



Research article

Identification and characterization of a phenyl-thiazolyl-benzoic acid derivative as a novel RAR/RXR agonist



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ABSTRACT

Objective: To identify an agonist of RXR α and RAR α with reduced undesired profiles of all-trans retinoic acid for differentiation-inducing therapy of acute promyelocytic leukemia (APL), such as its susceptibility to P450 enzyme, induction of P450 enzyme, increased sequestration by cellular retinoic acid binding protein and increased expression of P-glycoprotein, a virtual screening was performed.

Results and conclusion: In this study, a phenyl-thiazolyl-benzoic acid derivative (PTB) was identified as a potent agonist of RXR α and RAR α . PTB was characterized in nuclear receptor binding, reporter gene, cell differentiation and cell growth assays. PTB bound directly to RXR α and RAR α , but not to PPAR α , $\delta(\beta)$ or γ . PTB fully activated reporter genes with enhancer elements for RXR α /RXR α , and partially activated reporter genes with enhancer elements for RAR α /RXR α , PPAR $\delta(\beta)$ and PPAR γ . Furthermore, PTB induced differentiation and inhibited the growth of human APL cells. Thus, PTB is a novel dual agonist of RXR α and RAR α and works as both a differentiation inducer and a proliferation inhibitor to leukemic cells.

1. Introduction

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia with the t(15; 17) (q22; q21) chromosomal translocation which involves promyelocytic leukemia (PML) and retinoic acid receptor alpha (RAR α) genes and produces the two chimeric fusion proteins, PML-RAR α and reciprocal RAR α -PML [1, 2]. The PML-RAR α fusion protein exhibits dominant negative effects on both PML and RAR α pathways, prevents promyelocytes maturation and then leads to immature leukemic cells accumulation [3]. Moreover, PML fused to RAR α transforms a RAR-retinoid X receptor (RXR) heterodimer into an oncogenic PML-RAR α homodimer, and this enforced RAR α homo-dimerization is considered as a common mechanism to block transcription and differentiation by various RAR α fusion proteins [4].

In the late 1980s, the *all-trans* retinoic acid (ATRA)-based therapy, which induces hematological complete remission (CR) in APL patients [5], has dramatically advanced the treatment of APL. The ATRA-based therapy, initially classified as a differentiation therapy, is now regarded as a molecular-targeted therapy aimed at the pathogenic PML-RAR α [6].

Although ATRA has the beneficial effect on APL [7, 8, 9], an average duration of the hematological CR with ATRA is several months [10], and in some cases before reaching CR, APL acquires resistance against ATRA and then relapses within a short period [11]. There are a few mechanisms believed to explain the ATRA resistance [12, 13]. First, a continuous ATRA treatment causes a progressive reduction in plasma drug concentration, partly by increasing drug metabolism due to the induction of cytochrome P450 enzymes [14, 15, 16]. Second, increased levels of cellular retinoic acid binding protein (CRABP) in ATRA-resistant leukemic cells prevents ATRA to enter enough into the nucleus [17, 18]. Third, ATRA might be eliminated by P-glycoprotein, which is a transmembrane drug efflux pump involved in resistance to multiple chemotherapeutic agents and is increased in ATRA-resistant leukemic cells [16]. Furthermore, a missense mutation in RAR α region of PML-RAR α fusion gene has been identified in the APL cells of relapsed patients. The mutation located in the ligand-binding domain of RAR α prevents the interaction of PML-RAR α with ATRA and reverses the effect of ATRA on myeloid differentiation [19].

