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Diallyl Biphenyl-Type Neolignans Have a Pharmacophore of PPARα/γ Dual Modulators

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Abstract

Adiponectin secretion-promoting compounds have therapeutic potentials in human metabolic diseases. Diallyl biphenyl-type neolignan compounds, magnolol, honokiol, and 4-*O*-methylhonokiol, from a *Magnolia officinalis* extract were screened as adiponectin-secretion promoting compounds in the adipogenic differentiation model of human bone marrow mesenchymal stem cells (hBM-MSCs). In a target identification study, magnolol, honokiol, and 4-*O*-methylhonokiol were elucidated as PPARα and PPARγ dual modulators. Diallyl biphenyl-type neolignans affected the transcription of lipid metabolism-associated genes in a different way compared to those of specific PPAR ligands. The diallyl biphenyl-type neolignan structure provides a novel pharmacophore of PPARα/γ dual modulators, which may have unique therapeutic potentials in diverse metabolic diseases.

Key Words: Diallyl biphenyl-type neolignans, Adiponectin, Human bone marrow mesenchymal stem cells, Peroxisome proliferator-activated receptor α/γ

INTRODUCTION

Adiponectin, also referred to as an adipocyte complement-related protein of 30 kDa, is an anti-inflammatory adipocytokine mainly produced in mammalian adipocytes (Straub and Scherer, 2019). Hypoadiponectinemia has been reported in various metabolic diseases such as obesity, diabetes, and cardiovascular metabolic syndrome (Ahn *et al*., 2018; Waragai *et al*., 2018). Serum adiponectin levels are lower in diabetic and proinflammatory conditions than in healthy population (Kershaw and Flier, 2004). Adiponectin has been regarded as a key factor in not only decreasing the intracellular influx of non-esterified fatty acids but also suppressing hepatic glucose production (Yamauchi, *et al*., 2002; Chakrabarti, 2010). In addition, significant correlation has been reported between lower serum adiponectin levels and obesity-associated cancers (Dalamaga, *et al*., 2012). Therefore, adiponectin secretion-promoting compounds have therapeutic potential in metabolic diseases and cancer (Yamauchi and Kadowaki, 2008; Shin *et al*., 2009).

Human bone marrow-mesenchymal stem cells (hBM-MSCs) can be induced to differentiate into adipocytes by treatment with an adipogenesis-inducing cocktail containing insulin, dexamethasone and 3-isobutyl-1-methylxanthine (IBMX) (IDX condition) (Chen *et al*., 2016). The IDX condition is effective in inducing the adipogenic differentiation of murine preadipocyte cell lines such as 3T3-L1 cells. In contrast, approximately 10-20% of the hBM-MSC population has adipocyte phenotypes in response to the IDX condition (Noh, 2012). Diverse pharmacological reagents have been added to the IDX condition to improve the efficiency of adipogenic differentiation of hBM-MSCs (Byun *et al*., 2013). For example, PPAR modulators significantly promote adiponectin secretion during adipogenesis in hBM-MSCs (Byun *et al*., 2013). Adiponectin production is also upregulated by sulfonylurea anti-diabetic drugs during adipogenesis (Iwaki *et al*., 2003). Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin and indomethacin are widely known to increase adiponectin production in hBM-MSCs when treated with IDX (Shin *et al*., 2009; Ahn *et al*., 2018). Monoamine oxidase inhibitors (MAOIs) like moclobemide significantly promote adiponectin production during adipogenesis in hBM-MSCs, although its pharmacological

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mechanism has not been fully elucidated (Byun *et al*., 2013). There are many molecular and cellular targets responsible for the adiponectin secretion-promoting activity in hBM-MSCs. In this regard, a phenotype-based screening approach may be more efficient for screening adiponectin secretion-promoting compounds compared to molecular target-based screening methods. In a phenotype assay, adiponectin secretion-promoting compounds can be identified using the adipogenesis model of hBM-MSCs by supplementing test compounds to the IDX condition. When adiponectin secretion-promoting compounds are screened, target identification experiments primarily focus on investigating their effects on nuclear receptors such as glucocorticoid receptors (GR), PPARs, and liver X receptors (LXR) (Yu *et al*., 2017).

MATERIALS AND METHODS

Cell culture and differentiation

hBM-MSCs were purchased from Lonza (Walkersville, MD, USA) and cultured as previously described (Noh, 2012; Yu *et al*., 2017). hBM-MSCs were maintained in DMEM (1 g/L glucose) supplemented with 10% fetal bovine serum (FBS), antibiotics, and Glutamax™ (Invitrogen, Carlsbad, CA, USA). When hBM-MSCs were 100% confluent, adipocyte differentiation was induced by exchanging media with DMEM (4.5 g/L glucose) containing 10% FBS, insulin (10 μg/mL), dexamethasone (0.5 μM), and IBMX (0.5 mM) (IDX condition). Glibenclamide, troglitazone, Wy-14643, dexamethasone, insulin and IBMX were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol extracts of *M. officinalis* were obtained from SK Bioland Co (Osong, Korea). *N*-acetyl lanonaine, magnoflorine, and syringaresinol were obtained from the natural products-originated chemical archives in the Natural Products Research Institute at Seoul National University (Seoul, Korea). Honokiol, magnolol, and 4-*O*-methylhonokiol as an analytical standard were obtained from SK Bioland Co.

