약학석사학위논문

Adiponectin-secretion promoting compounds during adipogenesis in human mesenchymal stem cells as peroxisome proliferator-activated receptor modulators

중간엽줄기세포 지방분화 아디포넥틴 분비 촉진 화합물의 페록시솜증식체활성화수용체 조절 기능 연구

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ABSTRACT

Adiponectin-secretion promoting compounds during adipogenesis in human mesenchymal stem cells as peroxisome proliferator-activated receptor modulators

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Adiponectin, an adipocyte-derived cytokine, plays important roles in the regulation of inflammation, apoptosis, and metabolism. Adiponectin has therapeutic potentials to improve pathologic symptoms of various metabolic diseases like type 2 diabetes, obesity, and microvascular complication. The adipogenesis model of human bone marrow mesenchymal stem cells (hBM-MSCs) has been applied to screen novel adiponectin-secretion promoting compounds. Here, we focused on discovering potential compounds that promote adiponectin secretion in human bone

marrow mesenchymal stem cells (hBM-MSCs) by regulating adipocyte differentiation. We performed screening system to identify candidates from natural plant extracts and synthetic compounds. As results, we found that magnolol, honokiol, and methylhonokiol, major chemical components of *Magnolia officinalis* ethanol extracts promoted adipogenesis and increased adiponectin production in hBM-MSCs. Furthermore, new synthetic benzoimidazol derivatives were also identified to regulate adiponectin secretion effectively. Through molecular target identification research, we discovered that Magnolol, Honokiol, Methylhonokiol enhanced glucose and lipid metabolism by activating both Peroxisome proliferator-activated receptor alpha (PPARα) and PPARγ. Moreover, adiponectin-secretion promoting activity of benzoimidazol derivatives directly associated with PPARγ by high binding affinity. Based on different substituents, three of these derivatives, which the substituents of benzoimidazole were displaced to phenlycarbamolyl showed PPARγ partial agonism effect, while others showed PPARγ full agonism effect. In our study, we found various adiponectin-secretion promoting compounds as PPAR modulators which could be potential candidates in type 2 diabetes and related metabolic disorders therapy.

keywords : Adiponectin, Adipogenesis, Human bone marrow mesenchymal stem cells, Peroxisome proliferator-activated receptor

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Part I.

Adiponectin-promoting activity of *Magnolia Officinalis* in human mesenchymal stem cells as peroxisome proliferator-activated receptor dual modulators

Ⅰ. Introduction

Metabolic syndrome such as insulin resistance, fatty degeneration of liver and visceral fat obesity is a group of risk factors associated with the progression to type 2 diabetes and microvascular complications (1). Specially, type 2 diabetes which is main form of diabetes has become a principal threat to human health all over the world. And the number of people affected by diabetes is rapidly increasing nowadays (2). Adiponectin has received enormous attention for its potential actions associated with anti-diabetic (5). Adiponectin is a secretory protein exclusively produced by adipocytes, released at a high rate into circulation which regulates energy metabolism mainly by increasing insulin sensitivity (3, 4). Therefore, adiponectin is regarded as a crucial biomarker for insulin sensitivity, cardiovascular risk and inflammation, and also it plays a causal role in the diagnoses of type 2 diabetes and metabolic syndrome (3-6).

Magnolol, honokiol, and methylhonokiol are bioactive constituents extracted from *Magnolia officinalis* (7, 8). These compounds have been reported to have been used as traditional Chinese medicines exhibited various pharmacological effects for treatment of various diseases, such as neurosis, anxiety, and stroke (8, 9). In our study, we found that magnolol, honokiol and methylhonokiol significantly promoted adipogenesis and increased adiponectin production in hBM-MSCs. Several reports indicate that magnolol, honokiol and methylhonokiol may regulate glucose uptake as peroxisome proliferator-activated receptor gamma (PPARγ) modulators (10-15). The peroxisome proliferator-activated receptors (PPARs), a group of nuclear

receptor proteins that belong to a subfamily of the nuclear receptor superfamily of ligand-inducible transcription factors. PPARs are classified to three isotypes which are PPARα, PPARγ and PPARδ. They play essential roles in the regulation of cellular differentiation, development, metabolism and tumorigenesis of higher organisms. About metabolism, PPARs mainly control the expression of gene networks involved in adipogenesis, lipid metabolism, inflammation, and the maintenance of metabolic homeostasis (16, 17). We also found that PPARα and PPARγ dual agonists modulate cholesterol metabolism and fatty acid synthesis, may exhibit skin anti-aging through maintaining subcutaneous fat volume. From our study, we discovered that major chemical components of *Magnoliae* extracts: magnolol, honokiol and methylhonokiol regulate glucose and lipid metabolism by activating both PPARα and PPARγ.

