A novel peroxisome proliferator-activated receptor α/γ agonist, BPR1H0101, inhibits topoisomerase II catalytic activity in human cancer cells

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Peroxisome proliferator-activated receptor (PPAR) γ agonists are used clinically for treating diabetes mellitus and cancer. 2-Methyl-2[(1-{3-phenyl-7-propylbenzol[*d*]isoxazol-6-yl}oxy)propyl]-1H-4-indolyl) oxy]propanoic acid (BPR1H0101) is a novel synthetic indole-based compound, discovered through research to identify new PPARγ agonists, and it acts as a dual agonist for PPARγ and PPARα. Isobologram analysis demonstrated that BPR1H0101 is capable of antagonistic interaction with the topoisomerase (topo) II poison, VP16. A study of its mechanism showed that BPR1H0101 could inhibit the catalytic activity of topo II *in vitro*, but did not produce detectable topo II-mediated DNA strand breaks in human oral cancer KB cells. Furthermore, BPR1H0101 could inhibit VP16-induced topo II-mediated DNA cleavage and ataxia-telangiectasia-mutated phosphorylation in KB cells. The results suggest that BPR1H0101 can interfere with the topo II reaction by inhibiting catalytic activity before the formation of the intermediate cleavable complex; consequently, it can impede VP16-induced topo II-mediated DNA cleavage and cell death. This is the first identified PPARα/γ agonist that can serve as a topo II catalytic inhibitor, to interfere with VP16-induced cell death. The result might have relevance to the clinical use of the PPARα/γ agonist in combination chemotherapy. ***Anti-Cancer Drugs*** **19:151–158 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Anti-Cancer Drugs 2008, **19**:151–158

Keywords: peroxisome proliferator-activated receptor α/γ, topoisomerase II, VP16

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Received 20 March 2007 **Revised form accepted** 24 September 2007

Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily, with three ligand-activated isoforms: α, γ and δ. PPARγ is of the greatest interest among the three isoforms, as it is used pharmacologically in treating patients with type II diabetes mellitus [1] and atherosclerosis [2]. Moreover, agonist-mediated PPARγ activation exhibits antineoplastic effects through the inhibition of cell proliferation, induction of apoptosis, and terminal differentiation in many cancer cells [3–11]. A combination treatment of PPARγ agonists with other chemotherapeutic drugs or molecular targets has been shown to enhance their anticancer effects [12–14]. Taken together, these studies suggest that PPARγ could be a promising therapeutic target for the treatment of human cancers.

DNA topoisomerase (topo) II is an enzyme that plays a central role in manipulating DNA topology during transcription, replication, and chromosome conformational changes [15–19]. Topo II forms a covalent linkage to both strands of the DNA helix and catalyzes a transient DNA double-strand break (DSB), allowing for the passing of the intact double helix and religation of the cleaved DNA. This delicate and vulnerable process makes topo II an attractive target for anticancer drugs [20,21]. Agents that interfere with topo II are divided into topo poisons and catalytic inhibitors. Topo II poisons, such as etoposide (VP16), are effective anticancer agents that act by stabilizing topo II–DNA cleavage complexes, and by subsequently increasing the levels of topo II-mediated DNA strand breaks, thus converting this essential enzyme into a potent cellular toxin [21–23]. A second class of drugs that interferes with topo II activity also seems to have clinical potential [22,24]. In contrast to poisons, these agents act by inhibiting the catalytic activity of the enzyme without stimulation of DNA cleavage.

We recently designed and synthesized a series of indole-based compounds as novel PPARγ agonists, to evaluate

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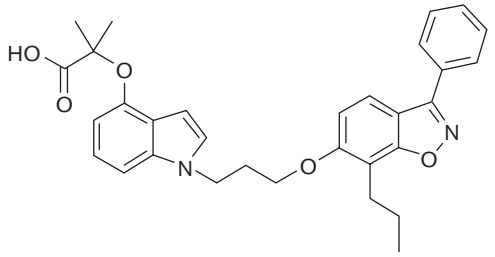
Fig. 1



Chemical structure of BPR1H0101.