Cell viability assay

A cell counting kit-8 assay (CCK-8, Dojindo, Kumamoto, Japan) was used to assess the cytotoxic effects of compounds on hBM-MSCs. Cells were plated in 48-well plates and incubated at 37°C in 5% CO_2. At 100% confluence, cells were treated with compounds for 5 days. To measure cell viability, CCK-8 solution was added to each culture well and after incubation, the absorbance was measured at 450 nm using a microplate reader (BioTek, Winooski, VT, USA).

Oil Red O and hematoxylin staining

The lipid accumulation was primarily assessed by Oil Red O (ORO) staining (Sigma-Aldrich). Differentiated adipocytes were rinsed twice with phosphate-buffered saline (PBS) and fixed with 10% formalin in PBS (pH 7.4) for 1 h. Fixed cells were washed once with 60% isopropanol and stained with 0.2% ORO solution for 10 min at 24°C and washed four times with tap water. To visualize the nucleus, differentiated hBM-MSCs were counterstained with hematoxylin reagent (Sigma-Aldrich) for 1 min and then washed four times with tapping water. The ORO-stained hBM-MSCs were observed using an inverted phase-microscope (Nikon Co., Tokyo, Japan).

Enzyme-linked immunosorbent assay (ELISA)

For quantitative measurement of adiponectin in cell culture supernatants, a Quantikine™ immunoassay kit (R&D Systems, Minneapolis, MN, USA) was used, and adiponectin concentrations were determined as previously described (Kim *et al*., 2018).

PPAR receptor binding assay

Lanthascreen™ time resolved fluorescence resonance energy transfer (TR-FRET) competitive binding assay kits (Invitrogen) were used to evaluate PPARα, PPARδ and PPARγ binding activities of chemical ligands as described (Ahn *et al*., 2018). All assay measurements were performed using a CLARIOstar plate reader (BMG LABTECH, Ortenberg, Germany).

Molecular docking simulation

The protein coordinates of PPARs were downloaded from the Protein Data Bank (PDB, https://www.rcsb.org/). Docking simulations were performed using AutoDock Vina 1.1.2 software (The Scripps Research Institute, La Jolla, CA, USA) against crystal structures of PPARα (PDB code 5HYK) and PPARγ (PDB code 3ADU) (Erickson *et al*., 2004; Trott and Olson, 2010). The PPAR crystal structure was prepared for docking simulations by removing the native ligand from the ligand-binding domain (LBD), followed by adding polar hydrogens using MGLTools 1.5.6 (The Scripps Research Institute). The ligand docking space, or center and size of the grid box, was determined based on the location of the native ligand in crystal structures. We focused on key amino acid residues of the hydrophilic and hydrophobic regions of PPAR LBDs for successful docking. Docking success was evaluated based on the lowest free energy value.

Total RNA isolation and quantitative real-time PCR (Q-RT-PCR)

Total RNA samples were prepared using Trizol reagent (Invitrogen). RNA samples were purified using a Qiagen RNeasy kit (Qiagen, Valencia, CA, USA). Sample RNA concentration was measured spectrophotometrically at 260/280 nm. RNA sample integrity was validated using an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). For cDNA synthesis, total RNA samples were reversely transcribed using the Superscript Reverse Transcriptase (RT) II Kit (Invitrogen). TaqMan Universal Master Mix II and Q-RT-PCR primer sets (Applied Biosystems, Foster City, CA, USA) were used to determine the transcription levels of acetyl-CoA carboxylase beta (ACACB, Hs00163715_m1), fatty acid desaturase 1 (FADS1, Hs01096545_m1), 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1, Hs00940429_m1) and lipoprotein lipase (LPL, Hs00173425_m1). Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 4333764F) was used to normalize sample variations. Q-RT-PCR was performed with an Applied Biosystems 7500 real-time PCR system (Applied Biosystems). Relative gene expression was quantified using the Pfaffl method (Pfaffl *et al*., 2002).

Statistical analysis

Experimental values are expressed as the mean ± standard deviation. Statistical analysis was performed using one-way ANOVA. *p*-values less than 0.05 were regarded as statistically significant.

RESULTS

In screening plant extract libraries, the methanol extract of *M. officinalis* was identified as a potent adipogenesis-inducing plant extract in hBM-MSCs, which was determined by ORO staining (Fig. 1A). To elucidate the adipogenesis-inducing active ingredients of *M. officinalis* extract, metabolites found in *M. officinalis* were selected from natural product-based chemical libraries. Among the *M. officinalis*–derived metabolites, *N*-acetyl lanonaine, honokiol, magnoflorine, magnolol, 4-*O*-methylhonokiol, and syringaresinol were examined to determine whether these compounds were responsible for the adipogenesis-inducing activity of the *M. officinalis* extract (Fig. 1B). When magnolol, honokiol, or 4-*O*-methylhonokiol at 10 μM each was added to the IDX, they significantly promoted adipogenesis in hBM-MSCs by 6.33, 4.04 and 3.74 fold, respectively, compared to that of the IDX control (Fig. 1B, 1C). Three diallyl biphenyl-type neolignans, magnolol, honokiol, and 4-*O*-methylhonokiol, also increased the size and number of lipid droplets in differentiated adipocytes (Fig. 1D).