RAR and RXR forms a heterodimer which plays important roles in

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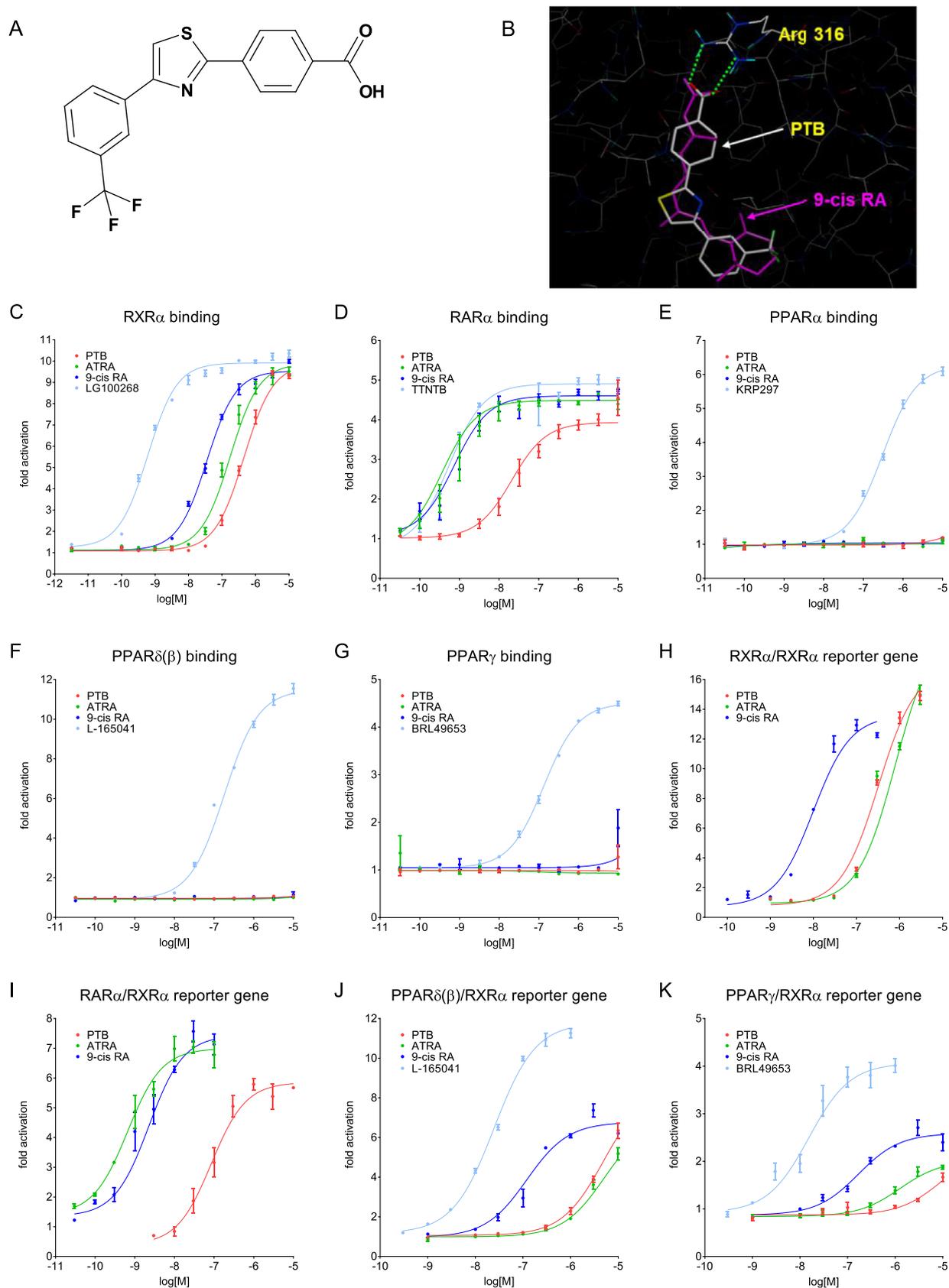


Fig. 1. A: The structure of 4-[4-(3-trifluoromethyl-phenyl)-thiazol-2-yl]-benzoic acid derivative (PTB). B: Modeled structure of PTB (atom color) in the ligand binding pocket of RXR α . 9-cis RA (magenta) is overlaid as a reference. C–G: The receptor profiles of PTB by TR-FRET binding assay. Values are expressed by mean \pm s.e.m. ($n = 3$). H–K: The receptor profile of PTB by reporter gene assay. Values are expressed by mean \pm s.e.m. ($n = 3$).

myelocyte differentiation and apoptosis, and the PML-RAR α fusion protein represses RAR/RXR signaling pathway [4]. In HL-60 cells that does not carry the typical translocation but has a capacity to differentiate, ligand-induced RAR α activation is enough to induce differentiation, whereas RXR α activation could induce apoptosis by downregulating Bcl-2 mRNA [20, 21]. Moreover, a combination of RXR and RAR α ligands could enhance differentiation synergistically in differentiation-resistant APL cell line [22].

In this study, a virtual screening was performed to identify an agonist of RXR α and RAR α with reduced undesired profiles of ATRA for the treatment of APL, and a phenyl-thiazolyl-benzoic acid derivative (PTB) was identified and characterized in binding, reporter gene, differentiation and growth assays.