their biological activities for further drug development. Among them, 2-Methyl-2[(1-{3-phenyl-7-propylbenzol [*d*]isoxazol-6-yl}oxy)propyl]-1H-4-indolyl)oxy] propanoic acid (BPR1H0101, Fig. 1) was identified as a potential lead against cancer cell growth, which could act as a dual agonist for PPARγ and PPARα. Isobologram analysis demonstrated that BPR1H0101 could prevent the effects of VP16-mediated cytotoxicity in cancer cells. Consequently, we proposed that topo II could act as a determinant for this antagonistic interaction. We showed, herein, that the PPAR agonists, BPR1H0101 and troglitazone, could inhibit topo II catalytic activity. We have further investigated the mechanism of inhibition of topo II by BPR1H0101, and have demonstrated that this compound did not induce a cleavable complex formation but inhibited the VP16-induced cleavable complex stabilization. This is the first identified PPARα/γ agonist that can serve as a topo II catalytic inhibitor to interfere with VP16-induced cell death. The result might have relevance to the clinical use of the PPARα/γ agonist in combination chemotherapy.

Methods

Drugs, enzymes, and chemicals

The compound BPR1H0101 [25] was synthesized by the Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes (Hsinchu, Taiwan). VP16, camptothecin (CPT), and troglitazone were from Sigma-Aldrich (St Louis, Missouri, USA). Purified human topo II was from TopoGEN Inc. (Port Orange, Florida, USA). Phosphospecific monoclonal antibodies against ataxia-telangiectasia-mutated (ATM) (Ser1981) and Chk1 (Ser317) were from Cell Signaling Technology Inc. (Danvers, Massachusetts, USA). Mouse antibody against β-actin was from Sigma-Aldrich. Polyclonal PPARα and PPARγ antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, California, USA).

In-vitro peroxisome proliferator-activated receptor ligand binding by scintillation proximity assay

To determine the binding affinity of the synthesized BPR1H0101 to PPARs, a scintillation proximity assay (SPA) was performed as described [26], with some modifications. Briefly, SPA binding assays were performed in binding buffer containing the recombinant ligand-binding domain of PPARα/γ/δ-linked glutathione *S*-transferase (GST) fusion protein, goat anti-GST antibodies, unlabeled BPR1H0101, and protein A-SPA beads. The receptor–ligand complex is bound to the bead through interaction between the GST-fusion receptor and anti-GST antibodies on the bead. As the scintillant is contained within the SPA bead, only the radiolabel that is attached to the bead is detected. After the addition of [^{3}H] rosiglitazone (American Radiolabeled Chemicals, St Louis, Missouri, USA) to each well, the plate was incubated at 15°C for 24 h while being shaken. Radioactivity was quantified in a Packard Topcount scintillation counter (Packard Instrument Company, Meriden, Connecticut, USA).

Peroxisome proliferator-activated receptor transactivation assay

PPAR transactivation assays were executed using luciferase reporter gene (*luc*) activity in Huh-7 transient transfectants. Transfections were performed 24 h after the seeding of Huh-7 cells at 1×10^5 cells/well in a 24-well plate. Transfection mixtures for each well contained a pcDNA3-GAL4/PPAR expression vector and a pUAS (5×)-tk-*luc* reporter vector. SV40-REN was used as an internal control for normalizing transfection efficiency. After 6 h of transfection, cells were further incubated for 24 h in the presence of various compounds at different concentrations. Luciferase activity in the cell extracts was determined by using luciferase assay kits in a SIRIUS-0 luminometer (Berthold Detection Systems, Pforzheim, Germany).

Cell cultures

Human oral cancer (KB) cells were obtained from the American Tissue Culture Collection (Manassas, Virginia, USA) and propagated in RPMI 1640 medium (Invitrogen Ltd, Gaithersburg, Maryland, USA) supplemented with 5% fetal bovine serum at 37°C in a humidified 5% CO_2 incubator.

Growth inhibition assay

KB cells in logarithmic phase were seeded into 96-well plates to adhere overnight and exposed to various concentrations of BPR1H0101 or VP16 in three replicate wells for 3 days. Control cells received dimethylsulfoxide at a concentration equal to that of drug-treated cells. The sulfurhodamine B assay was used to evaluate the test compounds on cell growth as described previously [27], as well as to determine the drug concentration that inhibited 50% of cell growth (IC_{50}).

Analysis of combined drug effects

Interaction of BPR1H0101 and VP16 in growth inhibition assays was analyzed using the isobologram method as

described previously [28]. The isobole has been used to evaluate the presence of synergism or antagonism in many fields. It requires experimental data for agents that are used alone and in different dose combinations at equally effective levels. When the dose–response curve with agents in combination displays a concave-up isobole, it indicates synergism between the two agents. Conversely, when the dose–response curve shows a concave-down isobole, it indicates antagonism between the two agents. If two agents do not interact (i.e. if their effects are additive) the line that intersects the point that corresponds with the combination is a straight line.