Next, we evaluated the effects of diallyl biphenyl-type neolignans on adiponectin production during adipogenesis in hBM-MSCs (Fig. 2). To show the concentration-dependent effect of diallyl biphenyl-type neolignans, cell viability was first determined after treating hBM-MSCs with magnolol, honokiol, and 4-*O*-methylhonokiol. Magnolol did not affected cell viability

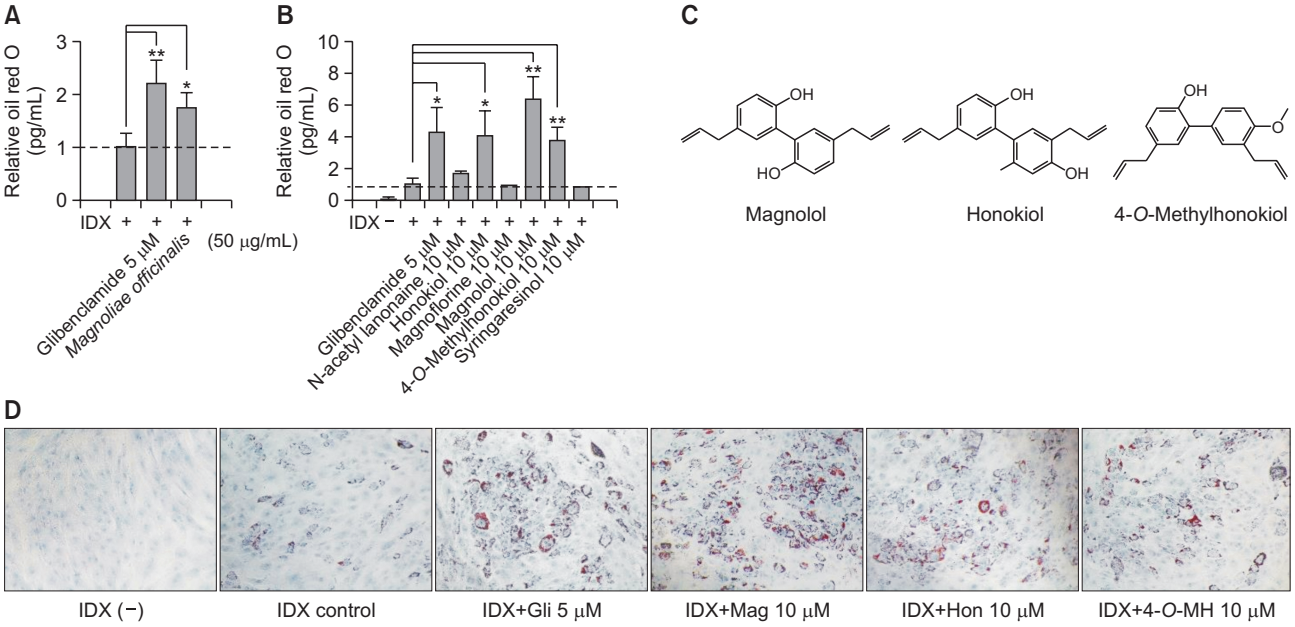

Fig. 1. Effects of natural products-derived compounds on adipogenesis in hBM-MSCs. (A) hBM-MSCs were cultured under the adipogenesis-inducing medium (IDX) with a *M. officinalis* ethanol extract. On day 5, lipid droplets in differentiated adipocytes were stained with ORO. The ORO was dissolved in isopropyl alcohol and the staining level was quantified at 500 nm by spectrometer. (B) Effects of the major compounds in *M. officinalis* on adipogenesis in hBM-MSCs were evaluated by ORO. (C) Chemical structures of diallyl biphenyl-type neolignans in *M. officinalis*, magnolol, honokiol and 4-*O*-methylhonokiol. (D) Differentiated adipocytes were visualized via ORO staining on the 5th day of adipogenesis induction. Values represent mean ± standard deviation (n=3). *$p \leq 0.05$ and **$p \leq 0.01$. IDX, adipogenesis-inducing media consisting of insulin, dexamethasone and 3-isobytyl-1-methylxanthine; Gli, glibenclimide; Mag, magnolol; Hon, honokiol; 4-*O*-MH, 4-*O*-methylhonokiol.

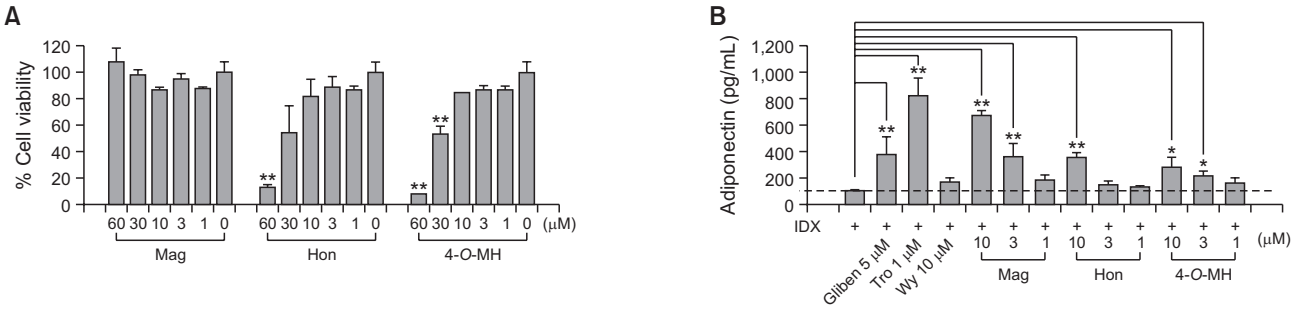

Fig. 2. Concentration-dependent effects of diallyl biphenyl-type neolignans on adiponectin production during adipogenesis in hBM-MSCs. (A) Cell viability was determined by CCK-8 assay. (B) Magnolol, honokiol and 4-*O*-methylhonokiol was co-treated with IDX medium. Cell culture supernatants were harvested on the 7th day and the amount of adiponectin secretion were measured. Values represent mean ± standard deviation (n=3). *$p \leq 0.05$ and **$p \leq 0.01$. Gliben, glibenclimide; Tro, troglitazone; Wy, wy-14643; Mag, magnolol; Hon, honokiol; 4-*O*-MH, 4-*O*-methylhonokiol.

