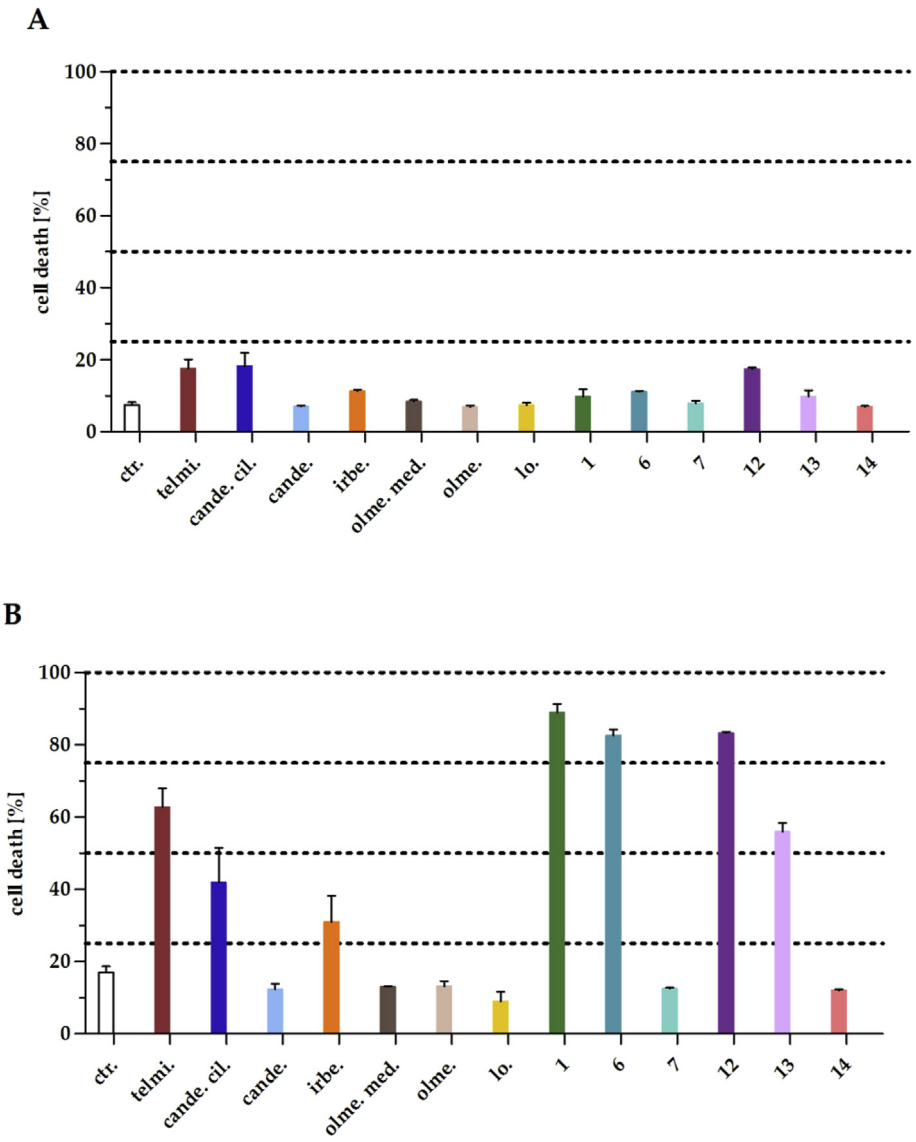


Fig. 4. Induction of cell death in K562-resistant cells, treated with telmisartan (telmi.), candesartan cilexetil (cande. cil.), candesartan (cande.), irbesartan (irbe.), losartan (lo.), olmesartan medoxomil (olme. med.), olmesartan (olme.), and the compounds **1**, **6**, **7**, **12**–**14** either without (A) or in combination with 1 μM of imatinib (B). Ctr. represents the vehicle treated control without (A) or with imatinib (1 μM, B). Dead cells were detected by PI FACS analyses. Data represent the mean +SEM of ≥4 independent experiments.

the efficiency of **13** at 5 and 10 μM.

2.2.4. Cytotoxicity assay

2.2.4.1. PI FACS analyses. The human non-malignant cell line HS-5 was used to exclude a general cytotoxicity of all compounds. PI FACS analyses were applied to detect cell death. None of the compounds showed cytotoxic properties up to a concentration of 10 μM. Due to control-like effects, they can be stated as non-cytotoxic (Fig. 6).

2.2.5. Immunoblot analyses

As a possible mode of action repression of the STAT5 activity is discussed. Inhibition of STAT5 was reported to be essential for TKI-induced cell death in resistant CML cells [23]. It is strongly involved in the development of imatinib resistance and the maintenance of insensitive stem cells [10,24]. Therefore, telmisartan as well as all synthesized compounds were investigated to inhibit the expression or the phosphorylation status of STAT5 at tyrosine 694/699, which is a hallmark for activated STAT5, by immunoblot analyses.

None of the compounds repressed the STAT5 expression in K562-resistant cells treated with imatinib (Fig. 7; see also Fig. S20). However, imatinib combined with the compounds **1**, **6**, or **12** and to a small extent also telmisartan or **13** repressed the phosphorylation status of STAT5 (Fig. 7).

In case of **7** and **14**, neither an influence on STAT5 nor on pSTAT5 regulation was observed. These results are in good agreement with those of the PI FACS analyses. It is very likely that regulation of the STAT5 expression is part of the mode of action regarding the investigated cell death modulators. The inhibition of STAT5, which is a guardian protein of quiescence and stemness of leukemia cells, by the investigated compounds might lead to eradication of the stem cell pool. However, participation of other targets or pathways cannot be excluded yet.

2.3. Discussion

Prost et al. [10] stated to overcome imatinib resistance by co-administration with the full PPARγ agonist pioglitazone.