Topoisomerase II-mediated DNA relaxation assay

Topo II catalytic activity was measured by a DNA relaxation assay, as described [29]. Reactions contained 2 U of purified human topo II enzyme, 0.2 μg of negatively supercoiled pBR322 plasmid, and 0.5 mmol/l ATP in a total of 20 μl of reaction buffer (50 mmol/l Tris–HCl, pH 8.0; 120 mmol/l KCl; 10 mmol/l $MgCl_2$; 0.5 mmol/l DTT) including different concentrations of BPR1H0101 at 37°C for 30 min. Reactions were terminated by adding a stop solution containing 1% sarcosine and were separated on agarose gels in 1 × TBE buffer (100 mmol/l Tris–borate, 2 mmol/l EDTA, pH 8.3). Photographs of the resulting ethidium bromide-stained agarose gel were taken under ultraviolet light.

Measurement of protein-linked DNA breaks by potassium–sodium dodecyl sulfate assay

Potassium–sodium dodecyl sulfate (K–SDS) coprecipitation assays were performed to study the effects of CPT, VP16, and BPR1H0101 on protein-linked DNA break (PLDB) formation. KB cells were labeled with [^{14}C] thymidine overnight and treated with the compounds at indicated concentrations (Fig. 3) for 30 min. The reactions were stopped by adding 1 ml of prewarmed lysis solution containing 1.25% SDS, 5 mmol/l EDTA (pH 8.0), and 0.4 mg/ml salmon sperm DNA into each sample. Cell lysates were passed five times through a 22-gauge needle with a 1-ml syringe, and then heated at 65°C for 10 min. The covalent enzyme–DNA complexes were precipitated by adding KCl (325 mmol/l final concentration) to each tube. As topoisomerases form covalent bonds with DNA, treatment with SDS does not disassociate DNA from the protein. When the protein is precipitated with KCl, the covalently linked DNA is also precipitated. Pellets were washed twice with wash buffer containing 10 mmol/l Tris–HCl (pH 8.0), 100 mmol/l KCl, 1 mmol/l EDTA (pH 8.0), and 0.1 mg/ml salmon sperm DNA. Results are expressed as the ratio of [^{14}C] DNA to the total counts/min of untreated cells.

Topoisomerase II-mediated DNA cleavage assay

Evaluation of topo II-mediated DNA DSBs was as described [30]. The induction of DNA cleavage in the presence of purified recombinant topo II was measured using ^{32}P-labeled linearized pBR322 DNA. The topo II-mediated DNA cleavage reactions were performed in reaction buffer (50 mmol/l Tris–HCl, pH 7.5; 100 mmol/l KCl; 0.5 mmol/l dithiothreitol; 0.5 mmol/l EDTA; and 30 μg/ml bovine serum albumin) with [α-^{32}P]dATP-labeled DNA, VP16, BPR1H0101, and 15 U of topo II. Reactions were incubated at 37°C for 15 min, terminated by the addition of 5 μl of 5% SDS and 0.5 mg/ml of proteinase K, and incubated for 30 min more at 50°C. Topo II-mediated DNA DSB causes smaller DNA fragments to be produced, which can be detected by autoradiography, as they retain their originally labeled 3′ ends.

Inhibition of peroxisome proliferator-activated receptor γ expression by using small interfering RNA

PPARγ expression was reduced by using 200 nmol/l small interfering RNA mixes (PPARγ siRNA ON-TARGET plus SMART pool; Dharmacon, Lafayette, Colorado, USA), transfected into 30% confluent KB cells using Lipofectamine 2000 (Invitrogen, Carlsbad, California, USA). Scrambled control siRNA was purchased from MDBio Inc. (Taipei, Taiwan). Suppression of PPARγ levels was verified 72 h after the transfection by immunoblot, using a polyclonal antibody specific for PPARγ.

Immunoblotting

Whole cell extracts were separated by 5 or 8% SDS–PAGE under reducing conditions and blotted onto an equilibrated polyvinylidene difluoride membrane (Amersham Biosciences, Buckinghamshire, UK). Blots were probed with the appropriate primary antibodies and with secondary antibodies conjugated with horseradish peroxidase. Bound antibodies were detected by enhanced chemiluminescence (Millipore Corporation, Billerica, Massachusetts, USA).