of hBM-MSCs up to 60 μM (Fig. 2A). In contrast, both honokiol and 4-*O*-methylhonokiol were cytotoxic to hBM-MSCs at 60 μM. At 30 μM, 54 and 53% of hBM-MSCs were viable when treated with honokiol and 4-*O*-methylhonokiol, respectively. When hBM-MSCs were co-treated with IDX and non-cytotoxic concentrations of diallyl biphenyl-type neolignans, adiponectin production was upregulated compared to that of the IDX control condition (Fig. 2B). Magnolol and 4-*O*-methylhonokiol increased adiponectin production during adipogenesis in hBM-MSCs in a concentration-dependent manner. Honokiol, cytotoxic at 30 μM, showed a significant effect on adiponectin production only at 10 μM. Therefore, diallyl biphenyl-type neolignan compounds contributed to the adipogenesis-promoting activity of *M. officinalis* methanol extract in hBM-MSCs.

In adipocytes, adiponectin production is primarily upregulated by PPARγ activation (Farmer, 2005). In fact, it has been reported that most adiponectin secretion-promoting drugs such as glibenclamide and indomethacin can directly bind to PPARγ (Lehmann *et al*., 1997; Fukuen *et al*., 2005). However, moclobemide, an adiponectin secretion-promoting MAOI, did not directly bind to PPARγ (Byun *et al*., 2013). PPARα and PPARδ also play a role in the regulation of adipogenesis. Other nuclear receptors like GR, estrogen receptor and LXRs can affect adiponectin production in mammalian adipocytes (Lefterova *et al*., 2014). We examined the binding activity of diallyl biphenyl-type neolignans with TR-FRET (time resolved fluorescence resonance energy transfer)-based receptor binding assay. At 10 μM, three diallyl biphenyl-type neolignans significantly replaced the receptor binding of labeled ligands to both PPARα and PPARγ whereas they did not affect PPARδ binding (Fig. 3A). Diallyl biphenyl-type neolignans did not change the labeled ligand binding activity of GR, ER, LXRα and LXRβ (data not shown). To confirm the specific receptor binding, the concentration–response relationships of diallyl biphenyl-type neolignans to PPARα, PPARγ and PPARδ were examined. Preliminary screening results showed, magnolol, honokiol, and 4-*O*-methylhonokiol to have significant and concentration-dependent competitive binding activities against both PPARα and PPARγ whereas PPARδ binding activity was not affected (Fig. 3B-3D). The Ki values of magnolol, honokiol, and 4-*O*-methylhonokiol for PPARα were 3.38, 4.60, and 5.56 μM, respectively, under the condition that the Ki value of a well-known PPARα agonist Wy-14643 was 9.40 μM (Fig. 3B). The Ki values of magnolol, honokiol and 4-*O*-methylhonokiol for the PPARγ binding were 0.22, 2.00, and 9.10 μM, respectively (Fig. 3C). The Ki value of a specific PPARγ agonist troglitazone was 0.07 μM. Therefore, the diallyl biphenyl-type neolignan compounds, magnolol, honokiol and 4-*O*-methylhonokiol can modulate both PPARα and PPARγ during adipogenesis in hBM-MSCs.

Next, the ligand binding modes of diallyl biphenyl-type neolignans against both PPARα and PPARγ were analyzed. The LBDs of all PPARs are generally explained as a Y-shaped structure consisting of three pockets (Xu *et al*., 1999). The first hydrophilic pocket is located between helix (H) 3 and H12 including the activation factor-2 (AF-2) domain. H3 and β-sheets form the hydrophobic pocket of PPAR LBDs. The entrance region of LBD is composed of hydrophilic and hydrophobic amino acid residues, forming an amphiphilic binding pocket. In general, PPAR full agonists occupy both hydrophilic and hydrophobic pockets around H3 and form hydrogen bonding with a tyrosine (Tyr) residue in H12 (Hughes *et al*., 2014). In contrast, partial agonists mainly interact with PPARs in hydrophobic or amphiphilic pockets (Bernardes *et al*., 2013; Garcia-Vallvé *et al*., 2015). Optimal ligand docking modes of magnolol, honokiol and 4-*O*-methylhonokiol were analyzed with a PPARα LBD (PDB, 5HYK) (Fig. 4). The docking free energy levels of three diallyl biphenyl-type neolignans were as potent as that of Wy-14643, showing that they have similar PPARα binding activity (Fig. 3, 4). In the hydrophilic pocket, amino acid residues Leu456 and Phe273 commonly contributed to the stabilization of the ligand docking structure of Wy-14643 and three diallyl biphenyl-type neolignans. The docking simulation showed that amino acid residues Tyr464 and Leu460 interacted with that of Wy-14643, a well-studied PPARα agonist. The interaction between Tyr464 and a ligand molecule is important in the stabilization of PPARα to recruit transcriptional co-activators (Xu *et al*., 2002). In contrast, diallyl biphenyl-type neolignans formed hydrophobic interactions with Leu460 whereas they did not interact with Tyr464.