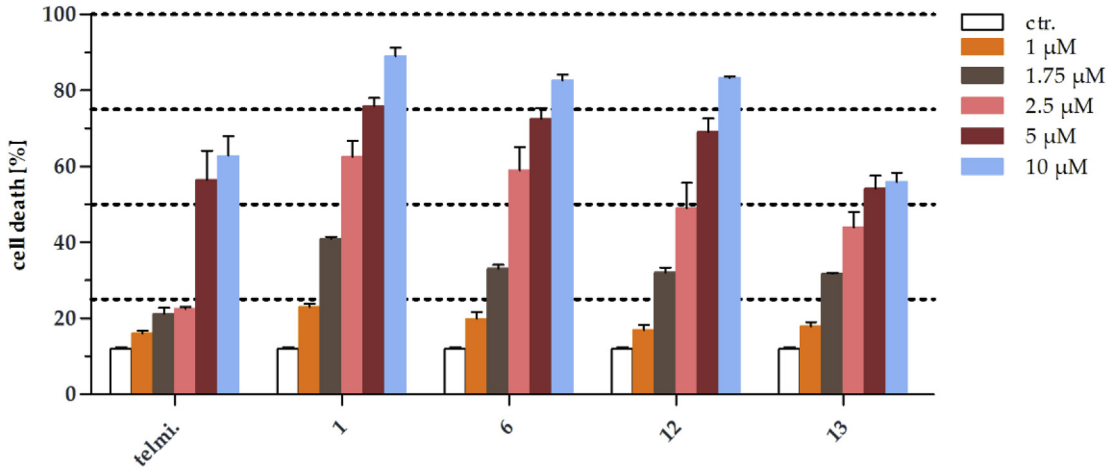


Fig. 5. PI FACS analyses of K562-resistant cells treated with 1 μM of imatinib in combination with increasing concentrations (1–10 μM) of telmisartan (telmi.), **1**, **6**, **12**, or **13**, respectively. The mean values +SEM of ≥4 independent experiments are shown.

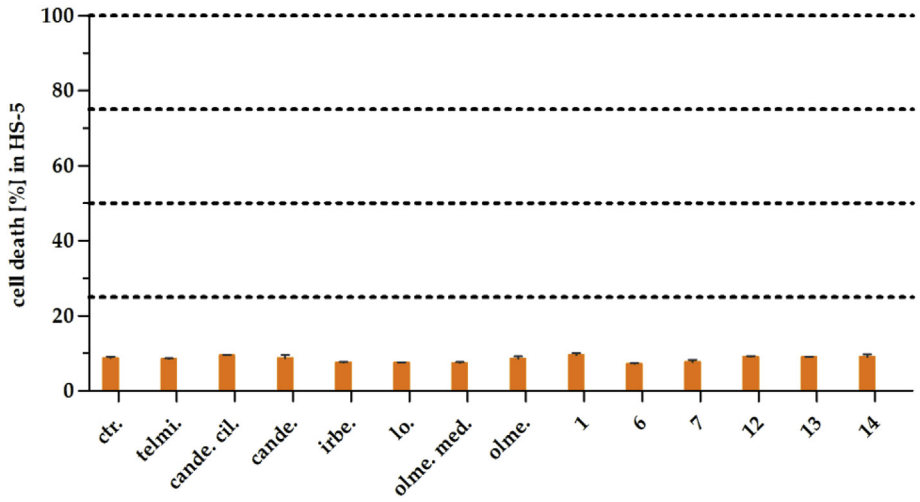


Fig. 6. Induction of cell death in HS-5 cells, treated with DMSO (ctr.), telmisartan (telmi.), candesartan cilexetil (cande. cil.), candesartan (cande.), irbesartan (irbe.), losartan (lo.), olmesartan medoxomil (olme. med.), olmesartan (olme.), **1**, **6**, **7**, and **12**–**14**, respectively. The mean values +SEM of ≥3 independent experiments are shown.

Therefore, we focused our attention on the potency of the partial PPARγ agonist telmisartan and related sartans (candesartan cilexetil and candesartan, irbesartan, losartan, olmesartan medoxomil as well as olmesartan). The purpose of this study was on the one hand the investigation of their PPARγ activating potential and on the other hand the determination of their cell death modulating effects. This work continues a recently published SAR study with 4-substituted derivatives of the lead 4'-((2-propyl-1*H*-benzo[*d*]imidazol-1-yl)methyl)-[1,1′-biphenyl]-2-carboxylic acid with the objective to establish a correlation between PPARγ activation and circumvention of imatinib resistance in K562-resistant cells.

Telmisartan and candesartan cilexetil were partial PPARγ agonists, all other sartans were more or less inactive. Also candesartan did not activate the receptor. This led to the question if a carboxyl group at the benzimidazole (either at position 4 or 7) generally decreases the binding. Accordingly, the lead was modified (compounds **7** and **14**) and the related methyl esters (**6**, **12**, **13**) were as well included in our investigations. Additionally, telmisartan was derivatized to an amide (**1**) following the positive results described in Ref. [12]. While **1** represented a partial PPARγ agonist, **6**, **12**, and **13** only marginally stimulated the gene expression. The carboxylic acid derivatives **7** and **14** were completely inactive.

All compounds were non-cytotoxic against COS-7 and HS-5 cells as well as K562 imatinib-resistant CML cells. When the compounds were tested together with imatinib on the K562-resistant cell line, telmisartan, candesartan cilexetil, irbesartan, **1**, **6**, **12**, and **13** abrogated the resistance. Especially in presence of **1**, **6**, and **12** (10 μM) imatinib caused maximum cytotoxic effects, even higher than in sensitive K562 cells (89% vs 46%).

