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SHORT COMMUNICATION

Design, Synthesis, and Biological Evaluation of Resveratrol Derivatives as PPARα Agonists

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Abstract The peroxisome proliferator-activated receptor subtype α (PPARα) was established as a molecular target in drug discovery research for new lipid-lowering drugs. Pterostilbene is a naturally occurring PPARα agonist that has been shown to lower plasma lipid concentrations via the activation of PPARα. In this study, various pterostilbene conjugates with methyl, amino acid, and pivaloxymethyl (POM) groups at the 4-OH position were synthesized, and the activating effect on PPARα were investigated. Of the conjugates investigated, 4-OMe-pterostilbene had lower activating effect than pterostilbene, but the pterostilbenes with either amino acid (4a and 4b) or POM moiety (5) showed a small but significant increase in PPARα activation of PPARα activity compared to pterostilbene. Therefore, the structure-activity relationship of the pterostilbene conjugates studied indicates that substitution of the free 4-OH moiety of pterostilbene with a nonmethyl group can increase PPARα agonistic activity. This finding warrants further investigation of the structure-activity relationship of the pterostilbene conjugates as potent PPARα agonists.

Keywords Agonist · Peroxisome proliferator-activated receptor alpha (PPARα) · Pivaloxymethyl (POM) · Pterostilbene

The peroxisome proliferator-activated receptors (PPARs) belong to a family of fatty acid-activated nuclear receptors that function as key regulators of glucose, lipid, and cholesterol metabolism (Kliewer et al., 1997). Moreover, the PPAR subtypes, PPARγ, α, and δ, have been established as molecular targets in drug discovery research for new antidiabetic agents, lipid-lowering drugs, and drugs that reverse cholesterol transport and affect high-density lipoprotein metabolism, respectively (Wilson and Wahli, 1997).

PPARα is expressed in the liver, heart, and muscle cells and is involved in the metabolism of fatty acids and lipids via the regulation of genes involved in fatty acid β-oxidation. A wide range of synthetic as well as naturally occurring agonists are known to bind to the large multifunctional ligand-binding region of PPARα (Xu et al., 1999). Ligands include fibrates, which are used to treat hypercholesterolemia due to their triglyceride-lowering and HDL-cholesterol-increasing effects; these drugs are synthetic agonists with low affinity for the PPARα receptor (Desai et al., 2006). Among the naturally occurring PPARα agonists is pterostilbene (2, Fig. 1), a naturally occurring dimethylether of resveratrol (1, Fig. 1), which has been shown to lower plasma lipid concentrations via activation of PPARα in animal models (Rimando et al., 2005). Recently, Mizuno *et al*., (2008) investigated the structure-activity relationship of certain pterostilbene derivatives and reported that, among the pterostilbene derivatives investigated, only pterostilbene phosphate increased PPARα activity (~6-fold). Moreover, the PPARα-activating effect of pterostilbene phosphate was only observed at high concentration (100 μM, suggesting that further structure-activity relationship studies are necessary to establish the PPARα-activating effects of the pterostilbene derivatives, including the introduction of polar as well as nonpolar substitutes to the free phenolic OH group of the parent compound (Fig. 1). In a previous study, we have shown that after conjugation with the naturally occurring flavonoid quercetin, both amino acid (Kim et al., 2009) and pivaloxymethyl (POM) (Kim et al., 2010) moieties can impart the quercetin conjugate with significantly enhanced biological activity. Therefore, in the present study, we sought to further investigate the structure-activity relationship of pterostilbene by introducing amino acid moieties (4a and 4b, Fig. 1) and a POM group (5, Fig. 1).

Herein, we report the synthesis and PPARα-activating effects of pterostilbene methyl ether (3), and certain pterostilbene conjugates with an amino acid (4a and 4b) and a POM moiety (5).

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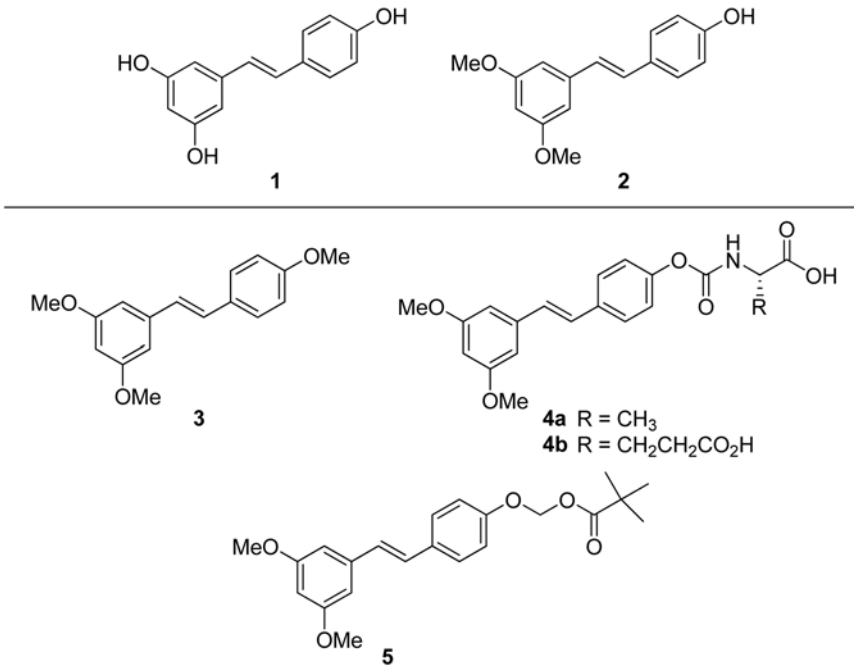

