A natural propenoic acid derivative activates peroxisome proliferator-activated receptor-β/δ (PPARβ/δ)

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ABSTRACT

Aims: Previous studies showed that natural prenyloxyphenylpropanoid derivatives have potent biological properties *in vivo*. Given the structural similarities between these compounds and known peroxisome proliferator-activated receptor (PPAR) agonists, the present study examined the hypothesis that propenoic acid derivatives activate PPARs.

Main methods: Chimeric reporter assays were performed to identify propenoic acid derivates that could activate PPARs. Quantitative polymerase chain reaction (qPCR) analysis of wild-type and *Pparβ/δ*-null mouse primary keratinocytes was performed to determine if a test compound could specifically activate PPARβ/δ. A human epithelial carcinoma cell line and primary mouse keratinocytes were used to determine the effect of the compound on cell proliferation.

Key findings: Three of the propenoic acid derivatives activated PPARs, with the greatest efficacy being observed with prenyloxycinnamic acid derivatives 4′-geranyloxyferulic acid (compound 1) for PPARβ/δ. Compound 1 increased expression of a known PPARβ/δ target gene through a mechanism that requires PPARβ/δ. Inhibition of cell proliferation by compound 1 was found in a human epithelial carcinoma cell line.

Significance: Results from these studies demonstrate that compound 1 can activate PPARβ/δ and inhibit cell proliferation of a human skin cancer cell line, suggesting that the biological effects of 4′-geranyloxyferulic acid may be mediated in part by activating this PPAR isoform.

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Introduction

Oxyprenylated natural products (isopentenyloxy-, geranyloxy- and the less spread farnesyloxy-compounds and their biosynthetic derivatives) represent a family of secondary metabolites that were previously considered as biosynthetic intermediates of *C*-prenylated derivatives. However, recent evidence suggests that these natural products are important, biologically active phytochemicals. More than 300 related molecules have been isolated from plants primarily belonging to the families of Rutaceae, Apiaceae, and Compositae, comprising common edible vegetables and fruits, as well as from fungi and bacteria. A wide variety of compounds containing a prenyloxy side chain were isolated and these comprise alkaloids, coumarins, flavonoids, cinnamic acids, benzoic acids, phenols, alcohols, aldehydes, anthraquinones, chalcones, lignans, xanthones, aceto- and benzophenones. Many of the isolated oxyprenylated natural products

were shown to have remarkable anti-cancer, anti-inflammatory, anti-microbial and anti-fungal effects using *in vitro* and *in vivo* models. The chemistry and pharmacology of this class of natural products have been recently reviewed (Epifano et al. 2007).

Peroxisome proliferator-activated receptors (PPARs) belong to a family of nuclear receptors comprising three family members: PPARα (NR1C1), PPARβ/δ (known also as PPARβ or PPARδ) (NR1C2), and PPARγ (NR1C3). In response to ligand activation, PPARs heterodimerize with the retinoid X receptor and bind to response elements, leading to increased transcription of target genes. Genes targeted by PPARs are typically involved in the regulation of lipid and glucose homeostasis, cell proliferation and differentiation, and inflammation (reviewed in Lee et al. 2003; Peraza et al. 2006; Peters et al. 2005, 2008; Peters and Gonzalez 2009). Each PPAR isoform displays a tissue-selective expression pattern. PPARα is predominantly found in the liver, heart, kidney and muscle and is involved in the regulation of fatty acid catabolism and inflammatory processes. PPARγ is expressed in adipose, macrophages and other cell types and is known to regulate adipocyte differentiation, sensitivity to insulin and inflammation. PPARβ/δ is expressed at high levels in liver, skin and large and small