These findings clearly demonstrate that CO_2CH_3 or $CONH_2$ groups (especially a derivatization of the 2-COOH group) are positive structural modifications, whereas COOH groups either at position 4 or 7 of the benzimidazole reduce the cell death modulating effects. A correlation with the PPARγ activation does not exist.

To get an insight into the mode of action, the influence on the STAT5/pSTAT5 expression in K562-resistant cells in the presence of imatinib was studied. The ability of telmisartan, **1**, **6**, **12**, and **13** to repress the STAT5 phosphorylation correlates with their potency to circumvent imatinib resistance in these cells.

In conclusion, no clear connection of overcoming imatinib resistance and activating PPARγ was observed. Moreover, the compounds themselves did not exhibit cytotoxic effects.

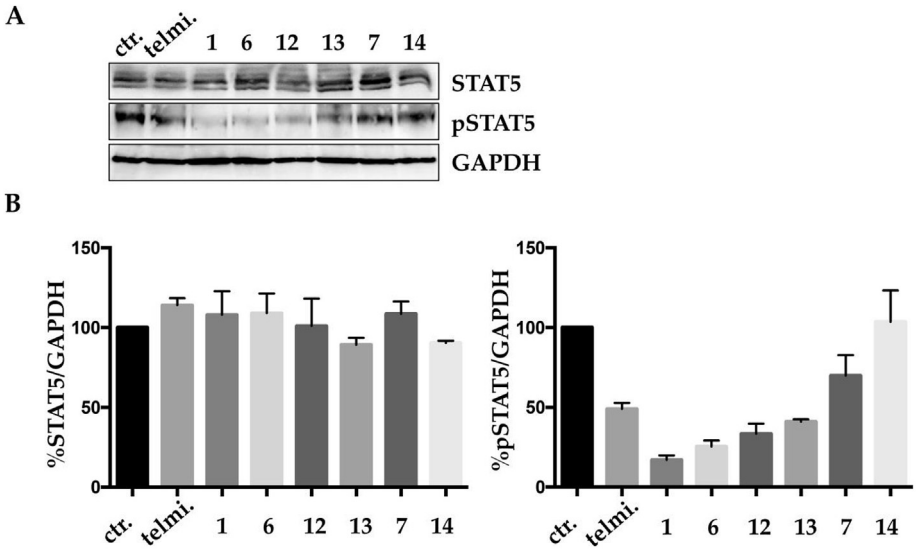


Fig. 7. STAT5 and pSTAT5 expression in K562-resistant cells treated with 1 μM of imatinib alone (ctr.) or together with 10 μM of telmisartan (telmi.), **1**, **6**, **12**, **13**, **7**, and **14** for 6 h (A). GAPDH served as loading control. Densitometric analyses (B) were performed with the ImageJ 1.48 software. Control cells were set as 100%.

Nevertheless, the proven repression of the STAT5 phosphorylation upon combination of imatinib with telmisartan, **1**, **6**, **12**, or **13** is related to the mode of action. This is in agreement with the theory of Rousselot et al. [18] and Schafranek et al. [23], stating the eradication of the CML stem cell pool by silencing its trigger STAT5. Consequently, an increased deep MR to imatinib is assumed.

3. Conclusions

The sartans are a very interesting class of drugs regarding their potential to overcome imatinib resistance. The representatives telmisartan and the prodrug candesartan cilexetil, but not candesartan itself, were efficient sensitizers to imatinib treatment in K562-resistant CML cells, whereas irbesartan, losartan, olmesartan medoxomil, and olmesartan were inactive.

The design of **6**, **12**, and **13** bearing substructures of each telmisartan and candesartan yielded highly active imatinib sensitizers. Their potency to treat imatinib-resistant CML cells was completely abrogated upon hydrolysis of the methyl esters to the carboxylic acids (**7** and **14**). However, the derivatization of telmisartan to its carboxamide derivative **1** resulted in a very effective compound.

Compound **1** showed moderate intrinsic activity towards PPARγ, while **6**, **7**, **12**–**14**, candesartan, irbesartan, losartan, olmesartan medoxomil, or olmesartan were inactive. Although **1**, telmisartan, and candesartan cilexetil acted by partial agonism at PPARγ, the mechanism of action is presumably dependent on the phosphorylation status of STAT5. Inhibition of this pro-survival transcription factor was proven for telmisartan and the developed imatinib sensitizers. Notably, none of the tested compounds exhibited cytotoxic properties.

Summing up, this study demonstrated that derivatives of telmisartan or candesartan are capable cell death modulators to overcome imatinib resistance. Regarding the optimization of the lead structure, a benzimidazole scaffold definitely is favored over an imidazole core and compounds bearing more than one carboxylic acid reveal no sensitizing effect.

Based on the established application of telmisartan and candesartan in clinical use, our findings might be beneficial to attain deep MR in CML patients.