II. Materials and Methods

1. Cell culture and differentiation

hBM-MSCs were purchased from Lonza (Walkersville, MD, USA) and cultured as previously described (18, 19). hBM-MSCs were cultured and maintained by DMEM with low glucose (1g/L glucose) supplemented with 10% FBS, supplemented with antibiotics and GlutamaxTM (Invitrogen, Carlsbad, CA, USA). When hBM-MSCs were in 100% confluence, adipocyte differentiation was induced, the medium was exchanged with DMEM with high glucose (4.5 g/L glucose) with 10% FBS, 10 μg/mL insulin, 0.5 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) (IDX condition). Dexamethasone, insulin, IBMX, glibenclamide, troglitazone, WY14643 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethanol extracts of plants were obtained from Korean Rural Development Administration. N-acetyl lanonaine, honokiol, magnoflorine, magnolol, methylhonokiol and syringaresinol were obtained from SK Bioland Co. to perform preliminary evaluation to find active compound in Magnoliae officinalis.

2. Cell counting kit-8 (CCK-8) assay

Cell counting kit-8 (CCK-8) assay (Dojindo laboratories, Kumamoto, Japan) was used to assess the cytotoxicity of compounds on hBM-MSCs. Cells were inoculated in 48-well plates and incubated at 37 ℃ in 5 % CO2. When cells in suspension

reach 100% confluence, cells were washed three times and treated with compounds in concentration-dependent manner. After incubation, the supernatant of each well was removed and CCK-8 solution (200 μl/well), diluted 20 fold with media, were added to each well and further incubated for 20 min at 37 ℃. The absorbance was measured at 450 nm using Epoch microplate spectrophotometer (BioTek Instruments, Inc). The cell viability was calculated by comparison with percentage of control.

3. Oil Red O and Hematoxylin staining

The level of adipocyte differentiation was assessed using Oil Red O stain from Sigma–Aldrich (St. Louis, MO, USA) as an indicator of intracellular lipid accumulation. Differentiated adipocytes were rinsed twice with phosphate-buffered saline (PBS), fixed with 10% formalin in PBS (pH 7.4) for 1 h, and then washed once with 60% isopropanol. Cultures were stained with 0.2% Oil Red O reagent for 10 min at room temperature and washed four times with H2O. To visualize the nucleus, the differentiated hBM-MSCs were counterstained with hematoxylin reagent from Sigma–Aldrich (St. Louis, MO, USA) for 1 min and then washed four times with H2O. The level of adipocyte differentiated was observed using an inverted phase-microscope.

4. 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 according to the manufacturer's instructions.

5. Nuclear receptor (NR) assays

LanthascreenTM's time resolved fluorescence resonance energy transfer (TR-FRET) competitive binding assay kits (Invitogen, Carlsbad, California, United States) were used to evaluate the PPARα, PPARδ, and PPARγ binding activities of chemical ligands. All assay measurements were performed according to the manufacturer's instructions using CLARIOstar (BMG LABTECH, Ortenberg, Germany).

6. Molecular docking simulation

Docking simulations were performed using both AutoDock Vina 1.1.2 software (The Scripps Research Institute, La Jolla, CA, USA) and Accelrys Discovery Studio (Dassault Systems, BIODIVA Corporation, San Diego, CA, USA) against crystal structures of PPARs (20, 21). The protein coordinate of PPAR was downloaded from the Protein Data Bank. The protein crystal structure was prepared for docking by removing the native ligand from the ligand-binding domain, followed by adding polar hydrogens using MGLTools 1.5.6 (The Scripps Research Institute). The Center of Grid box was calculated and the size of the grid box (docking space) was confined

by the native ligand. We focused on key amino acid residues of the hydrophilic and hydrophobic regions of the ligand-binding domain for successful docking. Docking success was evaluated based on the lowest affinity value.