Results

BPR1H0101 with potent peroxisome proliferator-activated receptor α/γ-binding and transactivation activity

The potency and selectivity of BPR1H0101 on the binding and activation of human PPARα, PPARγ, and PPARδ were determined by using SPA and a cell-based reporter gene assay (Table 1). BPR1H0101 was identified as a potent PPARα/γ dual agonist. The half-maximal effective concentrations (IC_{50}) of BPR1H0101 on transactivation of human PPARα and PPARγ were 9 and 220 nmol/l, respectively, and the concentrations required to displace 50% (IC_{50}) of tritiated ligand were 67 nmol/l for PPARα and 230 nmol/l for PPARγ. On comparison, the potencies of BPR1H0101 on PPARγ binding and transactivation are greater than that of troglitazone, a thiazolidinedione (TZD) type of PPARγ agonist (Table 1).

Table 1 In-vitro PPAR binding and transactivation of BPR1H0101 and troglitazone

	PPAR Binding[a] IC_{50} (nmol/l)			PPAR Transactivation[b] EC_{50} (nmol/l)		
Compound	α	γ	δ	α	γ	δ
BPR1H0101	67 ± 5	230 ± 20	6165 ± 350	9 ± 1	220 ± 10	ND
Troglitazone	ND	1352 ± 118	ND	ND	1610 ± 132	ND

In-vitro PPAR binding and transactivation of BPR1H0101 and troglitazone were measured by scintillation proximity assay and cell-based reporter gene transactivation assay, respectively.
[a]Concentrations of BPR1H0101 and troglitazone required to displace 50% of tritiated ligands of α, γ, and δ, respectively.
[b]Concentrations of BPR1H0101 and troglitazone that produced 50% of the maximal reporter activity in the α and γ receptors, respectively. Each value is the mean of three experiments ± SD (ND = not determined).
EC_{50}, term half-maximal effective concentrations; PPAR, peroxisome proliferator-activated receptor.

Antagonistic interaction of BPR1H0101 and VP16 in human oral epidermoid carcinoma KB cells

To determine whether the effects of BPR1H0101 on chemotherapeutic agent-induced cytotoxicity are synergistic, additive, or antagonistic, isobolograms at IC_{50} and a median effect plot were made. As shown in Fig. 2a and b, the IC_{50} values for BPR1H0101 and VP16 are 21.5 and 0.35 μmol/l, respectively, in KB cells. When treating cells with BPR1H0101 in combination with VP16, the dose–response curve showed a concave-down isobole that indicates antagonism between these two drugs (Fig. 2c). Therefore, we propose that topo II can act as a determinant for this antagonistic interaction.

BPR1H0101 inhibits topoisomerase II catalytic activity without cleavable complex formation

To investigate whether alteration of VP16 cytotoxicity by BPR1H0101 is correlated with changes in the catalytic activity of topo II, an ATP-dependent topo II-mediated DNA relaxation assay was performed. As shown in Fig. 3a, BPR1H0101 possessed potent activity in inhibiting topo II-mediated DNA relaxation. The dose-dependent progressive changes the linking numbers of supercoiled pBR322 to covalently closed circular DNA were inhibited by BPR1H0101 at concentrations ranging from 1 to 10 μmol/l, and in turn inhibited 50% of topo II catalytic activity at 5 μmol/l, with nearly 100% inhibition at 10 μmol/l (Fig. 3b). It is interesting to note that both the TZD (troglitazone) and non-TZD (BPR1H0101) type of PPARγ agonists inhibited topo II catalytic activity. The potency of BPR1H0101, however, is greater than that of troglitazone (Fig. 3a).

To clarify whether BPR1H0101 inhibits topo II-mediated DNA relaxation activity derived from stabilizing topo-DNA cleavable complexes like CPT or VP16, we performed a K–SDS coprecipitation assay to measure the production of PLDBs. As shown in Fig. 3c, after a 30-min exposure to increasing concentrations of CPT and VP16, the level of PLDBs increased in a concentration-dependent manner. BPR1H0101, however, did not produce detectable PLDBs in KB cells up to 25 μmol/l. These results strongly suggest that BPR1H0101 and VP16 act differently on topo II inhibition.