intestine (Girroir et al. 2008) and was recently shown to be an excellent target for metabolic syndrome (Barish et al. 2006). Due to their central role in lipid and glucose homeostasis, all three PPARs have been targeted for the prevention and treatment of dyslipidemias, metabolic syndrome, diabetes and cancer (reviewed in Evans et al. 2004; Lee et al. 2003; Michalik et al. 2004; Peters and Gonzalez 2009; Peters et al. 2008). Thus, it is not surprising that the search for novel, natural compounds capable of modulating the activity of PPARs is a field of growing interest. Towards this goal, the present studies were designed to evaluate whether natural and semi-synthetic prenyloxyphenylpropanoids activate PPARs to provide insight into the mechanism of biological action of these chemicals. The rationale for this evaluation was based on the recent finding that prenyloxyphenylpropanoids are chemopreventive in a rodent colon cancer model (Curini et al. 2006), and there is good evidence that activating PPARs can prevent colon tumorigenesis (Michalik et al. 2004; Peters and Gonzalez 2009; Peters et al. 2008). In particular, we focused on the following compounds (Fig. 1): prenyloxycinnamic acid derivatives 4′-geranyloxyferulic acid (compound 1) obtained from *Acronychia baueri* Schott., boropinic acid (compound 2) extracted from *Boronia pinnata* Sm., 4′-geranyloxy-*p*-coumaric acid (compound 3) obtained from *A. baueri*, 4′-isopentenyloxy-*p*-coumaric acid (compound 4) obtained from *Esenbeckia hieronymi*, and 4′-geranyloxy-dihydro-*p*-coumaric acid (compound 5) and 4′-isopentenyloxy-dihydro-*p*-coumaric acid (compound 6), which were both extracted from *Zanthoxylum pistaciflorum* Hayata (Rutaceae).

Materials and methods

Chemical synthesis

Compounds 1–6 (Fig. 1) were synthesized as previously described (Genovese et al. 2009). The purity of each compound was assessed by GC–MS (>99.5%).

Reporter assays

COS-1 cells (ATCC, Manassas, VA) were cultured in high-glucose Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Sigma, St. Louis, MO), 0.2 mg/ml streptomycin and 200 U/ml penicillin (Gibco, Grand Island, NY) on 10 cm culture dishes. When cells were ~80% confluent, they were transfected with plasmid DNA using lipofectamine reagent (Invitrogen, Carlsbad, CA) following the manufacturer's recommended

procedures. Previously described plasmids (Bility et al. 2004) containing the ligand-binding domain of mouse PPARα, PPARβ/δ or PPARγ fused to the DNA binding domain of the yeast transcription factor Gal4 under control of the SV40 promoter and another plasmid encoding the UAS-firefly luciferase reporter under control of the Gal4 DNA response element were used. After 6 h, the DNA–lipofectamine complex was removed, and the cells were allowed to recover for 1 h in culture medium. Following this recovery, the transfected COS-1 cells were split to 24 well plates. The following day, the cells were treated with culture medium containing the compounds of interest at concentrations of 10, 50, or 100 μM and a positive control (PPARα-50 μM Wy-14,643, PPARβ/δ-0.2 μM GW0742, or PPARγ-3 μM troglitazone) to evaluate PPAR activation. Six hours after treatment, the plates were washed and treated with 1X passive lysis buffer (Promega, Madison, WI), rocked for 1 h and then placed in a −80 °C freezer overnight. Luciferase activity was measured using the Luciferase reporter assay kit (Promega, Madison, WI) and a Turner TD-20/20 Luminometer (Turner BioSystems, Sunnyvale, CA) following the manufacturer's recommended procedures. The protein concentration of the cell lysate was determined using the BCA protein assay kit (Pierce, Rockford, Illinois) following the manufacturer's protocol. Luciferase activity was normalized to the protein concentration of each sample. The fold induction of normalized luciferase activity was calculated relative to DMSO (vehicle)-treated cells, and represents the mean of three independent samples per treatment group.