2. Results

2.1. Virtual screening

Virtual screening of a commercial database against the agonist-bound form of RXR α was performed using the docking program GLIDE (Schrödinger, LLC, New York, NY) and refined parameters. Through a post-docking analysis involving a visual inspection, a phenyl-thiazolyl-benzoic acid derivative (PTB; Key Organics Limited, Catalog No. 1G-433S) as shown in Fig. 1A was identified as one of the most promising compounds since it showed very good overlap with a known agonist 9-cis RA, and had excellent complementarity to the binding site as depicted in Fig. 1B.

2.2. The receptor selectivity profiles

Direct binding of PTB to RXR α and RAR α was evaluated by using TR-FRET assay. PTB showed agonistic activities for both RXR α and RAR α (Fig. 1C–D). Direct binding of PTB to any of PPARs was not observed (Fig. 1E–G). PTB has the highest affinity for RAR α among the nuclear receptors tested. EC₅₀ values of PTB to several nuclear receptors are shown in Table 1.

The reporter gene assays were carried out to examine functional effects of PTB in cellular systems. The nuclear receptors, RXR α /RXR α , RAR α /RXR α , PPAR δ (β)/RXR α and PPAR γ /RXR α , were tested in reporter gene assays. The results are shown in Fig. 1H–K. PTB fully activated RXR α /RXR α reporter gene, and partially activated RAR α /RXR α , PPAR δ (β)/RXR α and PPAR γ /RXR α reporter genes. EC₅₀ values were not able to be calculated in PPAR δ (β)/RXR α and PPAR γ /RXR α reporter genes because the signals did not reach plateau in PPAR δ (β)/RXR α and PPAR γ /RXR α reporter genes. The obtained EC₅₀ values and fold increase in activation at 10 μ M are listed in Table 1.

2.3. Effect of PTB on differentiation of APL cell lines

It is well known that RAR agonists induce the differentiation of APL cells. Therefore, the effect of PTB on RAR mediated induction of

differentiation in NB4 cells was examined. PTB-treated NB4 cells induced tetrazolium reduction ability, an indicator of differentiation, in a dose dependent manner (Fig. 2A). Calculated EC₅₀ value of PTB to induce differentiation based on the reduction activity of tetrazolium salt was 0.95 μ M.

To confirm the differentiation, PTB effects on CD11b expression on differentiated leukemia cell surface were examined by flow cytometry because it is known that differentiated NB4 and HL-60 cells express the CD11b antigen. PTB induced the expression of CD11b antigen in a dose dependent manner that is consistent with the tetrazolium reduction assay. The differentiation inducing profiles of PTB was almost the same between NB4 and HL-60 (Fig. 2B–C).

2.4. Anti-leukemic activity of PTB in vitro and in vivo

PTB inhibited proliferation of HL-60 cells with IC₅₀ value of 0.71 μ M (Fig. 2D), and inhibited NB4 subcutaneous tumor growth significantly by 44% at 20 mg/kg given orally once daily (Fig. 2E).

3. Discussion and conclusion

PTB was identified as a novel RXR α and RAR α agonist by virtual screening. It showed a very good structural overlap with a known agonist, 9-cis RA, and had excellent complementarity to the binding site of RXR. After we identified and characterized PTB in early 2000, we noted that PTB was also reported as a candidate of RAR α ligand in a literature [23], where PTB was identified as a possible RAR α ligand by virtual screening, docked into the binding pocket of RAR (important residues as R274, R278 and S289) and superimposed with the crystal structure of ATRA. However, the direct binding of PTB to RAR α was not investigated, although PTB activated the CAT reporter gene with RAR α , RAR β , RAR γ and RXR β .

In the nuclear receptor binding assay, PTB did not bind directly to PPAR δ (β) and PPAR γ , but in the cellular system, PTB partially activated PPAR δ (β)/RXR α and PPAR γ /RXR α reporter genes. Activation of these reporter genes by PTB seems to be due to its activity to RXR α . ATRA also did not bind directly to PPAR δ (β) and PPAR γ but activated PPAR δ (β)/RXR α and PPAR γ /RXR α reporter genes. ATRA is reported as a high affinity ligand for PPAR δ (β) and binds to PPAR δ (β) with nanomolar affinity [24]. Furthermore, they reported that ATRA did not activate PPAR γ reporter gene. However, their results were not reproduced and were not consistent with our results.