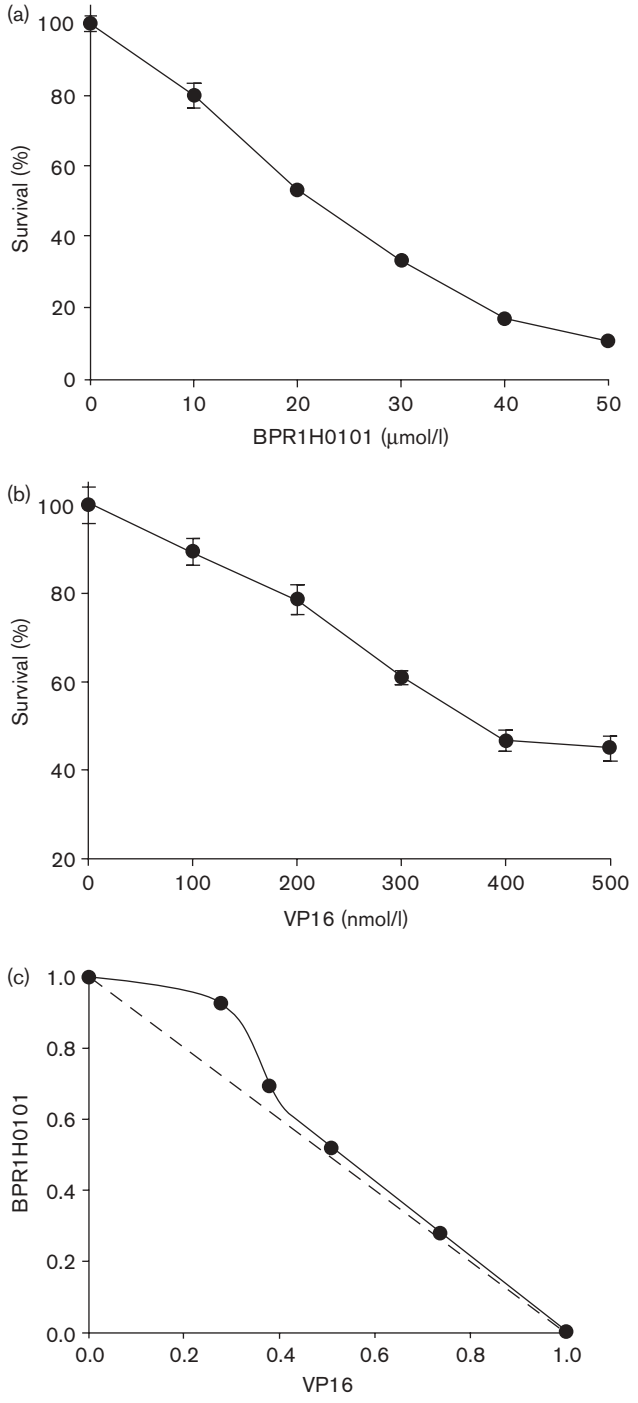
BPR1H0101 prevents VP16-induced topoisomerase II-mediated DNA cleavage and ataxia-telangiectasia-mutated Chk1 phosphorylation

Evidence that topo II catalytic inhibitors can prevent topo II poison-stimulated DNA cleavage [31] has been well documented. Therefore, we cotreated cells with BPR1H0101 and VP16 to evaluate the effect of BPR1H0101 on VP16-stimulated DNA cleavage. As shown in Fig. 4a, a baseline level of topo II-mediated DNA DSBs was observed in the absence of drug treatment. BPR1H0101 at concentrations of 1–25 μmol/l had no effect on this. This result is consistent with the lack of PLDB production in KB cells (Fig. 3c). Combined BPR1H0101 with VP16, however, diminished VP16-induced topo II-mediated DNA cleavage in a concentration-dependent manner.

In response to VP16-induced DSBs, we further verified whether the appearance of ATM and Chk1 phosphorylation could be blocked by BPR1H0101. The result showed a dramatic increase in ATM and Chk1 phosphorylation after treating the cells with VP16. BPR1H0101, however, did not induce ATM and Chk1 phosphorylation. Notably, BPR1H0101 inhibited VP16-induced phosphorylation of ATM and Chk1 in a concentration-dependent manner (Fig. 4b, scrambled siRNA). This result indicated that BPR1H0101 inhibited VP16-induced topo II-mediated DNA DSBs, and thus subsequently blocked ATM and Chk1 phosphorylation.