RNA analysis and quantitative real-time quantitative polymerase chain reaction

Primary mouse keratinocytes from wild-type and *Pparβ/δ*-null mice were isolated from 2-day-old neonates as described previously (Kim et al. 2006). Keratinocytes were cultured in low calcium (0.05 mM) Eagle's minimal essential medium with 8% chelexed fetal bovine serum at 37 °C and 7% CO_2 (Kim et al. 2006). The keratinocytes were cultured until they were approximately 80% confluent and were then treated with 0.1% DMSO, 0.2 μM GW0742, and two concentrations of compound 1 (10 μM or 100 μM). After 8 h of treatment, total mRNA was isolated from the cells using RiboZol and following manufacturer's recommended protocol (AMRESCO, Solon, OH). The mRNA encoding *Angiopoietin-like 4* (*Angptl4*), *acyl CoA oxidase* (*Aco*), or *Kruppel-like factor 4* (*Klf4*) was quantified using quantitative real-time polymerase chain reaction (qPCR) analysis. The cDNA was generated using 2.5 μg total RNA with MultiScribe Reverse Transcriptase kit (Applied Biosystems, Foster City, CA). Primers were
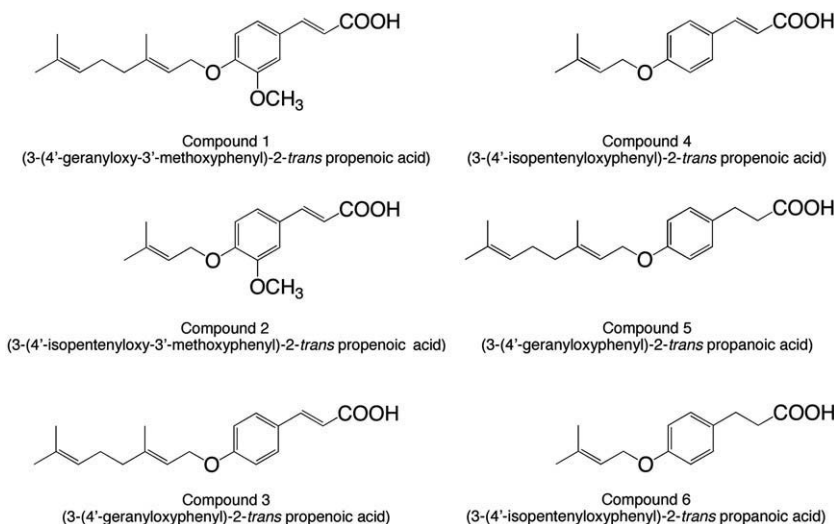

Fig. 1. Structure of compounds examined for PPAR activity.

designed for qPCR using the Primer Express software (Applied Biosystems, Foster City, CA). The sequence and GenBank accession number for the forward and reverse primers used to quantify mRNAs were: *Angptl4* (NM_020581) forward, 5′-TTCTCGCCTACCAGA-GAAGTTGGG-3′ and reverse, 5′-CATCCACAGCACCTACAACAGCAC-3′; *Aco* (NM_004035) forward, 5′-TGCTGATGAAGTATGCCCAGGTGA-3′ and reverse, 5′-TCCCACAAGGAAGGACCTGACAAA-3′; and *Klf4* (NM_004235) forward, 5′-TGACCAGGCACTACCGTAAACACA-3′ and reverse, 5′-TCTTCATGTGTAAGGCGAGGTGGT-3′. The mRNA was normalized to the gene encoding *glyceraldehyde 3-phosphate dehydrogenase* (*Gapdh*; BC083149) using the following primers: forward, 5′-GGTGGAGC-CAAAAGGGTCAT-3′ and reverse, 5′-GGTTCACACCCATCACAAACAT-3′. qPCR reactions were carried out using SYBR green PCR master mix (Finnzymes, Espoo, Finland) in the iCycler and detected using the MyiQ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). The following conditions were used for PCR: 95 °C for 15 s, 94 °C for 10 s, 60 °C for 30 s and 72 °C for 30 s and repeated for 45 cycles. The PCR included a no template control reaction to control for contamination and/or genomic amplification. All reactions had >95% efficiency. Relative expression levels of mRNA were normalized to *Gapdh* and analyzed for statistical significance using one-way analysis of variance (Prism 5.0, GraphPad Software Inc., La Jolla, CA).

Cell culture and cell proliferation analysis

A human epithelial carcinoma cell line (A431) was obtained from American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum at 37 °C and 5% CO_2. For proliferation assays, cells were plated on 12 well dishes at a density of ~40,000 cells per well 24 h prior to determining plating efficiency with a Z1 coulter particle counter® at time 0 (Beckman Counter, Hialeah, FL). After plating efficiency was determined cells were treated for 24, 48, and 72 h with 0.1% dimethyl sulfoxide control, 10 μM GW0742, 50 μM compound 1, or 100 μM compound 1. Culture medium was changed every 24 h. Cells were quantified every 24 h with a Z1 coulter particle counter®. Triplicate samples for each treatment were used for each time point for every treatment, and each replicate was counted three times.

Mouse primary keratinocytes from wild-type and *Pparβ/δ*-null mice were isolated and cultured as previously described (Borland et al. 2008) in medium with 0.1% dimethyl sulfoxide control, 1 μM GW0742, 25 μM compound 1 or 100 μM compound 1. Cell proliferation was quantified by counting three independent replicates per treatment at each timepoint using a Z1 coulter particle counter® (Beckman Counter, Hialeah, FL) as previously described (Borland et al. 2008).