PTB induced differentiation of both NB4 and HL-60 cells as detected by tetrazolium reduction assay and by CD11b expression analysis. The potency of PTB for the induction of cell differentiation was similar to those of ATRA and 9-cis RA. However, the minimum concentration of PTB required to induce differentiation was higher than that of ATRA or 9-cis RA. PTB potently inhibited proliferation of HL-60 cells. The ligand activation of RAR is sufficient to induce differentiation, whereas the ligand activation of RXR is essential for the induction of apoptosis in HL-60 cells [25]. Therefore, it is suggested that the effects of PTB on

Table 1
EC₅₀ values of PTB to nuclear receptors determined by binding and reporter gene assays.

EC ₅₀ [nM]		PTB	ATRA	9-cis RA
nuclear receptor binding	RXR α	454	175	35
	RAR α	21	0.36	0.73
	PPAR α	No binding	No binding	No binding
	PPAR δ (β)	No binding	No binding	No binding
	PPAR γ	No binding	No binding	No binding
reporter gene [fold activation at 10 μ M]	RXR α /RXR α	321 [15]	796 [15]	10 [12]
	RAR α /RXR α	86 [5.7]*	0.66 [6.8]	2.3 [7.1]
	PPAR δ (β)/RXR α	NA [6.3]	NA [5.2]	121 [6.2]*
	PPAR γ /RXR α	NA [1.7]	NA [1.9]*	168 [2.4]*

NA: not applicable.

* partial agonist.