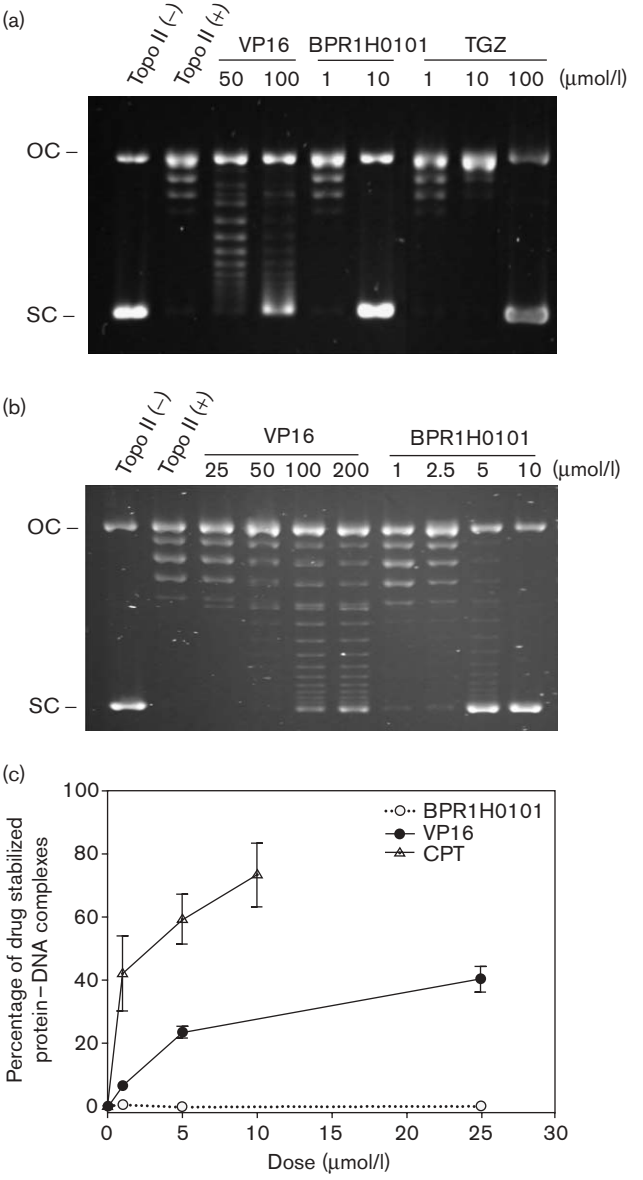
To assess whether BPR1H0101 prevents VP16-induced DNA damage through PPAR signaling, we evaluated the effects of BPR1H0101 in cells with decreasing PPARγ expression. As the KB cells only express the PPARγ isoform (Fig. 4b), we reduced the PPARγ levels in KB cells using siRNA. After siRNA transfection for 72 h, PPARγ levels were about 60% lower in the PPARγ siRNA-transfected cells, compared with those in scrambled siRNA-transfected cells (Fig. 4c). The reduced phosphorylation levels of ATM and Chk1 in cells transfected with PPARγ siRNA or scrambled siRNA were similar for a combined BPR1H0101/VP16 treatment (Fig. 4d). These findings suggest that the antagonistic effects of BPR1H0101 on VP16 might involve a PPARγ-independent mechanism.

Fig. 2



Antagonistic effects of BPR1H0101 on VP16-induced growth inhibition in KB cells by sulfurhodamine B assay. (a) Dose–response curve of BPR1H0101. (b) Dose–response curve of VP16. (c) Isobolograms for combinations of VP16 and BPR1H0101 in KB cells. Interaction between VP16 and BPR1H0101 at the point of IC_{50} were analyzed using the isobologram method [28]. Representative data from three independent experiments are shown. The dashed line on the graph connects the points at which the single drug concentrations give 50% inhibition. Combinations that have been plotted above this line are antagonistic.