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

Total RNA samples were isolated using Trizol's reagent (Invitogen, Carlsbad, CA, United States), followed by a purification step using the Qiagen RNeasy kit (Qiagen, Valencia, CA, United States). The concentration of RNA in each sample was determined spectrophotometrically at 260 nm. The integrity of each RNA sample was evaluated using Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). Total RNA from each sample was reversed transcribed to cDNA using the Superscript Reverse Transcriptase (RT) II Kit (Invitogen, Carlsbad, CA, United States). TaqMan Universal Master Mix II and Q-RT-PCR primer sets (Applied Biosystems, Foster City, CA, USA) were used according to the manufacturer's instructions to determine the transcription levels of adiponectin (ADIPOQ, Hs00605917_m1), fatty acid binding protein 4 (FABP4, Hs00609791_m1), 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 levels were quantified using equations from a mathematical model developed by Pfaffl (22).

8. Statistical analysis

Experimental values are expressed as the mean ± standard deviation. Statistical analysis was performed using one-way ANOVA. *P*-values of less than 0.05 and 0.01 were regarded as statistically significant.

III. Results

1. *Magnolia Officinalis* extracts promote adipogenesis in hBM-MSCs during adipogenesis.

In order to discover active natural products to improve adiponectin production, we used a model system for studying adipogenesis in hBM-MSCs. In a preliminary screening to find active natural products to promote adipocyte differentiation in hBM-MSCs. In preliminary experiments, glibenclamide (5 μM), a sulfonylurea-type antidiabetic drug to promote adipogenesis, was used as a positive control. Compared to the anti-diabetic drug glibenclamide, the activity of natural product ethanol extracts (50 μg/ml) was less potent in general. Among tested extracts, we found that the extracts of *Magnolia officinalis* showed significant activity to promote adipogenesis in hBM-MSCs. In order to identify active ingredients to explain the adiponectin promoting effect of *Magnolia officinalis*, we further investigated the effects of major chemical components of *Magnolia officinalis*, N-acetyl lanonaine, honokiol, magnoflorine, magnolol, methylhonokiol and syringaresinol. After 7 days in culture, the supernatants of cells were harvested and we analyzed adiponectin levels accumulated in cell culture supernatants during adipogenesis in hBM-MSCs by performing adiponectin ELISA. N-Acetyl lanonaine, magnolol, honokiol and methylhonokiol at 10 μM increased adiponectin production by 167%, 633%, 404% and 374%, respectively. In contrast, magnoflorine and syringaresinol had no effect

on adiponectin production during adipogenesis in hBM-MSCs compared to the level of adiponectin, in the IDX control.

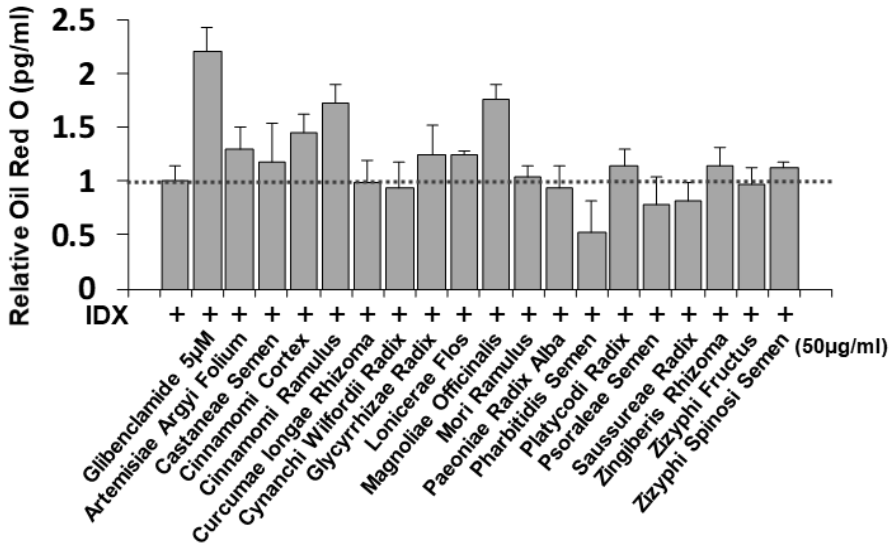


Figure 1. Evaluation of the adiponectin-promoting activity of natural products ethanol extracts during adipogenesis in hBM-MSCs.