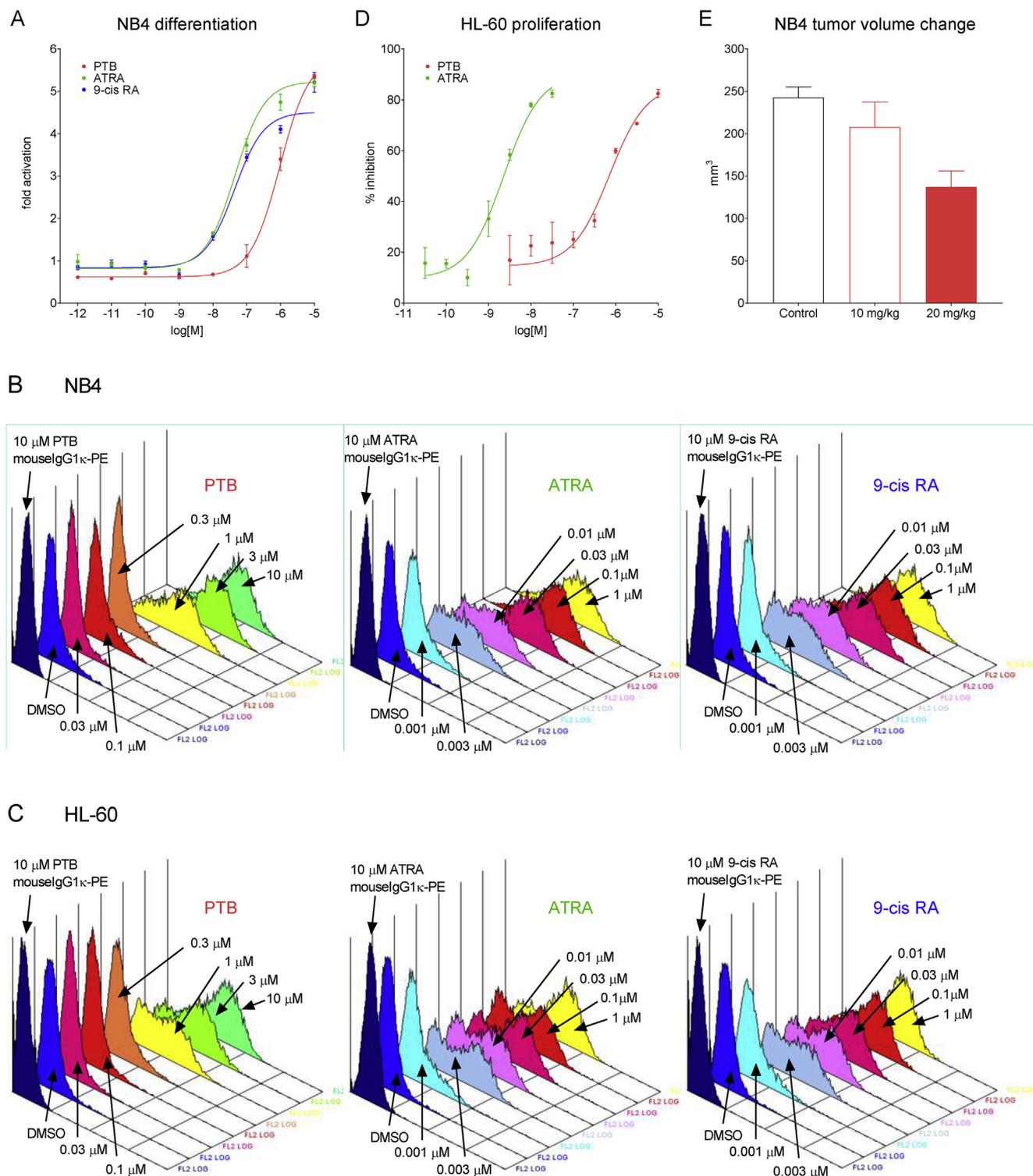


Fig. 2. A: Effect of PTB on NB4 differentiation detected by tetrazolium reduction. Values are expressed by mean \pm s.e.m. ($n = 3$). B–C: Effect of PTB, ATRA and 9-cis RA on cell differentiation induction detected by flow cytometry. The colors in histograms indicate the concentrations of compounds added into cell culture medium as described follows; dark blue: highest concentrations of compounds and cell-stained by R-PE-conjugated mouse IgG1, κ monoclonal immunoglobulin isotype control, blue: DMSO, light-blue: 0.001 μ M, somber light blue: 0.003 μ M, pink: 0.01 μ M, magenta: 0.03 μ M, red: 0.1 μ M, orange: 0.3 μ M, yellow: 1 μ M, light green: 3 μ M and green: 10 μ M. D: Effect of PTB and ATRA on HL-60 proliferation. Values are expressed by mean \pm s.e.m. ($n = 3$). E: Effect of PTB on NB4 xenograft tumor growth in vivo. Values are expressed by mean \pm s.e.m. ($n = 9$). **: $P = 0.0037$ versus 0.5% CMC (Dunnett's test following 1 way ANOVA).

induction of cell differentiation and inhibition of cell proliferation originate in its activity on RXR α and RAR α .

More recently, arsenic trioxide (ATO) has been the treatment of recurrent APL, and the combination of ATRA and ATO in frontline

therapy [26]. It would be also important to compare the effect of PTB to ATO and ATO/ATRA in APL models, in terms of efficacy as well as safety point of view.

In conclusion, PTB was identified as a dual agonist of RXR α and RAR α

and worked as both a differentiation inducer and a proliferation inhibitor to leukemic cells. Further characterization of PTB in patient-derived cells including ATRA-resistant cells, cellular toxicity assays, additional *in vivo* models, metabolic stability, pharmacokinetics and safety assessment, such as an effect on triglycerides through evaluation of LXR selectivity and SREBP1c induction, will be needed to show a possibility of its application to APL treatment.

4. Materials and methods

4.1. Nuclear receptor binding assay

PTB (Key Organics Ltd., Cat. No. 1G-433S; in 2003) was evaluated in nuclear receptor binding assay by TR-FRET method. The receptors tested in TR-FRET assays were RXR α , RAR α , PPAR α , PPAR δ (β) and PPAR γ . ATRA and 9-cis RA were tested for binding profiles and compared with PTB. RXR α agonist LG100268, RAR agonist TTNPB, PPAR α agonist KRP297, PPAR δ agonist L-165041 and PPAR γ agonist BRL49653 were used as positive controls of the ligand binding assay to RXR α , RAR α , PPAR α , PPAR δ (β) and PPAR γ , respectively. TR-FRET signals from europium to allophycocyanin were measured by ARVosx + L multilabel counter. The ratio of fluorescence intensity of 665 nm–615 nm was used as a TR-FRET signal for data analysis. EC₅₀ values were determined by non-linear curve fit analysis using GraphPad Prism.