4. Experimental section

4.1. Chemistry

4.1.1. General materials and methods

Compound **2**, all reagents as well as other chemicals were purchased from Sigma-Aldrich, TCI Chemicals, or Alfa Aesar and used without further purification. Telmisartan, irbesartan, losartan, candesartan cilexetil, and olmesartan medoxomil were isolated from tablets that were received from the pharmacy. The ester-prodrugs candesartan cilexetil and olmesartan medoxomil were hydrolyzed by established procedures and their analytical data was in accordance with the literature. The solvents dichloromethane (DCM), ethanol (EtOH), ethyl acetate (EA), and methanol (MeOH) were distilled before use, whereas petroleum ether (PE) was used directly. Ethylene glycol (EG), *N*,*N*-dimethylformamide (DMF), *N*,*N*-diisopropylethylamine (DIPEA), and tetrahydrofuran (THF) were purchased in appropriate quality. Column chromatography was performed applying classic standard procedures and/or medium pressure liquid chromatography. For the latter an Isolera One 3.0 Flash purification system (Biotage) was employed. Silica gel 60 (particle size 40–63 μm, 230–240 mesh) served as stationary phase. Thin layer chromatography (TLC) was carried out using Polygram SIL G/UV_{254} polyester foils covered with a 0.2 mm layer of silica gel and a fluorescence indicator (Macherey-Nagel). TLC plates were visualized with UV-light at 254 nm or 366 nm. Nuclear magnetic resonance spectra (NMR) were recorded using a 200 MHz Gemini (now Agilent), a 400 MHz Avance 4 Neo (Bruker), or a 600 MHz Avance II (Bruker) spectrometer. Deuterated dimethyl sulfoxide (DMSO-d_6) or acetone (acetone-d_6) were used as solvents (both from Eurisotop or Alfa Aesar). Chemical shifts (δ) are given in parts per million (ppm) and were referenced to the solvent peak or to tetramethylsilane (TMS) as an internal standard. 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). For the determination of purity, high performance liquid chromatography (HPLC) was applied, using a Shimadzu Nexera-i LC 2040C 3D 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. As stationary phase an RP-18 column (dimension 125 × 4 mm, 5 μm particle size, Knauer) was used and the chromatograms were analyzed with the program Lab-Solutions 5.86 (Shimadzu). Purity of ≥95% was assured for all compounds.

*4.1.2. Synthesis of 4'-((1,7′-dimethyl-2′-propyl-1H,3′H-[2,5′-bibenzo[d]imidazol]-3′-yl)methyl)-[1,1′-biphenyl]-2-carboxamide (**1**)*

Telmisartan (0.15 g, 0.3 mmol) was dissolved in anhydrous DMF (1 ml) and cooled in an ice bath. PyBOP (0.17 g, 0.3 mmol) dissolved in anhydrous DMF (1 ml) was added and the reaction mixture was stirred at 0 °C for 5 min. DIPEA (0.15 g, 1.2 mmol) was added, followed by an NH_4Cl (0.02 g, 0.3 mmol) suspension in anhydrous DMF (1 ml). It was stirred for another 30 min at 0 °C. The mixture was allowed to attain room temperature and stirring was continued for 16 h. Then, the solution was diluted with water to double the volume and it was basified to a pH of 9 with 1 N NaOH solution. The mixture was extracted with EA (3x) to remove HOBt and unreacted carboxylic acid. The organic phases were combined, washed with brine, dried over anhydrous Na_2SO_4 and filtered. EA was removed under reduced pressure. The resulting product was purified by column chromatography (DCM/MeOH, 95:5) and flash column chromatography with gradient elution (PE/EA, 7:3 to 0:1). Colorless solid, yield: 17%. ^{1}H NMR (400 MHz, DMSO-d_6): δ7.77–7.73 (m, 1H, Ar–H), 7.66–7.56 (m, 3H, Ar–H, NH), 7.50–7.34 (m, 6H, Ar–H, NH), 7.32 (dd, 1H, ^3J =7.6 Hz, ^4J =1.3 Hz, Ar–H), 7.30–7.19 (m, 3H, Ar–H), 7.17 (d, 2H, ^3J =8.3 Hz, Ar–H), 5.61 (s, 2H, NCH_2), 3.82 (s, 3H, NCH_3), 2.93 (t, 2H, ^3J= 7.6 Hz, $CH_2CH_2CH_3$), 2.63 (s, 3H, CH_3), 1.89–1.77 (m, 2H, $CH_2CH_2CH_3$), 1.01 (t, 3H, ^3J= 7.4 Hz, $CH_2CH_2CH_3$). ^{13}C NMR (101 MHz, DMSO-d_6): δ171.0, 156.2, 154.0, 142.7, 142.5, 139.7, 138.3, 137.4, 136.7, 135.95, 134.7, 129.8, 129.2, 128.7, 128.2, 127.5, 127.1, 126.4, 123.3, 123.2, 122.0, 121.8, 118.7, 110.4, 109.3, 46.0, 31.8, 28.7, 20.7, 16.5, 13.9. HRMS: *m/z*calculated for $C_{33}H_{31}N_5O$ $[M+H]^+$: 514.2601, found: 514.2653.

*4.1.3. Synthesis of methyl 1-((2'-(methoxycarbonyl)-[1,1′-biphenyl]-4-yl)methyl)-2-propyl-1H-benzo[d]imidazole-7-carboxylate (**6**)*

After dissolving **5** (2.56 g, 5.2 mmol) in EtOH (70 ml), $SnCl_2 \times 2$ H_2O (5.89 g, 26.1 mmol) was added and the mixture was heated to 80 °C for 14 h. Water was added to double the volume and a 1 N NaOH solution was used to obtain a pH of 9. The formed precipitate was sucked off and washed with EtOH. The filtrate was evaporated, the residue was suspended in water and extracted with DCM (3 ×). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure. Purification was performed by column chromatography (PE/EA, 1:1). Colorless solid, yield: 29%. ^{1}H NMR (400 MHz, DMSO-d_6): δ7.87 (dd, 1H, ^3J =8.0 Hz, ^4J =1.2 Hz, H4''), 7.70 (dd, 1H, ^3J =7.7 Hz, ^4J =1.5 Hz, H3), 7.62–7.55 (m, 1H, H5), 7.52 (dd, 1H, ^3J =7.6 Hz, ^4J =1.2 Hz, H6''), 7.50–7.42 (m, 1H, H4), 7.36 (dd, 1H, ^3J =7.8 Hz, ^4J =1.3 Hz, H6), 7.29–7.22 (m, 1H, H5''), 7.18 (d, 2H, ^3J =8.2 Hz, H2', H6'), 6.85 (d, 2H, ^3J =8.0 Hz, H3', H5'), 5.72 (s, 2H, NCH_2), 3.69 (s, 3H, 7''-CO_2CH_3), 3.51 (s, 3H, 2-CO_2CH_3), 2.90 (t, 2H, ^3J =7.5 Hz, $CH_2CH_2CH_3$), 1.90–1.75 (m, 2H, $CH_2CH_2CH_3$), 0.99 (t, 3H, ^3J =7.4 Hz, $CH_2CH_2CH_3$). ^{13}C NMR (101 MHz, DMSO-d_6): 168.5, 166.4, 157.7, 144.2, 140.7, 139.4, 136.3, 132.1, 131.5, 130.8, 130.4, 129.3, 128.3, 127.5, 125.7, 124.2, 123.1, 120.9, 116.7, 52.3, 51.7, 47.7, 28.9, 20.2, 13.8. HRMS: *m/z* calculated for $C_{27}H_{26}N_2O_4$ $[M+H]^+$: 443.1965, found: 443.2003.