hBM-MSCs were cultured in 24 well plates under IDX supplemented medium and co-treated with natural product ethanol extracts at 50 μg/ml. After treating natural product ethanol extracts, in every two or three day, media were exchanged. At the 5th days in culture, lipid droplets in differentiated adipocytes were stained with Oil Red O (ORO). We dissolved the ORO in isopropyl alcohol, and the level of staining was quantified at 500 nm by spectrometer. Data were normalized by setting the control as 1. Glibenclamide (5 μM) was used as a positive control. Results are the mean ± standard deviation (SD) of three measurements (n=3).

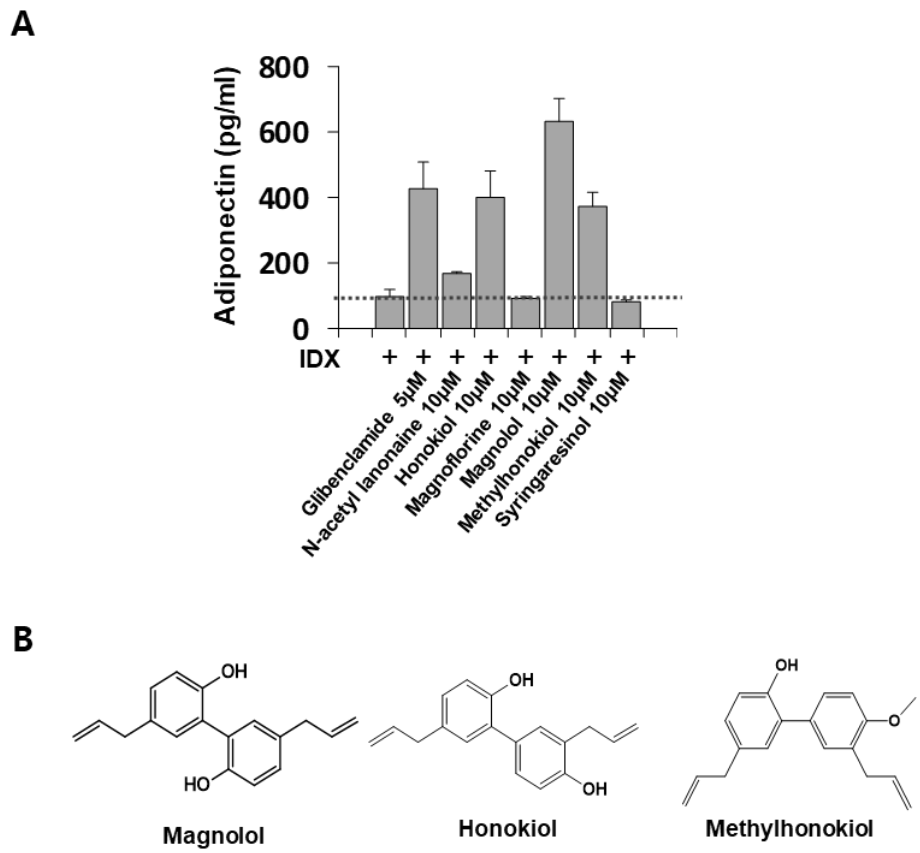


Figure 2. The effect of major chemical components of Magnolia extracts on adiponectin production during adipogenesis in hBM-MSCs.

Due to significant adiponectin-promoting effect of *Magnolia officinalis* extracts, the major compounds of *Magnolia officinalis* were evaluated by ELISA on the adiponectin-producing activities in hBM-MSCs at 10 μM during adipogenesis. (A). Chemical structures of magnolol, honokiol and methylhonokiol the major compounds of *Magnolia officinalis* (B). Glibenclamide (5 μM) was used as a positive control. Results are the mean ± standard deviation (SD) of three measurements (n=3).