Results

Activation of PPARs by substituted prenyloxyphenylpropanoids

A series of differentially substituted prenyloxyphenylpropanoids (compounds 1–6) with structural similarities to PPAR ligands were examined for their ability to activate mouse PPARs. The two propenoic acid compounds (compounds 1 and 2) contain a methoxyphenyl group activated mouse PPARs with the greatest efficacy. Compound 1 activated PPARα, PPARβ/δ and PPARγ, and the efficacy was greater for PPARβ/δ as compared to PPARα and PPARγ (Fig. 2). Compound 2 also activated all three PPARs, and the efficacy was greater for PPARα and PPARβ/δ as compared to PPARγ (Fig. 1). Compound 3 activated PPARα at concentrations greater than 50 μM (Fig. 2). Compounds 4–6 did not cause a significant increase in PPAR activity (Fig. 1). Interestingly, compound 5 significantly repressed PPARα and PPARβ/δ activity in a dose dependent manner (Fig. 2). Since compound 1 caused a significant increase in PPARβ/δ activity with the greatest efficacy, this compound was examined further in other assays.

Compound 1 causes a PPARβ/δ-dependent increase in gene expression

Activation of PPARβ/δ with the known PPARβ/δ ligand GW0742 caused a marked increase in expression of mRNA encoding *Angptl4* in wild-type mouse keratinocytes that was not seen in similarly treated *Pparβ/δ*-null keratinocytes (Fig. 3A). Culturing wild-type mouse keratinocytes with compound 1 at a concentration of 10 μM caused a modest increase in the expression of *Angptl4* mRNA, an effect that was not found in *Pparβ/δ*-null keratinocytes (Fig. 3A). Culturing wild-type mouse keratinocytes with compound 1 at a concentration of 100 μM caused a marked increase in the expression of *Angptl4* mRNA comparable to that observed with GW0742, and this change was not observed in *Pparβ/δ*-null keratinocytes (Fig. 3A). Culturing wild-type keratinocytes in the presence of compound 1 caused no change in expression of the PPARα target gene *Aco* or the PPARγ target gene *Klf4* (Fig. 3B, C).

Compound 1 inhibits cell proliferation of a human epithelial carcinoma cell line

Ligand activation of PPARβ/δ has been shown to inhibit cell proliferation in many different cell types including cancer cells (Peters and Gonzalez 2009). Since compound 1 can activate PPARβ/δ in a mouse primary keratinocytes, cell proliferation was examined in a human epithelial carcinoma cell line in response to compound 1. No differences in cell proliferation were observed after 24 or 48 h between any of the treatment groups (Fig. 4A). However, inhibition of cell growth was observed in A431 cells after 72 h following treatment with 100 μM compound 1, similar to results observed with the positive control PPARβ/δ ligand GW0742 (Fig. 4A). To determine if inhibition of cell proliferation by compound 1 was mediated by PPARβ/δ, cell proliferation was examined in wild-type and *Pparβ/δ*-null mouse primary keratinocytes. Ligand activation of PPARβ/δ with 1 μM GW0742 caused inhibition of cell proliferation in wild-type keratinocytes after 48 and 72 h of culture, and this effect was not found in similarly treated *Pparβ/δ*-null mouse primary keratinocytes (Fig. 4B). No change in cell proliferation was found in keratinocytes following treatment with 25 μM compound 1. However, inhibition of cell proliferation was found in wild-type keratinocytes after 24 h of treatment with 100 μM compound 1 and this effect was not found in similarly treated *Pparβ/δ*-null keratinocytes (Fig. 4B). Interestingly, after 48 and 72 h of culture, inhibition of cell proliferation was observed in both genotypes of keratinocytes treated with 100 μM compound 1 (Fig. 4B).

Discussion

One of the main representative natural compounds belonging to the class of prenyloxyphenylpropanoids, auraptene (Fig. 5), was previously shown to activate both PPARα and PPARγ (Kuroyanagi et al. 2008; Takahashi et al. 2008). In HepG2 cells, auraptene increased PPARα-dependent reporter activity and increased expression of well-characterized PPARα target genes (Takahashi et al. 2008). Since the increase in PPARα target gene expression was only observed in HepG2 cells that over-expressed PPARα, this demonstrates some specificity for this PPAR isoform in mediating this effect. Similarly, others also showed that auraptene could activate both PPARα and PPARγ based on reporter assays and modulation of both PPARα and PPARγ target gene expression (Kuroyanagi et al. 2008). Interestingly, in these studies, the changes in expression of known PPARγ targets including aP2 and MCP1 were diminished in the presence of a PPARγ antagonist demonstrating that PPARγ was required for these effects (Kuroyanagi et al. 2008). These observations collectively support the idea that PPARs may mediate the effect of natural prenyloxyphenylpropanoids.