Fig. 3

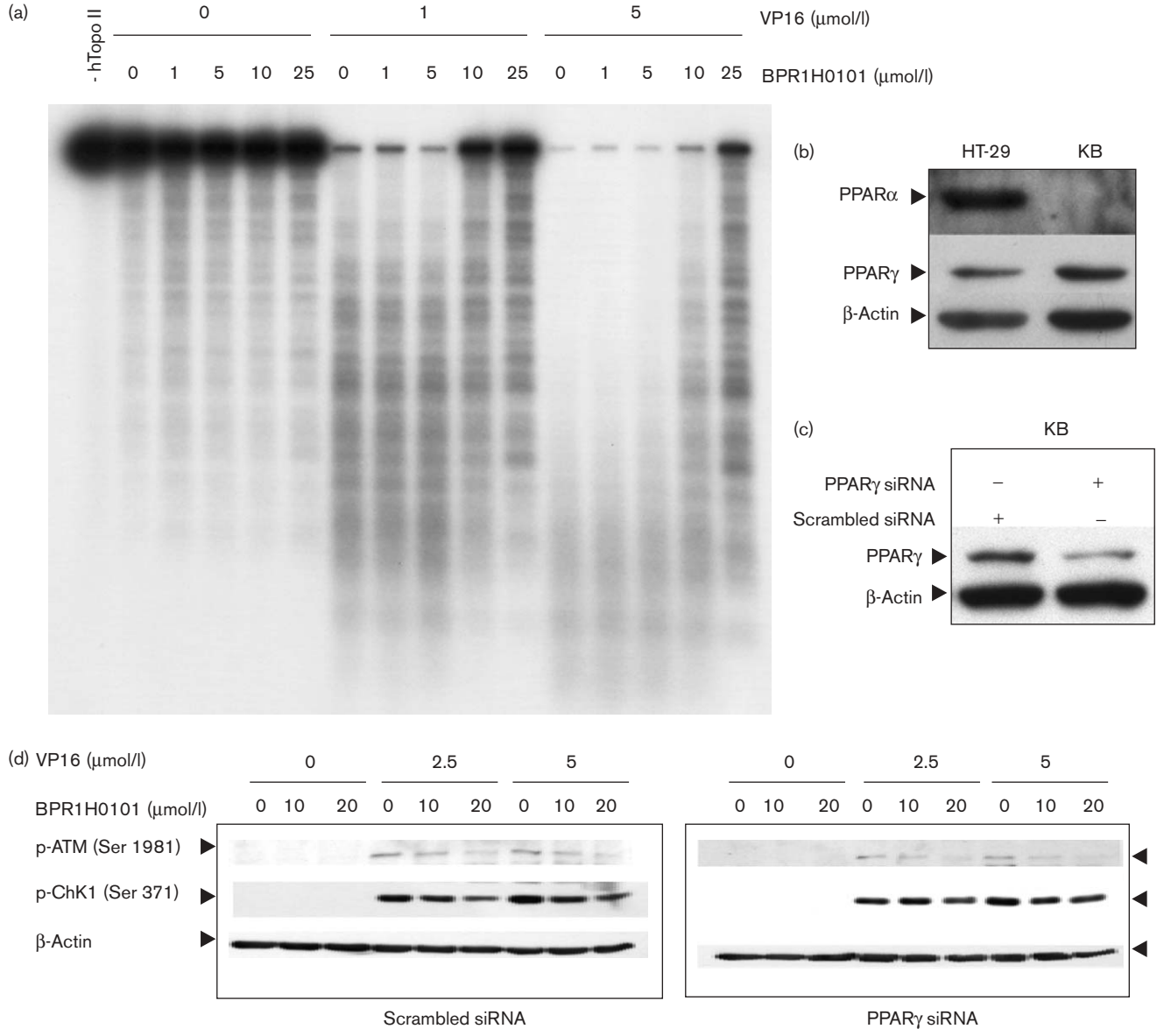


Effects of PPAR agonists on topo II catalytic activity and PLDB production in KB cells. (a) Inhibition of the conversion of supercoiled pBR322 DNA to its relaxed form by purified human topo II in the presence of the indicated concentrations of VP16, BPR1H0101, and troglitazone. (b) VP16 and BPR1H0101 inhibited topo II-mediated DNA relaxation. OC, open circular; SC, supercoiled. (c) Drug-induced PLDB production was measured using a potassium–sodium dodecyl sulfate coprecipitation assay. PLDB, protein-linked DNA break; PPAR, peroxisome proliferator-activated receptor.

Discussion

Both PPARα and PPARγ have been investigated over the past decade for their pivotal roles in the regulation of lipid, glucose metabolism, and adipocyte differentiation [1,32,33]. Ligands of PPARα andPPARγ are used clinically for treating hyperlipidemia, diabetes mellitus,

Fig. 4



Effect of BPR1H0101 on VP16-induced topo II-mediated DNA double-strand breaks and phosphorylation of ATM and Chk1 in KB cells. (a) Effect of BPR1H0101 on VP16-induced topo II-mediated DNA double-strand breaks in KB cells. DNA double-strand break assays were performed by incubating 3′-end-labeled pBR322 linear DNA with the indicated concentrations of BPR1H0101, VP16, or combinations of both compounds in the presence of 15 U of purified human topo II [30]. (b) Relative PPAR isoform expressions in KB and HT-29 cells by immunoblot. The HT-29 was used as a positive control for PPARα expression. (c) PPARγ levels in KB cells were examined after 72 h of siRNA transfection by immunoblot. (d) Effects of BPR1H0101 on VP16-induced ATM and Chk1 phosphorylation in siRNA-transfected KB cells. After transfection with scrambled siRNA or PPARγ siRNA for 48 h, cells were treated with VP16 alone or in combination with BPR1H0101 for another 24 h. Cell lysates were prepared and subjected to an immunoblot with phospho-ATM, phospho-Chk1 and β-actin. ATM, ataxia-telangiectasia-mutated; PPAR, peroxisome proliferator-activated receptor.