*4.1.4. N-Alkylation of compound **11** to methyl 1-((2'-(methoxycarbonyl)-[1,1′-biphenyl]-4-yl)methyl)-2-propyl-1H-benzo[d]imidazole-4-carboxylate (**12**)*

11 (0.10 g, 0.4 mmol) was dissolved in anhydrous DMF (0.5 ml). NaH (0.01 g, 0.5 mmol) was added in small portions and the mixture was stirred for 30 min. Then, methyl 4'-(bromomethyl)-[1,1′-biphenyl]-2-carboxylate (0.15 g, 0.5 mmol) was added and stirring was continued for 12 h at room temperature. The reaction mixture was treated with ice water to double the volume and it was neutralized using 1 N HCl. After extracting with EA (3 ×), the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure. Purification was performed by column chromatography (PE/EA, 7:3). Colorless solid, yield: 56%. ^{1}H NMR (400 MHz, acetone-d_6): δ7.81–7.74 (m, 2H, H3, H5''), 7.67 (dd, 1H, ^3J =8.1 Hz, ^4J =1.1 Hz, H7''), 7.62–7.56 (m, 1H, H5), 7.50–7.44 (m, 1H, H4), 7.40 (dd, 1H, ^3J =7.7 Hz, ^4J =1.3 Hz, H6), 7.31–7.23 (m, 3H, H2', H6', H6''), 7.17 (d, 2H, ^3J =8.1 Hz, H3', H5'), 5.64 (s, 2H, NCH_2), 3.93 (s, 3H, 4''-CO_2CH_3), 3.56 (s, 3H, 2-CO_2CH_3), 2.95 (t, 2H, ^3J =7.6 Hz, $CH_2CH_2CH_3$), 1.92–1.82 (m, 2H, $CH_2CH_2CH_3$), 1.03 (t, 3H, ^3J =7.4 Hz, $CH_2CH_2CH_3$). ^{13}C NMR (101 MHz, acetone-d_6): 169.3, 167.6, 157.9, 142.9, 142.3, 141.6, 137.8, 136.9, 132.3, 132.2, 131.4, 130.4, 129.7, 128.3, 127.1, 124.8, 122.2, 121.8, 115.1, 52.1, 51.9, 47.2, 21.9, 14.3. HRMS: *m/z*calculated for $C_{27}H_{26}N_2O_4$ $[M+H]^+$: 443.1965, found: 443.1977.

4.1.5. General procedure for the hydrolysis of the bis-methyl esters

The respective bis-methyl ester was dissolved in THF and EtOH (each ~1–2 ml/mmol). 2 N KOH (~3–4 ml/mmol) was added and the reaction mixture was heated to 70 °C for 16 h. The solvents were removed under reduced pressure and the residue was suspended in water. 6 N HCl was used to obtain a pH of 1. The mixture was extracted with DCM (3 ×), the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure. Purification was performed by column chromatography (DCM/MeOH, 9:1).

*4.1.5.1. 1-((2'-Carboxy-[1,1′-biphenyl]-4-yl)methyl)-2-propyl-1H-benzo[d]imidazole-7-carboxylic acid **(7)**.* From **6** (0.12 g, 0.3 mmol), in THF and EtOH (each 0.5 ml) with 2 N KOH (1 ml). Colorless solid, yield: 36%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.94 (br s, 2H, 2 × COOH), 7.86 (dd, 1H, ^3J =8.0 Hz, ^4J =1.2 Hz, H4''), 7.69 (dd, 1H, ^3J =7.6 Hz, ^4J =1.4 Hz, H3), 7.64 (dd, 1H, ^3J =7.6 Hz, ^4J =1.2 Hz, H6''), 7.56–7.49 (m, 1H, H5), 7.45–7.39 (m, 1H, H4), 7.33–7.19 (m, 4H, H6, H2', H6', H5''), 6.88 (d, 2H, ^3J =8.2 Hz, H3', H5'), 5.90 (s, 2H, NCH_2), 2.87 (t, 2H, ^3J =7.5 Hz, $CH_2CH_2CH_3$), 1.88–1.75 (m, 2H, $CH_2CH_2CH_3$), 0.97 (t, 3H, ^3J =7.4 Hz, $CH_2CH_2CH_3$). ^{13}C NMR (101 MHz, DMSO-d_6): 169.5, 167.7, 157.4, 140.4, 139.7, 136.5, 132.4, 132.2, 130.8, 130.5, 129.1, 128.6, 127.3, 125.7, 124.9, 122.8, 121.0, 117.9, 54.9, 47.6, 28.9, 20.1, 13.8. HRMS: *m/z* calculated for $C_{25}H_{22}N_2O_4$ $[M+H]^+$: 415.1652, found: 415.1714.