It was recently shown that auraptene and another structurally related compound, 4′-geranyloxyferulic acid (compound 1; Figs. 1, 5),
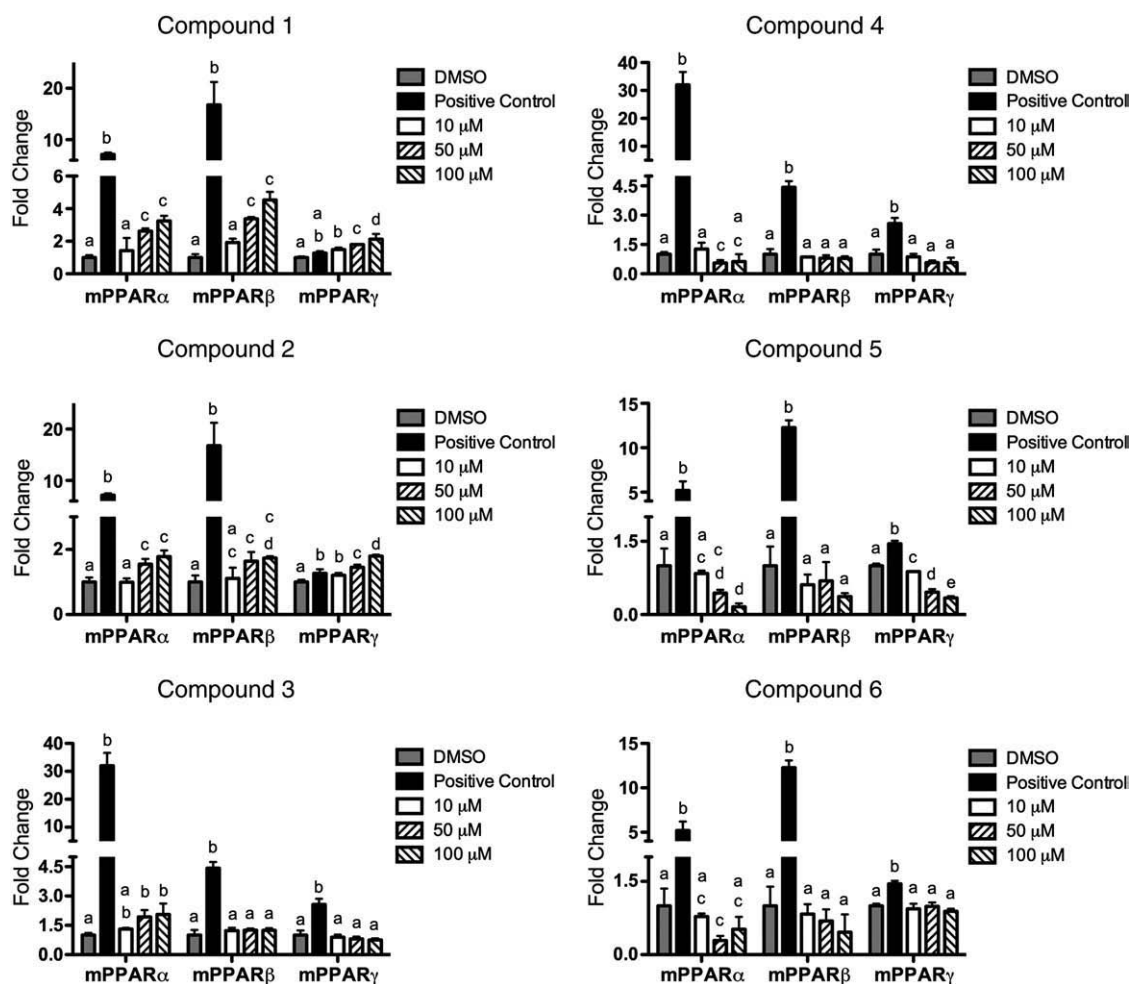
Fig. 2. Activation of mouse PPARα, PPARβ/δ, and PPARγ by differentially substituted prenyloxyphenylpropanoids. Transfected COS-1 cells containing chimeric mouse PPAR reporter plasmids were treated with the indicated concentration of prenyloxyphenylpropanoid compounds or positive control (50 μM Wy-14,643 (Wy), 0.2 μM GW0742, or 3 μM troglitozone). The fold induction of normalized luciferase activity was calculated relative to DMSO (vehicle)-treated cells and represents the mean of three independent samples per treatment group; P values were calculated with a one-way ANOVA using Tukey's multiple comparison test. Values within a row with different superscripts are significantly different at $P \leq 0.05$.