and atherosclerosis [2,34,35]. Aside from the metabolic actions, the anticancer activity of PPARα/γ ligands has been found both *in vitro* and in animal models of cancer [3,6,8,11,36,37]. These findings prompted us to further study the PPARα/γ agonists as a potential treatment for cancer.

BPR1H0101 is a novel synthetic indole-based PPARα/γ agonist (Table 1). The results of our research have clearly demonstrated that BPR1H0101 can inhibit purified topo II activity, after we performed the topo II-mediated DNA relaxation assay and topo II-mediated DNA cleavage assay (Fig. 3a and b). The inhibition seems to be specific to

topo II, because topo I was not inhibited by BPR1H0101 (data not shown). Drugs that interfere with topo II can be categorized as topo poisons and catalytic inhibitors. Topo II catalytic inhibitors, which are composed of a variety of structurally diverse compounds, disturb the activity of topo II by interfering with the different steps in the catalytic cycle of the enzymes, without trapping the covalent cleavable complex [24]. BPR1H0101 neither stimulated the formation of the cleavable complex in KB cells (up to 25 μmol/l) nor did it cause strand scission of DNA *in vitro* by purified topo II (Figs 3c and 4a). This is different from the topo II targeting anticancer drug, VP16, which causes enzyme-induced DNA cleavage.

ATM is a phosphatidylinositol 3-kinase-related kinase activated by autophosphorylation on Ser1981 in response to DNA damage that is induced by topo II poison. Activated ATM signals a cascade of events leading to the phosphorylation of several substrates involved in the DNA damage checkpoints, such as Chk1 and Chk2 [38–40]. A previous study had demonstrated that topo II catalytic inhibitors could prevent topo II poison-stimulated DNA cleavage [31]. A consistent hypothesis is that BPR1H0101 could inhibit VP16-induced DNA strand breaks, and thus subsequently block ATM and Chk1 phosphorylation (Fig. 4a and d). Accumulating evidence suggests that PPARγ agonists such as troglitazone and rosiglitazone mediate PPARγ-independent antitumor effects [41]. According to the RNA interference study, our result also indicated herein that BPR1H0101 attenuates VP16-induced DNA damage through PPARγ-unrelated pathway. Indeed, as subsequent reduction of ATM and Chk1 phosphorylation was also found in the combined BPR1H0101 with VP16 treatment of PPARγ siRNA-transfected cells, these findings suggest that the topo II inhibition effect of BPR1H0101 is, at least in part, carried out through a PPARγ-independent mechanism.

The process of drug-induced topo II catalytic inhibition can be quite different, depending on which steps of the topo II catalytic action are targeted. Merbarone, for example, acts by blocking DNA cleavage [42,43], and bisdioxopiperazine acts by inhibiting the intrinsic ATPase of topo II [44–46]. Bisdioxopiperazine derivatives trap the enzyme in the closed clamp form conformation around the DNA, and this might generate a physical barrier on the chromosome that impedes the actions of other DNA enzymes. Therefore, drugs that act at or before the DNA cleavage step are likely to attenuate the actions of topo II poisons. On the basis of our results, it is possible that BPR1H0101 interacted with topo II before the topo II-mediated DNA cleavage, thereby preventing the stabilization of the DNA–topo II complex induced by VP16. These results suggest that BPR1H0101 might affect the early steps in the catalytic cycles of the enzyme reaction.

In conclusion, we demonstrated for the first time that a PPAR agonist, BPR1H0101, is capable of inhibiting topo II catalytic activity without stimulation of cleavable complex formation. Furthermore, these effects might be involved in PPARγ-independent pathways. This finding provides valuable information on the clinical use of the PPARα/γ agonists in combination chemotherapy and might be significant in clinical cancer research.

Acknowledgements

This work was supported in part by grants from the National Health Research Institutes, Taipei, Taiwan (NHRI intramural Grant CA-096-PP-03), the National Science Council, Taipei, Taiwan (NSC 96-2752-B-400-001-PAE), and the C.Y. Foundation for Advancement of Education, Sciences and Medicine.

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