*4.1.5.2. 1-((2'-(Methoxycarbonyl)-[1,1′-biphenyl]-4-yl)methyl)-2-propyl-1H-benzo[d]imidazole-4-carboxylic acid **(13)**.* From **12** (0.07 g, 0.2 mmol), in THF and EtOH (each 0.2 ml) with 2 N KOH (0.6 ml). Colorless solid, yield: 75%. ^{1}H NMR (400 MHz, DMSO-d_6): δ7.88 (d, 1H, ^3J =7.4 Hz, H5''), 7.70 (d, 1H, ^3J =7.4 Hz, H3), 7.59–7.52 (m, 2H, H5, H7''), 7.49–7.42 (m, 1H, H4), 7.34 (d, 1H, ^3J =7.6 Hz, H6), 7.26–7.17 (m, 3H, H2', H6', H6''), 7.07 (d, 2H, ^3J =7.9 Hz, H3', H5'), 5.59 (s, 2H, NCH_2), 3.45 (s, 3H, CO_2CH_3), 3.15 (t, 2H, ^3J =7.8 Hz, $CH_2CH_2CH_3$), 1.15–0.94 (m, 2H, $CH_2CH_2CH_3$), 0.43 (t, 3H, ^3J =7.1 Hz, $CH_2CH_2CH_3$). ^{13}C NMR (101 MHz, DMSO-d_6): δ168.3, 167.2, 157.4, 140.7, 139.9, 135.9, 134.8, 131.5, 130.7, 130.4, 129.3, 128.5, 128.4, 127.5, 126.2, 124.6, 112.1, 51.7, 46.0, 13.3. HRMS: *m/z* calculated for $C_{26}H_{24}N_2O_4$ $[M+H]^+$: 429.1809, found: 429.1832.

*4.1.6. Hydrolysis of compound **13** to 1-((2′-carboxy-[1,1′-biphenyl]-4-yl)methyl)-2-propyl-1H-benzo[d]imidazole-4-carboxylic acid (**14**)*

13 (0.04 g, 0.1 mmol) was suspended in EG (0.2 ml) and 6 N KOH

(2 ml) was added before heating to 160 °C for 30 h. The reaction mixture was diluted with water to double the volume. 6 N HCl was used to reach a pH of 1. The mixture was extracted with DCM (3 ×), the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure. Purification was performed by column chromatography (DCM/MeOH, 9:1). Colorless solid, yield: 44%. ^{1}H NMR (600 MHz, DMSO-d_6): δ8.09 (d, 2H, ^3J =8.4 Hz, H5'', H7''), 7.74 (dd, 1H, ^3J =7.7 Hz, ^4J =1.4 Hz, H3), 7.69–7.60 (m, 1H, H6''), 7.60–7.54 (m, 1H, H5), 7.49–7.44 (m, 1H, H4), 7.37–7.26 (m, 5H, H6, H2', H3', H5', H6'), 5.88 (s, 2H, NCH_2), 1.76–1.63 (m, 2H, $CH_2CH_2CH_3$), 0.97 (t, 3H, ^3J =7.3 Hz, $CH_2CH_2CH_3$). ^{13}C NMR (151 MHz, DMSO-d_6): 206.5, 169.4, 165.6, 156.9, 140.8, 140.3, 132.1, 131.0, 130.5, 129.3, 128.9, 127.5, 126.7, 117.8, 56.0, 30.7, 21.2, 18.6, 13.6. HRMS: *m/z*calculated for $C_{25}H_{22}N_2O_4[M+H]^+$: 415.1652, found: 415.1681.

4.2. Biology

4.2.1. General cell culture methods

The monkey kidney-derived cell line COS-7 (ATCC) was cultured as monolayer culture in Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/l glucose and 584 mg/l L-glutamine (GE Healthcare), without phenol red and sodium pyruvate. It was supplemented with fetal calf serum (FCS 10%, Sigma-Aldrich). The human bone marrow/stroma cell line HS-5 (ATCC) was maintained applying the above mentioned medium with 100 U/ml penicillin and 100 μg/ml streptomycin (both Lonza).

The myelogenous leukemia cell lines K562 and K562-resistant were received from Ernesto Yague. K562-resistant cells were originally described as doxorubicin-resistant subclone of K562 cells (termed KD225 by Hui et al. [25]). These cell lines were cultivated in Roswell Park Memorial Institute (RPMI) 1640 medium (Lonza) supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Incubation of the cells was performed in a humidified atmosphere (5% CO_2/95% air) at 37 °C and all cell lines were passaged twice a week. The final concentration of DMSO never exceeded 0.1% in cell based assays and vehicle treated controls (ctr., DMSO) were always included.

4.2.2. PPARγ transactivation assay

A dual-luciferase reporter assay (Promega) was employed according to the manufacturer's protocol. COS-7 cells were seeded in 96-well plates (10^4 cells per well) in triplicates and incubated. After 24 h, the cells were transiently transfected with TransIT-LT1 (MoBiTec) and the three plasmids pGal5-TK-pGL3 (90 ng), pGal4-hPPARgDEF (9 ng), as well as pRenilla-CMV (3 ng) in phosphate-buffered saline (PBS), followed by further incubation for 7 h. Subsequently, pioglitazone, the ARBs telmisartan, candesartan cilexetil, candesartan, irbesartan, olmesartan medoxomil, olmesartan, losartan, the compounds **1**, **6**, **7**, **12**–**14**, or vehicle (DMSO), respectively, 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 overnight. The appropriate buffers were added and it was shaken for 30 min to complete lysis. Firefly-luminescence was measured with the multimode plate reader EnSpire (PerkinElmer) and normalized with the activity of the co-transfected pRenilla-CMV [26].