Fig. 1 Structures of resveratrol (1), pterostilbene (2), and pterostilbene conjugates (3–5).

Pterostilbene (2) was synthesized through the Wittig reaction (Pettit et al., 2002) of the phosphonium salt (7) and *p*-methoxymethyloxybenzaldehyde (9), which were synthesized from the commercially available precursors 3,5-dihydroxybenzoic acid (6) and *p*-hydroxybenzaldehyde (8) (Fig. 2). The Wittig reaction produced a mixture of *cis*/*trans* isomers which were separated by column chromatography on silica gel with the *cis*-isomer being eluted first at 42% yield followed by the *trans*-isomer. The identification of *cis*- and *trans*- stereochemistries were performed by comparison of the coupling constants of the vinyl protons in their ^{1}H NMR spectra (12.5 and 16.2 Hz for *cis* and *trans* isomers, respectively). Alkylation of pterostilbene (2) with methyl iodide, activated amino acid *tert*-butyl esters (11a and 11b), and POM-iodide (under basic conditions) were used to synthesize the corresponding methyl ether (3), amino acid (4a and 4b), and POM conjugates (5), respectively.

In accordance with previously reported cell-based assay protocols of Cho et al. (2005), the synthesized pterostilbene conjugates (3~5) were evaluated for their activity as PPARα agonists in HEK 293 cells at 10 μM. Briefly, HEK 293 cells were seeded into COSTAR tissue culture-treated clear bottom 24-well plates at a density of 1.5×10^5 cells/well in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% charcoal dextran-treated fetal bovine serum (FBS). The cells were then transfected with a PPARα expression plasmid, a PPRE reporter vector, and a pRL (renilla luciferase control reporter) vector by using a transfection reagent. Resveratrol (1), pterostilbene (2), and the pterostilbene conjugates (3~5) dissolved in dimethyl sulfoxide (DMSO) (10 μM) were added to the media. After incubation for 12 h under CO_2, the cells were lysed, and the amount of firefly luciferase activity detected was normalized to renilla luciferase activity. The extent of induction of luciferase activity was calculated relative to that in the vehicle (DMSO)-treated cells, and each result is the mean of three independent experiments. A comparison of the PPARα-activating effects of the pterostilbene conjugates with those of resveratrol (1) and pterostilbene (2) is shown in Fig. 3.

All pterostilbene derivatives investigated in the present study increased PPARα activity in the cell-based assay used (Fig. 3). In particular, methyl-substitution around the resveratrol (1, FI = 8.5, Fig. 3) scaffold provided the corresponding pterostilbene derivatives pterostilbene (2, FI = 17.1) and 4-methylpterostilbene (3, FI = 15.2) with increased PPARα agonistic activity. However, it should be noted that while pterostilbene (2), a 3,5-dimethyl ether of resveratrol, showed a two-fold increase in PPARα activation (FI = 17.1) compared to resveratrol (1, FI = 8.5), further methylation at the 4-position of the pterostilbene scaffold resulted in slightly low induction of PPARα activity (3, FI = 15.2). In contrast, the pterostilbenes conjugated with amino acids (4a and 4b) or POM moieties (5) showed small but significant increases in PPARα activation. Moreover, the pterostilbene-amino acid conjugates (4a and 4b) showed a similar level of PPARα activation regardless of the amino acid side chain (FI = 18.4 for 4a [R = CH_3], FI = 18.9 for 4b [R = $CH_2CH_2CO_2H$]). Finally, the pterostilbene-POM conjugate (5) showed the highest activation, with a 19-fold increase.