have potent chemopreventive activities in a rodent colon cancer model (Tanaka et al. 2010). There is compelling evidence that activating either PPARγ and/or PPARβ/δ can be chemopreventive in colon cancer (Harman et al. 2004; Marin et al. 2006; Michalik et al. 2004; Peters and Gonzalez 2009; Peters et al. 2008). Thus, the present study examined the idea that 4′-geranyloxyferulic acid and other five natural prenyloxycinnamic acids may activate PPARs (Fig. 1). Initial screening for PPAR activity from these six compounds with three different concentrations (10, 50, and 100 μM) showed that only compounds with a methoxy group in position 3 of the aromatic ring are able to activate PPARs with some specificity for PPARβ/δ, while the other four compounds lacking this moiety are essentially unable to activate PPARs. This outcome provides a basis to begin to establish a structure–activity relationship. Although all of the six compounds examined have a lipophilic side chain differing only in the length, the presence of a long hydrophobic chain resembling that of fatty acids, does not appear to be a crucial structural requirement for activating PPARs in the case of prenyloxycinnamic acids. This is of interest because a long hydrophobic side chain is a common structural characteristic of fatty acids, which are well known natural ligands of PPARs (Forman et al. 1997). This may be due to the fact that all compounds tested are planar rigid molecules with low conformational mobility, which may prevent the interaction between the oxyterpenyl side chain and the hydrophobic cluster that represents a peculiar feature of all isoforms of PPARs (O'Sullivan 2007). On the other hand, the presence of a methoxy group in the aromatic portion of each molecule seems to provide some selectivity towards PPARβ/δ. This moiety could allow for interaction with a key portion of the receptor ligand-binding domain allowing the lipophilic chain to be oriented in such a way to interact with the hydrophobic cluster of these receptors. Thus, a methoxy 3-substituted aromatic ring might be used in the future as a structural requirement for the design and synthesis of novel selective PPARβ/δ ligands. Comparing the structure of compounds 1 and 2 with that of the selective PPARβ/δ ligand GW0742 (Fig. 5), some structural similarities can be observed. For example, the presence of an oxyacetic acid moiety and a lipophilic group in position 4 of GW0742 closely resemble the α,β-unsaturated carboxylic acid and the O-side chain of compounds 1 and 2, respectively. Moreover it is of interest to note that although sharing some structural similarities with auraptene, like the terpenyloxy side chain, auraptene is reported to activate only PPARα and PPARγ, while compound 1 (4′-geranyloxyferulic acid) and compound 2 (boropinic acid) was capable of activating PPARα and PPARβ/δ, with greater efficacy for PPARβ/δ.