4.2.3. Determination of metabolic activity

COS-7 cells were seeded in 96-well plates (2×10^3 cells per well) in triplicates and incubated for 24 h. Pioglitazone, the ARBs telmisartan, candesartan cilexetil, candesartan, irbesartan, olmesartan medoxomil, olmesartan, losartan, the compounds **1**, **6**, **7**, **12**–**14**, or vehicle (ctr., DMSO) were added in different concentrations (10 μM or 20 μM), respectively, before further incubation for 72 h. A modified MTT assay (EZ4U kit, Biomedica) was employed to analyze the metabolic activity according to the manufacturer's protocol. Unspecific staining was excluded by subtracting the optical density of the FCS-containing medium and the substrate. Metabolic activity in the absence of the compounds (ctr., DMSO) was set to 100% and served as internal reference [12].

4.2.4. Determination of cell death by flow cytometry

Cell death was measured according to the previously described procedure [27]. K562 or K562-resistant cells (2×10^5 cells per well) and HS-5 cells (0.5×10^5 cells per well) were seeded in 24-well plates. The ARBs telmisartan, candesartan cilexetil, candesartan, irbesartan, olmesartan medoxomil, olmesartan, losartan, the compounds **1**, **6**, **7**, **12**–**14**, or vehicle (ctr., DMSO) were added in the respective concentrations, followed by an incubation for 72 h. After harvesting of the cells, they were stained with PI/Triton-X100 for 2 h at 4 °C. Forward/sideward scatter analyses were conducted using a CytomicsFC-500 Beckman Coulter. Stained nuclei in the sub-G1 marker window were considered to represent dead cells. The tested compounds were proven to be soluble in the applied concentrations by microscopy.

4.2.5. Western blot analyses

The protein extracts were prepared as previously described [27]. Immunoblots were incubated with antibodies specific to STAT5 (#94205, Cell Signaling) or pSTAT5 (#05–886R, Merck) and GAPDH (#2118, Cell Signaling). Prior to the development of the blots by enhanced chemiluminescence (GE Healthcare) and the measurement with the AutoChemi detection system (BioRad Laboratories), incubation with anti-rabbit horseradish-peroxidase-conjugated secondary antibody (GE Healthcare) was performed. Densitometric analyses were conducted with the ImageJ 1.48 software and control cells were set as 100%.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declarations of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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References

[1] R. Hehlmann, A. Hochhaus, M. Baccarani, Chronic myeloid leukaemia, Lancet 370 (2007) 342–350.
[2] J.F. Apperley, Part I: mechanisms of resistance to imatinib in chronic myeloid