4.2. Reporter gene assay

PTB was evaluated in the reporter gene assays with RXR α , RAR α , PPAR α , PPAR δ (β) and PPAR γ . ATRA and 9-cis RA were also tested and compared with PTB. PPAR δ agonist L-165041 and PPAR γ agonist BRL49653 were used as positive controls of the reporter gene assay against RXR α /RXR α , RAR α /RXR α , PPAR δ (β)/RXR α and PPAR γ /RXR α , respectively. HEK-293 cells were co-transfected with reporter plasmid, expression plasmid and reference plasmid using LF2000. The reporter plasmids code firefly luciferase DNA sequence and the reference plasmids code renilla luciferase DNA sequence. Transfected HEK-293 cells were inoculated into 96-well plate and incubated at 37°C for 6 h until compounds were added. After a further 20-h incubation, luciferase activities were measured using Dual-luciferase assay system by ARVosx + L. The firefly luciferase activity was normalized with the renilla luciferase activity as a standard. EC₅₀ values were determined by non-linear curve fit analysis using the GraphPad Prism software. NA: not applicable because the signals did not reach plateau and EC₅₀ values were not able to be calculated.

4.3. NB4 differentiation detected by tetrazolium reduction

NB4 cells (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) suspended in 100 μ L culture medium were inoculated in a 96-well plate at 40,000 cells/well and 100 μ L medium containing 1 pM to 10 μ M of PTB, ATRA or 9-cisRA was added. Final concentration of DMSO was adjusted to 0.1%. After a 72-h incubation at 37°C, the extent of cell differentiation was measured by tetrazolium reduction ability. Culture medium containing 20% WST-8 and 10 μ M PMA was added to 100 μ L of cell suspension for differentiation assay. After a 1-h incubation at 37°C, the values of OD₄₅₀ – OD₆₃₀ were measured using a microtiter plate reader. The degrees of cell differentiation (net differentiation) were normalized by cell numbers in each wells according to the following formula: $100 \times (\text{Test values of } [OD_{450} - OD_{630}] / \text{cell number}) / (\text{DMSO control values of } [OD_{450} - OD_{630}] / \text{cell number})$. EC₅₀ values of the tested compounds were determined by non-linear curve fit analysis using GraphPad Prism.

4.4. Cell differentiation induction detected by flow cytometry

NB4 and HL-60 cells (Dainippon Pharmaceutical Co., Ltd.) were

inoculated at 2×10^6 cells/well of 6-well plate in 2 mL of medium and 500 μ L of medium containing 0.01–10 μ M of PTB, 0.001–1 μ M of ATRA or 9-cis RA was added. Final concentration of DMSO was adjusted to 0.1%. After a 72-h incubation at 37°C, cells were collected and stained by R-PE-conjugated mouse anti-human CD11b/Mac-1 monoclonal antibody (Becton Dickinson, Cat. No. 555388) or the R-PE-conjugated mouse IgG1, κ monoclonal immunoglobulin isotype control (Becton Dickinson, Cat. No. 555749). Flow cytometry was performed by an EPICS ELITE and the results were analyzed using EPICS ELITE EXPO32 software. Differentiated cells were identified as the CD11b-positive cells.

4.5. HL-60 proliferation

HL-60 cells suspended in 100 μ L of medium were seeded in a 96-well plate at 40,000 cells/well and 100 μ L of medium containing of 0.003–10 μ M of PTB or 0.03–30 nM of ATRA was added. Final concentration of DMSO was adjusted to 0.1%. After a 72-h incubation at 37°C, cell numbers were measured using CellTiter-Glo™ luminescent cell viability assay. Percentages of the net growth were calculated according to the following formula: $100 \times (\text{Test cell numbers} - 40,000) / (\text{DMSO cell numbers} - 40,000)$. GI₅₀ values were determined by non-linear curve fit analysis using GraphPad Prism.

4.6. NB4 xenograft tumor growth *in vivo*

The animal experimental procedures described in this study were approved by Animal Welfare Committee in Novartis Institutes for BioMedical Research Tsukuba. A 100 μ L of NB4 cell suspension containing 3×10^6 cells was inoculated subcutaneously into the left flank of mice. Treatment was started when tumor volumes had reached approximately 70 mm³. PTB was suspended in 0.5% CMC and administered orally once daily for 7 days. Tumor volume was calculated according to the formula: length x width²/2.