2. The adiponectin promoting effects of magnolol honokiol and methylhonokiol during adipogenesis in hBM-MSCs.

In order to test the concentration-dependent effect of magnolol honokiol and methylhonokiol, we evaluate the cell viability in hBM-MSCs. According to the result of CCK-8 assay, we found that Magnolol had no cytotoxicity from 60 μM to 1 μM. However, both honokiol and methylhonokiol had cytotoxicity at 60 μM and 30 μM. The data showed that the cell viability of honokiol is 13% at 60 μM and 54% at 30 μM compared to the 100% vehicle. And the cell viability of methylhonokiol is 8% at 60 μM and 53% at 30 μM compared to the 100% vehicle. Therefore, we compared the activity of adipogenesis in a concentration-dependent manner at 10 μM, 3 μM and 1 μM. In the results of ORO analysis, magnolol honokiol and methylhonokiol all significantly increased the number of differentiated hBM-MSCs. In quantitative analysis of adiponectin production, we found that magnolol significantly promoted adiponectin levels in cell culture supernatants during adipogenesis in hBM-MSCs compared to the anti-diabetic drug glibenclamide at 5 μM and troglitazone at 1 μM. 10μM of honokiol and methylhonokiol also significantly increased adiponectin production by 354% and 282% compared to 100% IDX control . However, at lower concentrations (1 μM) these compounds did not show significant adiponectin promoting activity. What's more, in comparison with the effects of glibenclamide at 5 μM which was 374% compared to 100% IDX control, 10 μM of magnolol was more potent to promote adiponectin production in hBM-MSCs by 671%, but less potent than troglitazone which increased adiponectin production by 824%.

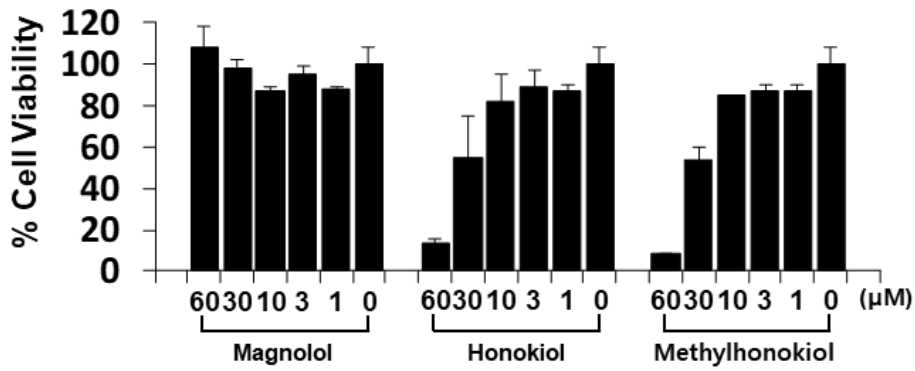


Figure 3. Cell viability of magnolol, honokiol and methylhonokiol in hBM-MSCs.

hBM-MSCs were cultured and then treated with magnolol, honokiol and methylhonokiol in a dose-dependent manner (60 μM, 30 μM, 10 μM, 3 μM, 1 μM) for 5days, the period is same as adipogenesis process . Cell viability was measured by CCK-8 assay. After 20min incubation with CCK-8 solution, the O.D. value was quantified at 450nm. The ratio of cells surviving in each concentration and compounds was calculated relative to the vehicle. Values are expressed as mean ± S.D. (n=3).