Results from these studies clearly establish that 4′-geranyloxyferulic acid can increase expression of the PPARβ/δ target gene *Angptl4* and that this effect requires a functional receptor since this change was not found in keratinocytes lacking expression of PPARβ/δ. This observation is consistent with reporter assays and provides convincing evidence that PPARβ/δ can mediate the biological effects
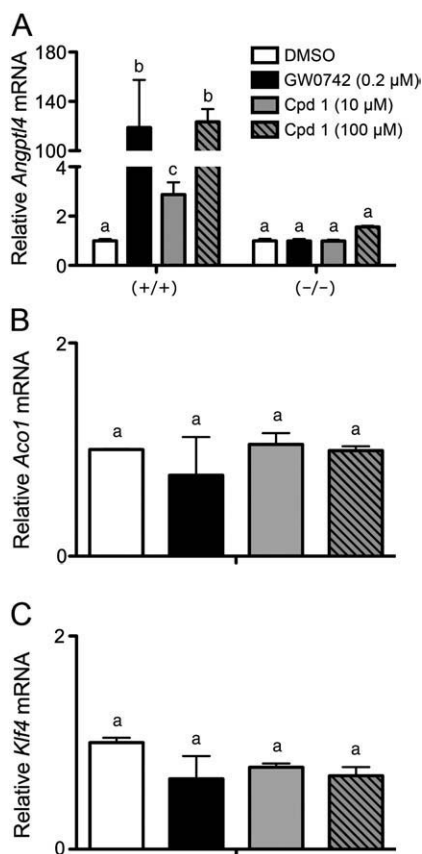
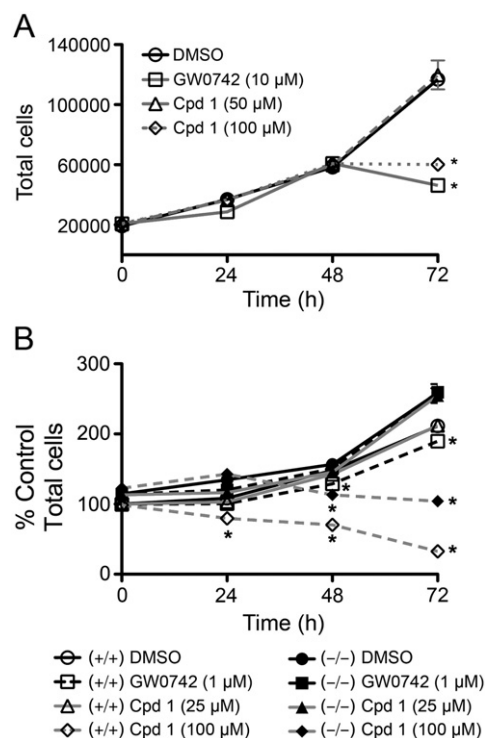**Fig. 3.** Activation of PPARβ/δ target gene expression in mouse primary keratinocytes Wild-type (+/+) and/or Pparβ/δ-null (−/−) keratinocytes were cultured as described and treated with the indicated concentration of 4′-geranyloxyferulic acid (compound 1; Cpd 1) or a positive control (0.2 μM GW0742). (A) PPARβ/δ-dependent increase in *Angiopoietin-like 4* (*Angplt4*), (B) lack of change in expression of the PPARα target gene *acyl CoA oxidase I* (*Aco1*) in wild-type keratinocytes, and (C) lack of change in expression of the PPARγ target gene *Kruppel-like factor 4* (*Klf4*) in wild-type keratinocytes. Values represent the mean ± SEM and were calculated from three independent samples per treatment. Values with different letters are significantly different at $P<0.05$.

of 4′-geranyloxyferulic in a cell type that expresses high levels of PPARβ/δ (Girroir et al. 2008). To examine the functional significance of the ability of 4′-geranyloxyferulic acid to activate PPARβ/δ in an epithelial cell, the effect of this compound on cell proliferation was examined in a human epithelial carcinoma cell line. Consistent with results from previous studies (reviewed in Peters and Gonzalez 2009), activating PPARβ/δ in A431 cells caused inhibition of cell proliferation, similar to that observed with the PPARβ/δ ligand GW0742. However, inhibition of cell proliferation was also found in primary keratinocytes lacking expression of PPARβ/δ. Thus, the inhibitory effects of 4′-geranyloxyferulic acid are likely influenced by other PPARβ/δ-independent mechanisms as well.

Conclusions

Results from these studies clearly establish for the first time that 4′-geranyloxyferulic acid (compound 1) can activate PPARβ/δ in keratinocytes and inhibit cell proliferation of a human epithelial carcinoma cell line. Since PPARβ/δ-dependent inhibition of cell proliferation is also observed in keratinocytes after 24 h of treatment, these findings are consistent with previous studies showing that PPARβ/δ inhibits cell proliferation in mouse and human keratinocytes/skin (Borland et al. 2008; Burdick et al. 2007; Chong et al. 2009; Kim et al. 2004, 2006, 2005; Kippenberger et al. 2001; Man et al. 2007; Michalik et al. 2001; Peters et al. 2000). However, the growth