Declarations

Author contribution statement

Chie Koshiishi, Takanori Kanazawa, Shinji Hatakeyama: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Eric Vangrevelinghe: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Toshiyuki Honda: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare the following conflict of interests: All authors are or were employees of Novartis Pharma at the time of the work was carried out.

Additional information

The datasets used in the current study are available from the corresponding author by request.

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References

- [1] J.D. Rowley, H.M. Golomb, C. Dougherty, 15/17 translocation, a consistent chromosomal change in acute promyelocytic leukaemia, *Lancet* 1 (8010) (1977) 549–550.
- [2] R.A. Larson, K. Kondo, J.W. Vardiman, A.E. Butler, H.M. Golomb, J.D. Rowley, Evidence for a 15;17 translocation in every patient with acute promyelocytic leukemia, *Am. J. Med.* 76 (5) (1984) 827–841.
- [3] A. Melnick, J.D. Licht, Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia, *Blood* 93 (10) (1999) 3167–3215.
- [4] R.J. Lin, T. Sternsdorf, M. Tini, R.M. Evans, Transcriptional regulation in acute promyelocytic leukemia, *Oncogene* 20 (10) (2001) 7204–7215.
- [5] M.E. Huang, Y.C. Ye, S.R. Chen, J.R. Chai, J.X. Lu, L. Zhou, L.J. Gu, Z.Y. Wang, Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia, *Blood* 72 (2) (1988) 567–572.
- [6] R. Ohno, N. Asou, K. Ohnishi, Treatment of acute promyelocytic leukemia: strategy toward further increase of cure rate, *Leukemia* 17 (8) (2003) 1454–1463.
- [7] A. Kanamaru, Y. Takemoto, M. Tanimoto, H. Murakami, N. Asou, T. Kobayashi, K. Kuriyama, E. Ohmoto, H. Sakamaki, K. Tsubaki, All-trans retinoic acid for the treatment of newly diagnosed acute promyelocytic leukemia. Japan Adult Leukemia Study Group, *Blood* 85 (5) (1995) 1202–1206.
- [8] A. Takeshita, H. Sakamaki, S. Miyawaki, T. Kobayashi, K. Kuriyama, O. Yamada, H. Oh, T. Takenaka, N. Asou, R. Ohno, Significant reduction of medical costs by differentiation therapy with all-trans retinoic acid during remission induction of newly diagnosed patients with acute promyelocytic leukemia, *J. Adult Leuk Study Group. Canc.* 76 (4) (1995) 602–608.
- [9] P. Fenau, C. Chastang, C. Chomienne, S. Castaigne, M. Sanz, H. Link, B. Löwenberg, M. Fey, E. Archim-Baud, L. Degos, Treatment of newly diagnosed acute promyelocytic leukemia (APL) by all transretinoic acid (ATRA) combined with chemotherapy: the European experience, *Eur. APL Group. Leuk Lymphoma.* 16 (5-6) (1995) 431–437.
- [10] M.S. Tallman, C. Nabhan, J.H. Feusner, J.M. Rowe, Acute promyelocytic leukemia: evolving therapeutic strategies, *Blood* 99 (3) (2002) 759–767.
- [11] R. Ohno, H. Yoshida, H. Fukutani, T. Naoe, T. Ohshima, T. Kyo, N. Endoh, T. Fujimoto, T. Kobayashi, A. Hiraoka, H. Mizoguchi, Y. Kadera, H. Suzuki, M. Hirano, H. Akiyama, N. Aoki, H. Shindo, S. Yokomaku, Multi-institutional study of all-trans-retinoic acid as a differentiation therapy of refractory acute promyelocytic leukemia. Leukaemia Study Group of the Ministry of Health and Welfare, *Leukemia* 7 (11) (1993) 1722–1727.
- [12] R.P. Warrell Jr., H. de Thé, Z.Y. Wang, L. Degos, Acute promyelocytic leukemia, *N. Engl. J. Med.* 329 (3) (1993) 177–189.
- [13] L. Degos, H. Dombret, C. Chomienne, M.T. Daniel, J.M. Micléa, C. Chastang, S. Castaigne, P. Fenau, All-trans-retinoic acid as a differentiating agent in the treatment of acute promyelocytic leukemia, *Blood* 85 (10) (1995) 2643–2653.