leukaemia, Lancet Oncol. 8 (2007) 1018–1029.
[3] D.M. Ross, S. Branford, J.F. Seymour, A.P. Schwarer, C. Arthur, P.A. Bartley, C. Slader, C. Field, P. Dang, R.J. Filshie, A.K. Mills, A.P. Grigg, J.V. Melo, T.P. Hughes, Patients with chronic myeloid leukemia who maintain a complete molecular response after stopping imatinib treatment have evidence of persistent leukemia by DNA PCR, Leukemia 24 (2010) 1719–1724.
[4] Ø. Bruserud, E. Aasebø, M. Hernandez-Valladares, G. Tsykunova, H. Reikvam, Therapeutic targeting of leukemic stem cells in acute myeloid leukemia - the biological background for possible strategies, Expet Opin. Drug Discov. 12 (2017) 1053–1065.
[5] S.M. Graham, H.G. Jorgensen, E. Allan, C. Pearson, M.J. Alcorn, L. Richmond, T.L. Holyoake, Primitive, quiescent, philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro, Blood 99 (2002) 319–325.
[6] A. Hochhaus, R.A. Larson, F. Guilhot, J.P. Radich, S. Branford, T.P. Hughes, M. Baccarani, M.W. Deininger, F. Cervantes, S. Fujihara, C.E. Ortmann, H.D. Menssen, H. Kantarjian, S.G. O'Brien, B.J. Druker, Long-term outcomes of imatinib treatment for chronic myeloid leukemia, N. Engl. J. Med. 376 (2017) 917–927.
[7] K.K.W. To, W.K.K. Wu, H.H.F. Loong, PPARgamma agonists sensitize PTEN-deficient resistant lung cancer cells to EGFR tyrosine kinase inhibitors by inducing autophagy, Eur. J. Pharmacol. 823 (2018) 19–26.
[8] T. Kozako, S. Soeda, M. Yoshimitsu, N. Arima, A. Kuroki, S. Hirata, H. Tanaka, O. Imakyure, N. Tone, S.i. Honda, S. Soeda, Angiotensin II type 1 receptor blocker telmisartan induces apoptosis and autophagy in adult T-cell leukemia cells, FEBS Open Bio 6 (2016) 442–460.
[9] E. Samukawa, S. Fujihara, K. Oura, H. Iwama, Y. Yamana, T. Tadokoro, T. Chiyo, K. Kobayashi, A. Morishita, M. Nakahara, H. Kobara, H. Mori, K. Okano, Y. Suzuki, T. Himoto, T. Masaki, Angiotensin receptor blocker telmisartan inhibits cell proliferation and tumor growth of cholangiocarcinoma through cell cycle arrest, Int. J. Oncol. 51 (2017) 1674–1684.
[10] S. Prost, F. Relouzat, M. Spentchian, Y. Ouzegdouh, J. Saliba, G. Massonnet, J.-P. Beressi, E. Verhoeyen, V. Raggueneau, B. Maneglier, S. Castaigne, C. Chomienne, S. Chretien, P. Rousselot, P. Leboulch, Erosion of the chronic myeloid leukaemia stem cell pool by PPARγ agonists, Nature 525 (2015) 380–383.
[11] M. Goebel, M. Clemenz, B. Staels, T. Unger, U. Kintscher, R. Gust, Characterization of new PPARγ agonists: analysis of telmisartan's structural components, ChemMedChem 4 (2009) 445–456.
[12] A.M. Schoepf, S. Salcher, P. Obexer, R. Gust, Overcoming imatinib resistance in chronic myelogenous leukemia cells using non-cytotoxic cell death modulators, Eur. J. Med. Chem. (2020) 111748.
[13] K. Oura, T. Tadokoro, S. Fujihara, A. Morishita, T. Chiyo, E. Samukawa, Y. Yamana, K. Fujita, T. Sakamoto, T. Nomura, H. Yoneyama, H. Kobara, H. Mori, H. Iwama, K. Okano, Y. Suzuki, T. Masaki, Telmisartan inhibits hepatocellular carcinoma cell proliferation in vitro by inducing cell cycle arrest, Oncol. Rep. 38 (2017) 2825–2835.
[14] S. De la Iglesia Inigo, C.E. Lopez-Jorge, M.T. Gomez-Casares, A. Lemes Castellano, P. Martin Cabrera, J. Lopez Brito, A. Suarez Cabrera, T. Molero Labarta, Induction of apoptosis in leukemic cell lines treated with captopril, trandolapril and losartan: a new role in the treatment of leukaemia for these agents, Leuk. Res. 33 (2009) 810–816.
[15] M. Shan, K.E. Carlson, A. Bujotzek, A. Wellner, R. Gust, M. Weber, J.A. Katzenellenbogen, R. Haag, Nonsteroidal bivalent estrogen ligands: an application of the bivalent concept to the estrogen receptor, ACS Chem. Biol. 8 (2013) 707–715.
[16] D.J. Kahl, K.M. Hutchings, E.M. Lisabeth, A.J. Haak, J.R. Leipprandt, T. Dexheimer, D. Khanna, P.-S. Tsou, P.L. Campbell, D.A. Fox, B. Wen, D. Sun, M. Bailie, R.R. Neubig, S.D. Larsen, 5-Aryl-1,3,4-oxadiazol-2-ylthioalkanoic acids: a highly potent new class of inhibitors of rho/myocardin-related transcription factor (MRTF)/serum response factor (SRF)-mediated gene transcription as potential antifibrotic agents for scleroderma, J. Med. Chem. 62 (2019) 4350–4369.
[17] K.J. Kieser, D.W. Kim, K.E. Carlson, B.S. Katzenellenbogen, J.A. Katzenellenbogen, Characterization of the pharmacophore properties of novel selective estrogen receptor downregulators (SERDs), J. Med. Chem. 53 (2010) 3320–3329.
[18] P. Rousselot, S. Prost, J. Guilhot, L. Roy, G. Etienne, L. Legros, A. Charbonnier, V. Coiteux, P. Cony-Makhoul, F. Huguet, E. Cayssials, J.-M. Cayuela, F. Relouzat, M. Delord, H. Bruzzoni-Giovanelli, L. Morisset, F.-X. Mahon, F. Guilhot, P. Leboulch, C.M.L.G. on behalf of the French, Pioglitazone together with imatinib in chronic myeloid leukemia: a proof of concept study, Cancer 123 (2017) 1791–1799.
[19] B. Apsel Winger, N.P. Shah, PPARgamma: welcoming the new kid on the CML stem cell block, Canc. Cell 28 (2015) 409–411.
[20] B. Yousefi, N. Samadi, B. Baradaran, V. Shafiei-Irannejad, N. Zarghami, Peroxisome proliferator-activated receptor ligands and their role in chronic myeloid leukemia: therapeutic strategies, Chem. Biol. Drug Des. 88 (2016) 17–25.
[21] S.C. Benson, H.A. Pershadsingh, C.I. Ho, A. Chittiboyina, P. Desai, M. Pravenec, N. Qi, J. Wang, M.A. Avery, T.W. Kurtz, Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity, Hypertension 43 (2004) 993–1002.
[22] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[23] L. Schafranek, E. Nievergall, J.A. Powell, D.K. Hiwase, T. Leclercq, T.P. Hughes, D.L. White, Sustained inhibition of STAT5, but not JAK2, is essential for TKI-induced cell death in chronic myeloid leukemia, Leukemia 29 (2015) 76–85.
[24] W. Warsch, K. Kollmann, E. Eckelhart, S. Fajmann, S. Cerny-Reiterer, A. Holbl, K.V. Gleixner, M. Dworzak, M. Mayerhofer, G. Hoermann, H. Herrmann, C. Sillaber, G. Egger, P. Valent, R. Moriggl, V. Sexl, High STAT5 levels mediate imatinib resistance and indicate disease progression in chronic myeloid leukemia, Blood 117 (2011) 3409–3420.
[25] R.C. Hui, R.E. Francis, S.K. Guest, J.R. Costa, A.R. Gomes, S.S. Myatt, J.J. Brosens, E.W. Lam, Doxorubicin activates FOXO3a to induce the expression of multidrug resistance gene ABCB1 (MDR1) in K562 leukemic cells, Mol. Canc. Therapeut. 7 (2008) 670–678.
[26] E. Raspe, L. Madsen, A.M. Lefebvre, I. Leitersdorf, L. Gelman, J. Peinado-Onsurbe, J. Dallongeville, J.C. Fruchart, R. Berge, B. Staels, Modulation of rat liver apolipoprotein gene expression and serum lipid levels by tetradecylthioacetic acid (TTA) via PPARalpha activation, J. Lipid Res. 40 (1999) 2099–2110.
[27] S. Salcher, M. Hermann, U. Kiechl-Kohlendorfer, M.J. Ausserlechner, P. Obexer, C10ORF10/DEPP-mediated ROS accumulation is a critical modulator of FOXO3-induced autophagy, Mol. Canc. 16 (2017) 95.