Although three diallyl biphenyl-type neolignans interacted with PPARα LBD via a similar structural form, magnolol, ho-

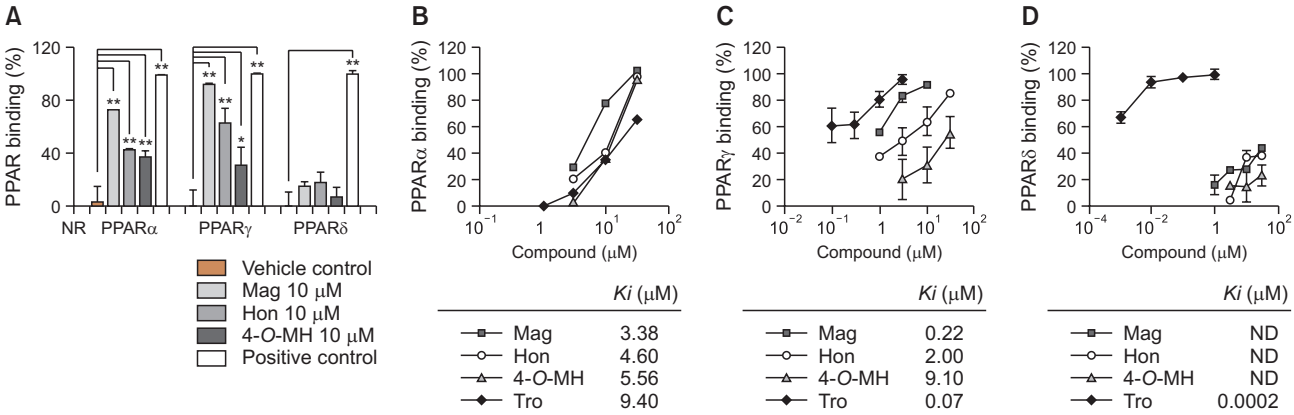

Fig. 3. PPAR binding activities of diallyl biphenyl-type neolignans. (A) TR-FRET competitive binding assay of PPAR subtypes were performed at 10 μM of diallyl biphenyl-type neolignans. TR-FRET competitive binding activities of diallyl biphenyl-type neolignans were determined for PPARα (B), PPARγ (C) and PPARδ (D). Values represent mean ± standard deviation (n=3). *p≤0.05 and **p≤0.01. Gliben, glibenclimide; Tro, troglitazone; Wy, wy-14643; Mag, magnolol; Hon, honokiol; 4-*O*-MH, 4-*O*-methylhonokiol.

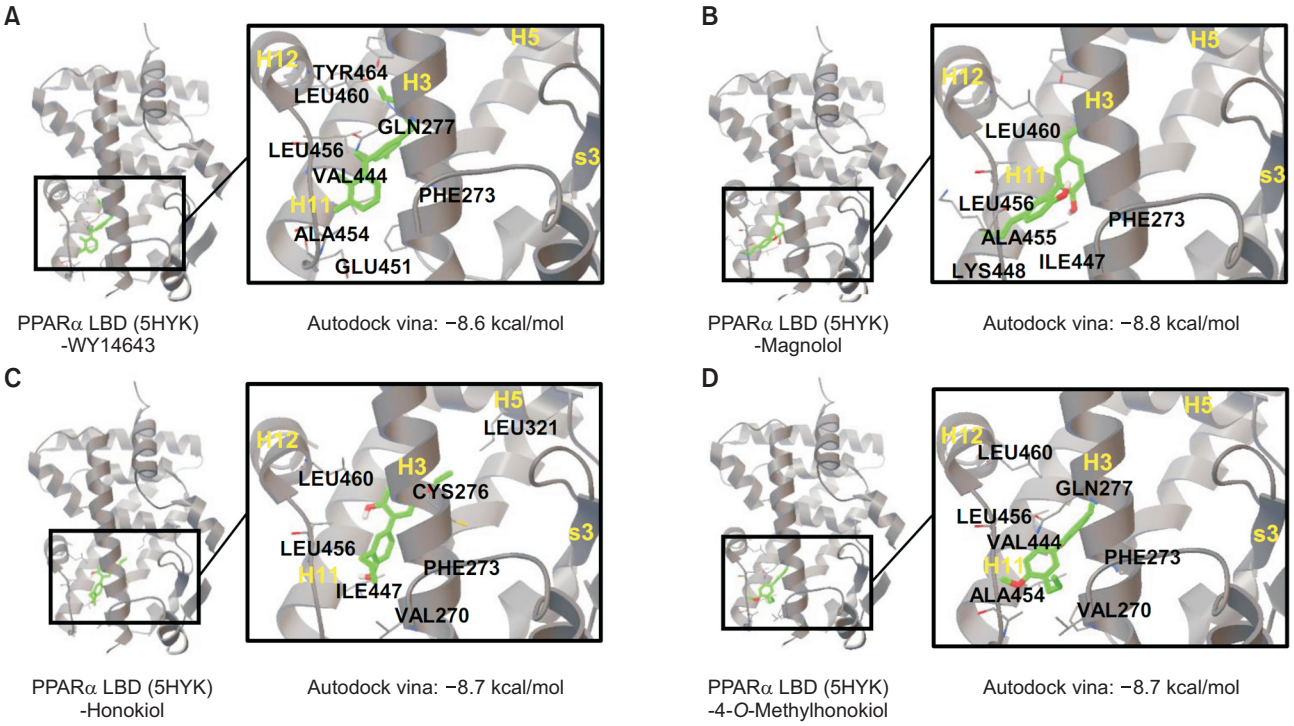

Fig. 4. Molecular docking analysis of Wy-14643 (A), magnolol (B), honokiol (C) and 4-*O*-Methylhonokiol (D) against the PPARα-LBD. Key amino acid residues involved in the PPARα binding pocket have been labeled. Docking simulations of the selective compounds were performed using AutoDock Vina 1.1.2 software (The Scripps Research Institute).