- [14] J. Muindi, S.R. Frankel, W.H. Miller Jr., A. Jakubowski, D.A. Scheinberg, C.W. Young, E. Dmitrovsky, R.P. Warrell Jr., Continuous treatment with all-trans retinoic acid causes a progressive reduction in plasma drug concentrations: implications for relapse and retinoid "resistance" in patients with acute promyelocytic leukemia, *Blood* 79 (2) (1992) 299–303.
- [15] J.F. Muindi, C.W. Young, Lipid hydroperoxides greatly increase the rate of oxidative catabolism of all-trans-retinoic acid by human cell culture microsomes genetically enriched in specified cytochrome P-450 isoforms, *Cancer Res.* 53 (6) (1993) 1226–1229.
- [16] M. Kizaki, H. Ueno, Y. Yamazoe, M. Shimada, N. Takayama, A. Muto, H. Matsushita, H. Nakajima, M. Morikawa, H.P. Koefler, Y. Ikeda, Mechanisms of retinoid resistance in leukemic cells: possible role of cytochrome P450 and P-glycoprotein, *Blood* 87 (2) (1996) 725–733.
- [17] L. Delva, M. Cornic, N. Balitrand, F. Guidez, J.M. Micléa, A. Delmer, F. Teillet, P. Fenau, S. Castaigne, L. Degos, Resistance to all-trans retinoic acid (ATRA) therapy in relapsing acute promyelocytic leukemia: study of in vitro ATRA sensitivity and cellular retinoic acid binding protein levels in leukemic cells, *Blood* 82 (7) (1993) 2175–2181.
- [18] P.C. Adamson, J.F. Boylan, F.M. Balis, R.F. Murphy, K.A. Godwin, L.J. Gudas, D.G. Poplack, Time course of induction of metabolism of all-trans-retinoic acid and the up-regulation of cellular retinoic acid-binding protein, *Cancer Res.* 53 (3) (1993) 472–476.
- [19] W. Ding, Y.P. Li, L.M. Nobile, G. Grills, I. Carrera, E. Paietta, M.S. Tallman, P.H. Wiernik, R.E. Gallagher, Leukemic cellular retinoic acid resistance and missense mutations in the PML-RARalpha fusion gene after relapse of acute promyelocytic leukemia from treatment with all-trans retinoic acid and intensive chemotherapy, *Blood* 92 (4) (1998) 1172–1183.
- [20] K. Mehta, T. McQueen, N. Neamati, S. Collins, M. Andreeff, Activation of retinoid receptors RAR alpha and RXR alpha induces differentiation and apoptosis, respectively, in HL-60 cells, *Cell Growth Differ.* 7 (2) (1996) 179–186.
- [21] N. Agarwal, K. Mehta, Possible involvement of Bcl-2 pathway in retinoid X receptor alpha-induced apoptosis of HL-60 cells, *Biochem. Biophys. Res. Commun.* 230 (2) (1997) 251–253.
- [22] F. Pendino, C. Dudognon, F. Delhommeau, T. Sahaoui, M. Flexor, A. Bennaceur-Griscelli, M. Lanotte, E. Ségal-Bendirdjian, Retinoic acid receptor alpha and retinoid-X receptor-specific agonists synergistically target telomerase expression and induce tumor cell death, *Oncogene* 22 (57) (2003) 9142–9150.
- [23] M. Schapira, B.M. Raaka, H.H. Samuels, Abagyan R in silico discovery of novel retinoic acid receptor agonist structures, *BMC Struct. Biol.* 1 (2001) 1.
- [24] N. Shaw, M. Elholm, N. Noy, Retinoic acid is a high affinity selective ligand for the peroxisome proliferator-activated receptor beta/delta, *J. Biol. Chem.* 278 (43) (2003) 41589–41592.
- [25] L. Nagy, V.A. Thomázy, G.L. Shipley, L. Fésüs, W. Lamph, R.A. Heyman, R.A. Chandraratna, P.J. Davies, Activation of retinoid X receptors induces apoptosis in HL-60 cell lines, *Mol. Cell. Biol.* 15 (7) (1995) 3540–3551.
- [26] M.A. Sanz, D. Grimwade, M.S. Tallman, B. Lowenberg, P. Fenau, E.H. Estey, T. Naoe, E. Lengfelder, T. Büchner, H. Döhner, A.K. Burnett, F. Lo-Coco, Management of acute promyelocytic leukemia : recommendations from an expert panel on behalf of the European LeukemiaNet, *Blood* 113 (9) (2009) 1875–1891.