In summary, this structure-activity relationship study of synthesized pterostilbene conjugates indicates that substitution of the free 4-OH moiety of the pterostilbene scaffold with a nonmethyl group can increase PPARα agonistic activity. This

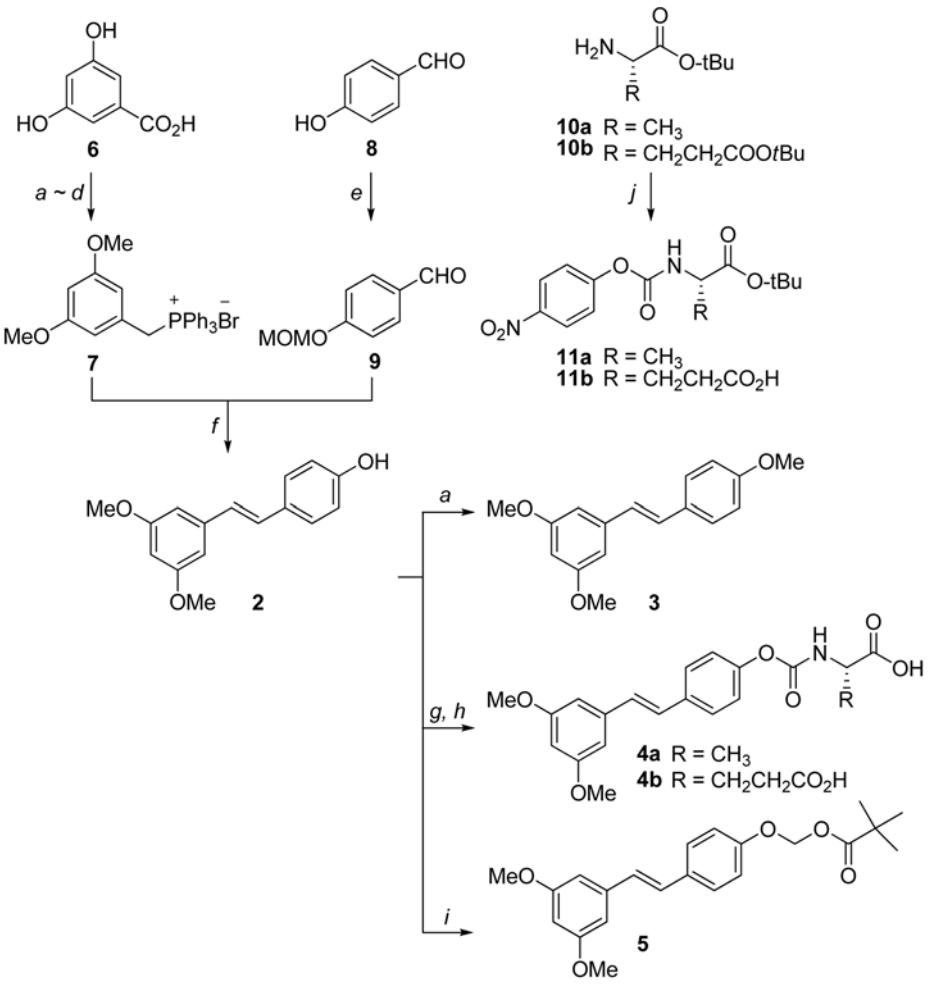

Fig. 2 Synthesis of the pterostilbene conjugates **3**–**5**. *Reagents and conditons*: a) Me_2SO_4, K_2CO_3, acetone, 65°C; b) LAH, THF, 0°C to rt; c) CBr_4, PPh_3, 0°C to rt; d) PPh_3, toluene, 110°C; e) MOMCl, K_2CO_3, acetone, rt; f) i) n-BuLi, THF, −78°C; ii) TFA, CH_2Cl_2, 0°C to rt; g) **11a** or **11b**, DIPEA, DMF, 0°C to rt; h) TFA, CH_2Cl_2, 0°C to rt; i) POMI, K_2CO_3, acetone, rt; j) $(4\text{-}NO_2\text{-}PhO)_2CO$, DIPEA, THF/DMF, 0°C to rt.

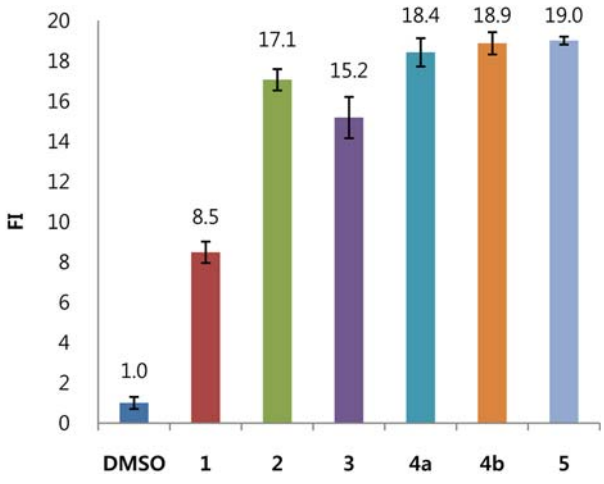

Fig. 3 Effects of 10 μM resveratrol (1), pterostilbene (2), and pterostilbene derivatives (3–5) on PPARα activation in a cell-based assay (DMSO = Control, FI = Fold induction).

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