nokiol and 4-*O*-methylhonokiol had different docking profiles to PPARγ LBD (PDB, 3ADU) (Fig. 5). In a PPARγ docking simulation, troglitazone showed a typical U-shaped structure in the lowest energy conformation (Fig. 5A). Like troglitazone, magnolol formed a hydrogen bond with Tyr473 in H12 in the hydrophilic pocket, which is important in interactions between PPARγ and transcriptional coactivators (Fig. 5B). However, honokiol and 4-*O*-methylhonokiol were located in the hydrophobic binding pocket of the PPARγ LBD in the lowest energy conformations. The free energy levels of diallyl biphenyl-type neolignans were correlated to Ki values determined in the PPAR binding assay. The lack of honokiol and 4-*O*-methylhonokiol in the Tyr473 interaction can explain why magnolol had more potent adiponectin secretion-promoting activity compared to those of honokiol and 4-*O*-methylhonokiol. Therefore, the ligand docking models explained the PPARα/γ binding and adiponectin secretion-promoting activities of diallyl biphenyl-type neolignans. Notably, the docking models of diallyl biphenyl-type neolignans to PPARγ LBD showed that magnolol was similar to the docking structure of PPARγ full agonists whereas honokiol and 4-*O*-methylhonokiol resembled PPARγ partial agonists.

In contrast to a full agonist, a partial agonist functions as an antagonist by competing with a full agonist (Ahn *et al.*, 2018). To validate the different PPARγ docking models, the competitive effects of magnolol, honokiol and 4-*O*-methylhonokiol on troglitazone were investigated during adipogenesis in hBM-MSCs (Fig. 5E). Magnolol additively increased adiponectin production when co-treated with 0.1 μM of troglitazone whereas honokiol and 4-*O*-methylhonokiol did not change the effect of troglitazone on adiponectin production. This result supported the conclusion that magnolol functioned as a PPARγ agonist. Notably, honokiol and 4-*O*-methylhonokiol did not antagonize the adiponectin secretion-promoting activity of troglitazone. The lack of antagonism by honokiol and 4-*O*-methylhonokiol may be associated with their PPARα modulating activity.

Next, we evaluated whether the PPARα/γ dual modulation of diallyl biphenyl-type neolignans had differentiate functional outcomes compared to those of specific PPARα or PPARγ agonists (Fig. 6). After the induction of adipocyte differentiation in hBM-MSCs, diallyl biphenyl-type neolignans were added to the differentiated adipocytes to evaluate the transcription of lipid metabolism-associated genes (Fig. 6A). Troglitazone significantly increased the gene transcription of acetyl-CoA carboxylase (ACACB), one of enzymes to regulate fatty acid biosynthesis, whereas Wy-14643 did not affect the ACACB gene transcription (Fig. 6B). Magnolol upregulated the gene transcription of ACACB but honokiol and 4-*O*-methylhonokiol had no effect on the mRNA levels of ACACB. The effect of PPAR modulators on lipoprotein lipase (LPL) resulted in a profile similar to that shown with ACACB (Fig. 6C). Notably, the dual activation of PPARα and PPARγ showed different effects on the gene transcription of 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) and fatty acid desaturase 1 (FADS1) (Fig. 6D, 6E). The single treatment of Wy-14643 or troglitazone did not change the gene transcription of HMGCS1 and FADS1. In contrast, the co-treatment of Wy-14643 and troglitazone significantly increased the mRNA levels of both HMGCS1 and FADS1 in the differentiated adipocytes. Diallyl biphenyl-type neolignans also upregulated the gene transcription of both HMGCS1 and FADS1 (Fig. 6D, 6E). In this regard, compared