Fig. 4. Effect of 4′-geranyloxyferulic acid on cell growth in mouse primary keratinocytes and a human epithelial carcinoma cell line. Wild-type (+/+) and Pparβ/δ-null (−/−) keratinocytes or A431 cells were cultured as described and treated with the indicated concentration of 4′-geranyloxyferulic acid (compound 1; Cpd 1) or a positive control (GW0742). (A) Compound 1 (4′-geranyloxyferulic acid) inhibits cell proliferation in a human epithelial carcinoma cell line. A431 cells were cultured for 72 h in the presence or absence of compound 1 (4′-geranyloxyferulic acid) or GW0742 at the indicated concentrations. Cell number was quantified every 24 h. (B) Primary keratinocytes were cultured for 72 h in the presence or absence of compound 1 (4′-geranyloxyferulic acid) or GW0742 at the indicated concentrations. Cell number was quantified every 24 h. 1 μM GW0742 inhibits cell proliferation in (+/+) mouse primary keratinocytes but not in (−/−)mouse primary keratinocytes after 48 and 72 h. 100 μM compound 1 (4′-geranyloxyferulic acid) inhibits cell proliferation in (+/+) mouse primary keratinocytes but not in (−/−)mouse primary keratinocytes after 24 h. 100 μM compound 1 (4′-geranyloxyferulic acid) inhibits cell proliferation in both genotypes after 48 and 72 h. Values represent the mean ± SEM and were calculated from three independent samples per treatment. *Significantly less than control at $P \leq 0.05$.

inhibitory effects of 4′-geranyloxyferulic acid are also likely mediated by other PPARβ/δ-independent mechanisms since inhibition of cell proliferation is found in keratinocytes lacking expression of PPARβ/δ after 48 and 72 h of exposure. It is likely that the PPARβ/δ-
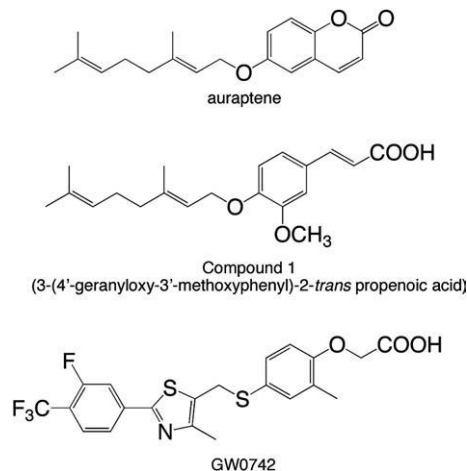
Fig. 5. Comparison of structures between auraptene, GW0742 and compound 1 (4′-geranyloxyferulic acid).

independent inhibition of cell proliferation induced by geranyloxyferulic acid (compound 1) mask PPARβ/δ-dependent mechanisms that also facilitate inhibition of cell growth. Nevertheless, natural prenyloxycinnamic acids represent a new and novel class of PPARβ/δ ligands. This class of compounds are easily synthesized from widely available and non-toxic materials using inexpensive approaches resulting in high-yielding, environmentally friendly compounds. Alternatively, the active natural products can be easily extracted from edible fruits and vegetables. Results from these studies provide a rationale to develop new semi-synthetic prenyloxycinnamic acids capable of activating PPARβ/δ.

While the role of PPARβ/δ in carcinogenesis remains controversial, there is a large body of evidence that this receptor can prevent tumorigenesis including colon cancer (reviewed in Peters and Gonzalez 2009; Peters et al. 2008). These observations are consistent with the known ability of PPARβ/δ to mediate the induction of terminal differentiation, inhibit cell growth and modulate anti-inflammatory activities (reviewed in Kilgore and Billin 2008; Peters and Gonzalez 2009; Peters et al. 2008). This is of particular interest because it was recently demonstrated that 4′-geranyloxyferulic acid inhibits azoxymethane-induced colon cancer in mice through mechanisms that include attenuation of inflammatory signalling (Tanaka et al. 2010). While the present studies did not specifically examine the chemopreventive effects of this compound, whether the mechanism of the reported colon cancer chemopreventive action of 4′-geranyloxyferulic acid is mediated (at least in part) by activation of PPARβ/δ should be examined since it has been shown that activating PPARβ/δ with GW0742 inhibits azoxymethane-induced colon cancer in mice through a mechanism that requires PPARβ/δ (Hollingshead et al. 2008; Marin et al. 2006). If future studies demonstrate a definitive role for PPARβ/δ in mediating the chemopreventive effects of 4′-geranyloxyferulic acid, development and characterization of more active and selective agents based on the structure of prenyloxycinnamic acid that can activate PPARβ/δ, and other inhibitory pathways, could lead to more effective chemopreventive strategies.

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