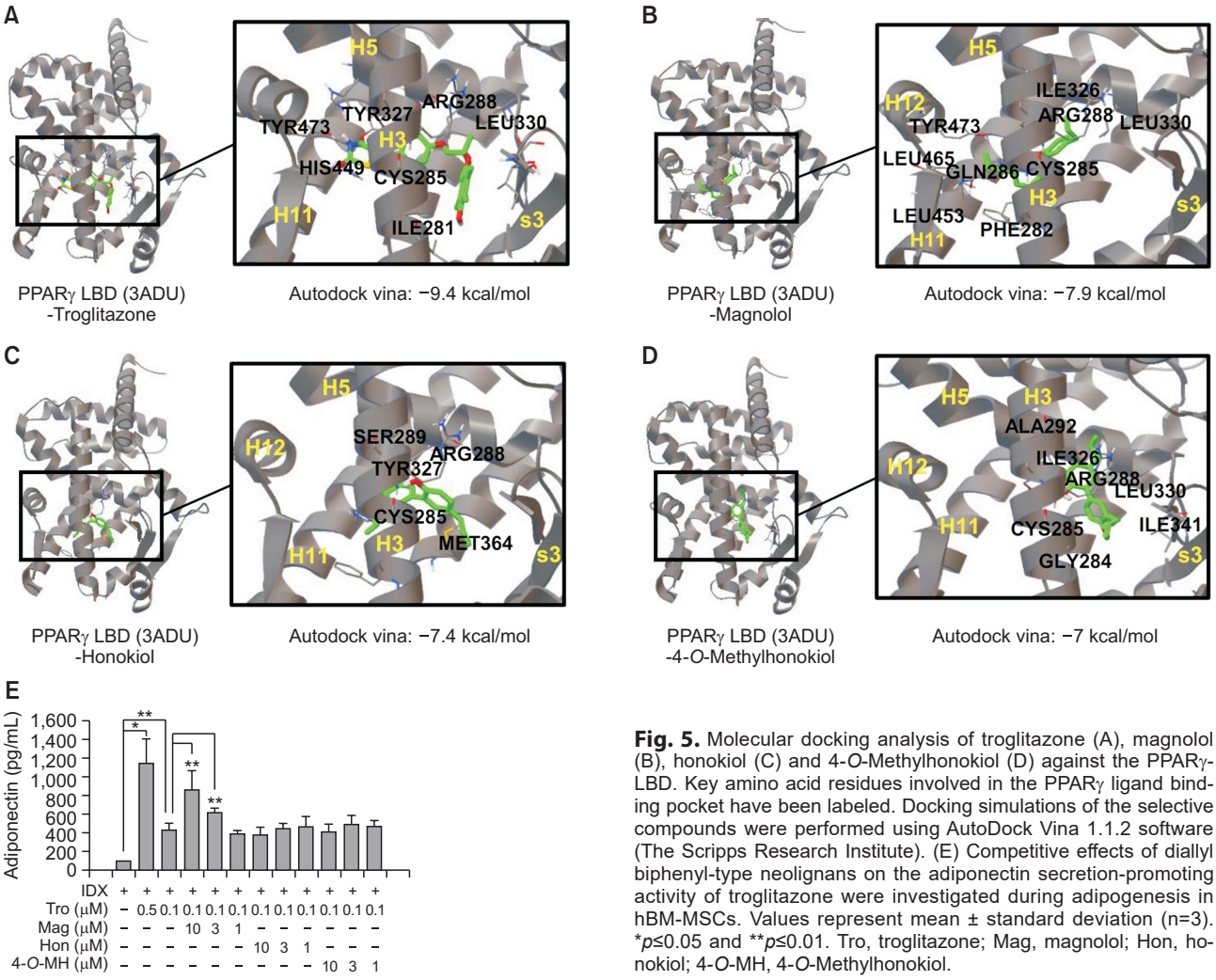

Fig. 5. Molecular docking analysis of troglitazone (A), magnolol (B), honokiol (C) and 4-*O*-Methylhonokiol (D) against the PPARγ-LBD. Key amino acid residues involved in the PPARγ ligand binding pocket have been labeled. Docking simulations of the selective compounds were performed using AutoDock Vina 1.1.2 software (The Scripps Research Institute). (E) Competitive effects of diallyl biphenyl-type neolignans on the adiponectin secretion-promoting activity of troglitazone were investigated during adipogenesis in hBM-MSCs. Values represent mean ± standard deviation (n=3). *$p \leq 0.05$ and **$p \leq 0.01$. Tro, troglitazone; Mag, magnolol; Hon, honokiol; 4-*O*-MH, 4-*O*-Methylhonokiol.

to the effect of specific PPAR or PPARγ agonists, diallyl biphenyl-type neolignans have unique features with a different regulatory role in metabolism as PPARα/γ dual modulators.

DISCUSSION

Adiponectin secretion-promoting compounds have diverse therapeutic potentials in metabolic diseases. Diallyl biphenyl-type neolignans, detected in various medicinal plants like *M. officinalis*, promoted adiponectin production during adipogenesis in hBM-MSCs. This study showed that the adiponectin secretion-promoting activity of three diallyl biphenyl-type neolignans, magnolol, honokiol and 4-*O*-methylhonokiol, was associated with PPARα/γ dual modulation. It has been reported that magnolol, honokiol, and 4-*O*-methylhonokiol can regulate glucose uptake via PPARγ-dependent pathways (Atanasov *et al*., 2013; Liang *et al*., 2015). Here, it was first demonstrated that these diallyl biphenyl-type neolignans directly bound to PPARα as well as PPARγ. Importantly, the docking simulation and the competitive functional study supported that magnolol functioned as a PPARγ full agonist whereas honokiol and 4-*O*-methylhonokiol were PPARγ partial agonists.

PPARs regulate diverse metabolic pathways and also affect cellular pathways associated with inflammation and immune function (Wang *et al*., 2014). Recently, many PPARα/γ dual modulators have been studied because of their therapeutic potential in type 2 diabetes and nonalcoholic steatohepatitis (NASH) (Henry *et al*., 2009; Jain *et al*., 2018). However, most PPARα/γ dual modulators have been withdrawn from clinical development due to unexpected side effects on the cardiovascular or hepatic system (Lincoff *et al*., 2014). Some of these PPARα/γ dual modulators were derived based on the pharmacophore of thiazolidinediones (TZDs) and therefore may have side effects similar to those of TZD PPARγ agonists (Home, 2011; Gross *et al*., 2017). Safer PPARα/γ dual modulators have been designed by synthesizing novel compounds which have different pharmacophores from that of TZDs. For example, the glitazar family of PPARα/γ dual modulators glitazar family has a tyrosine scaffold. In this regard, diallyl biphenyl-type neolignans provide a novel pharmacophore for a PPARα/γ dual modulator, different from that of TZD or other classes of PPARα/γ dual modulators.

In adipocytes differentiated from hBM-MSCs, diallyl biphenyl-type neolignan PPARα/γ dual modulators have a different effect on the gene transcription of lipid metabolic enzymes.

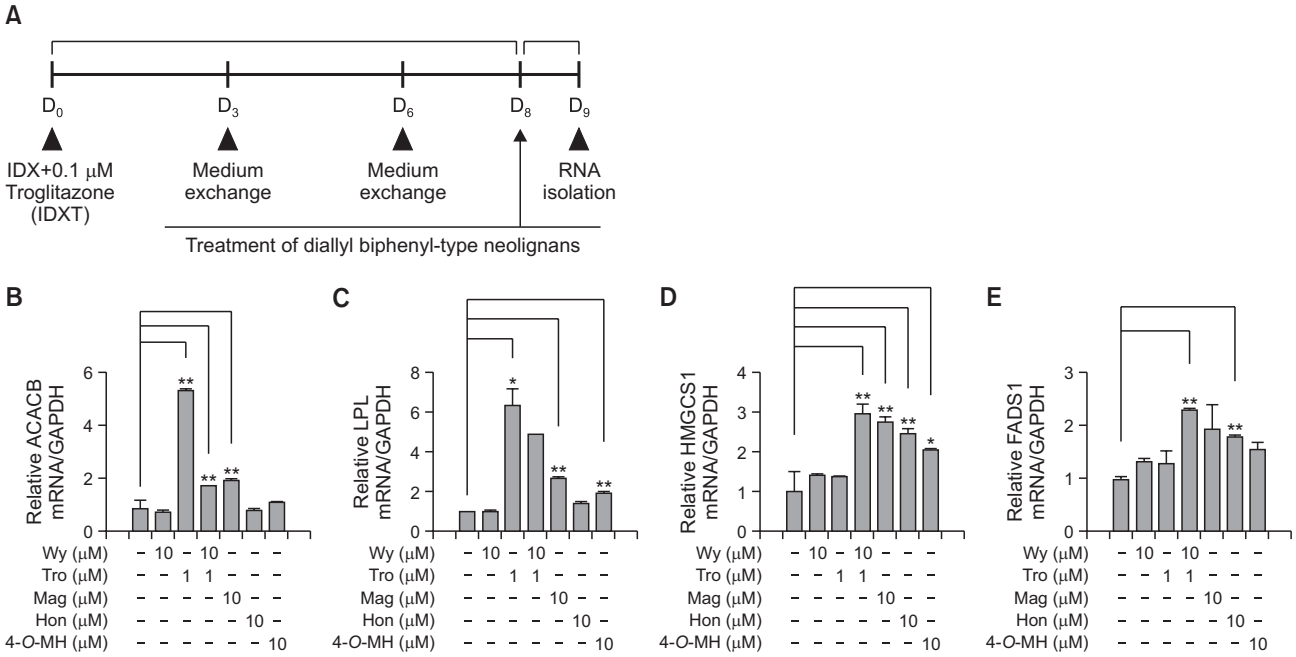

Fig. 6. Evaluation of diallyl biphenyl-type neolignans as a PPARα/γ dual modulators. (A) The experimental design to prepare differentiated adipocytes from hBM-MSCs. (B) Diallyl biphenyl-type neolignans in low glucose DMEM were treated to adipocytes differentiated from hBM-MSCs. On day 9, total RNA samples were isolated and Q-RT-PCR was performed for ACACB (B), LPL (C), HMGCS1 (D), FADS1 (E). GAPDH was used as an internal control. Values represent mean ± standard deviation (n=3). *p≤0.05 and **p≤0.01. Wy, wy-14643; Tro, troglitazone; Mag, magnolol; Hon, honokiol; 4-*O*-MH, 4-*O*-methylhonokiol.

Similar to troglitazone, magnolol affected the gene transcription of ACACB and LPL. However, two other diallyl biphenyl-type neolignans, honokiol and 4-*O*-methylhonokiol, had no effect on the mRNA levels of ACACB and LPL in the differentiated adipocytes. Notably, three diallyl biphenyl-type neolignans regulate HMGCS1 and FADS1 in common. In the docking analysis of PPARγ LBD, honokiol and 4-*O*-methylhonokiol showed the binding mode of PPARγ partial agonists whereas magnolol showed that of a PPARγ full agonist. Partial agonists are defined as having a specific receptor, and generally antagonize the pharmacological effect of full agonists, although partial agonists can also activate the receptor function themselves (Ahn *et al*., 2018). The partial agonist potential of honokiol and 4-*O*-methylhonokiol may explain why they had no effect on gene transcription on ACACB and LPL. Therefore, diallyl biphenyl-type neolignan structure may provide a diverse set of pharmacophores for functionally different PPARα/γ dual modulators. Recently, two molecules of magnolol were co-crystallized in PPARγ LBD (Zhang *et al*., 2011). Currently, no PPARγ LBD co-crystal structure with honokiol or 4-*O*-methylhonokiol has been reported. Further studies should be directed to explain the pharmacological difference of PPARα/γ dual modulation of diallyl biphenyl-type neolignans in terms of full or partial agonists against PPARγ.

In conclusion, diallyl biphenyl-type neolignans are adiponectin secretion-promoting compounds with a PPARα/γ dual modulator activity. The dual modulation profile of magnolol was different from that of other diallyl biphenyl-type neolignan compounds, honokiol and 4-*O*-methylhonokiol. Based on the docking model, three diallyl biphenyl-type neolignan compounds affected PPARα with the same interaction profile. However, magnolol was a PPARγ full agonist whereas honokiol and 4-*O*-methylhonokiol were PPARγ partial agonists. This difference may explain why diallyl biphenyl-type neolignan compounds showed different effects on the gene transcription of lipid metabolic enzymes.

CONFLICT OF INTEREST

Authors, So Hun Lee, Kang Hyuk Lee, and Song Seok Shin are employee of SK Bioland Inc. The other authors have no conflicts of interest.

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