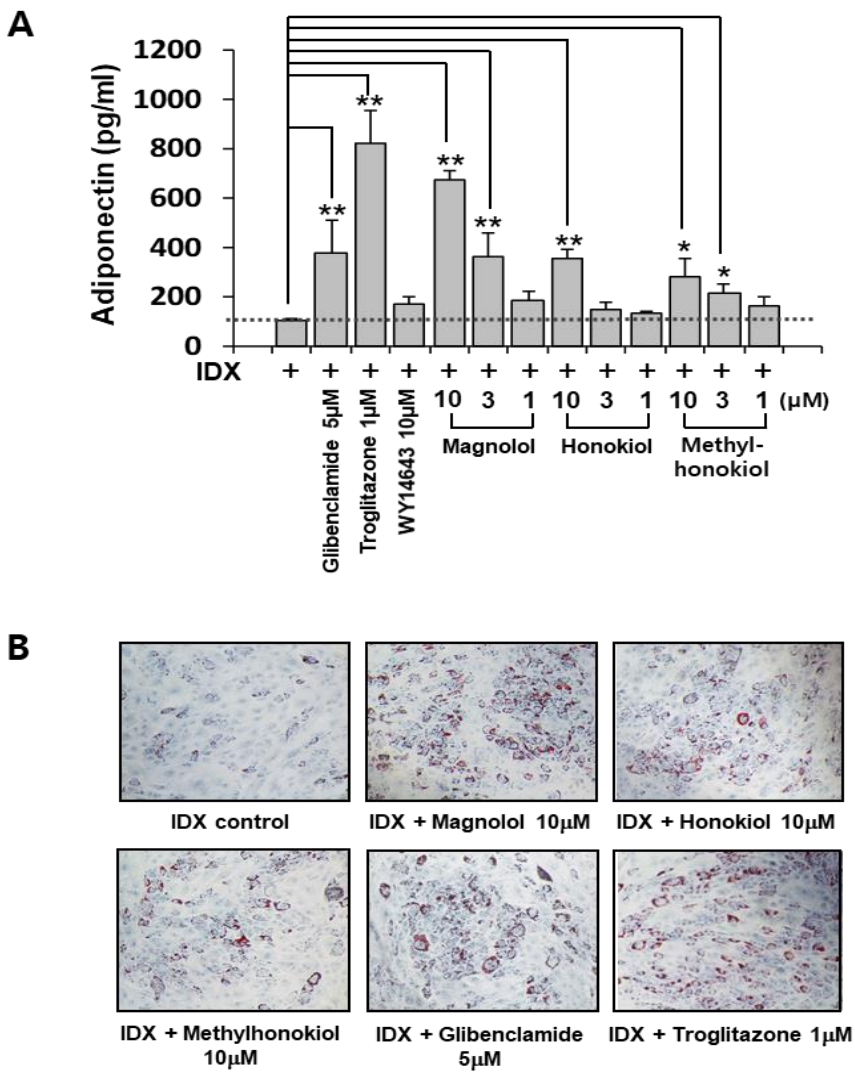


Figure 4. Concentration-dependent effects of magnolol, honokiol and methylhonokiol on adiponectin production during adipogenesis in hBM-MSCs. hBM-MSCs were treated with increasing concentrations (10 μM, 3 μM, 1 μM) of magnolol, honokiol and methylhonokiol in IDX supplemented medium. The cell

culture supernatants were harvested on the 7th day in culture and used to measure the amount of adiponectin secretion. Troglitazone, Wy-14643, and glibenclamide were used as positive controls (A). Relative adiponectin levels were normalized to IDX control as 100.Values represent mean ± SD (n=3). * $p < 0.05$ and ** $p < 0.01$. The levels of lipid accumulation of differentiated hBM-MSCs were qualitatively measured using Oil Red O staining. The extent of lipid accumulation was assessed relative to IDX control and positive control, troglitazone and glibenclamide (B).

3. Target deconvolution of magnolol honokiol and methylhonokiol

PPARs (peroxisome proliferator-activated receptors) are involved in the process of adipocyte differentiation, so we speculated that the adiponectin-promoting potential of magnolol honokiol and methylhonokiol could be due to the activation of the PPAR signaling pathway. In order to identify if the adiponectin-promoting activity had correlation with PPAR molecular target, TR-FRET NR competitive binding assays were performed to do molecular targets screening with magnolol honokiol and methylhonokiol first. As the figure showed, magnolol honokiol and methylhonokiol all had high binding affinity with both PPARα and PPARγ at 10 μM. According to the screening results, we determined the concentration–response relationship of magnolol honokiol and methylhonokiol in terms of their binding activity to PPARα, PPARγ, and PPARδ. Consistent with the literature, the agonists Troglitazone bound specifically to PPARγ and Wy-14643 bound specifically to PPARα. Compared to positive controls, as anticipated, the magnolol honokiol and methylhonokiol displayed significant competitive binding activities in a concentration-dependent manner. The K_i values of magnolol honokiol and methylhonokiol were 3.38, 4.60 and 5.56 for PPARα and 0.22, 2.00 and 9.10 for PPARγ respectively. Therefore, we speculated that the competitive and selective activation of PPARα and PPARγ suggests that the binding activities of the two PPAR isotypes are positively correlated with the adipogenic potential of magnolol honokiol and methylhonokiol during adipogenesis in hBM-MSCs.

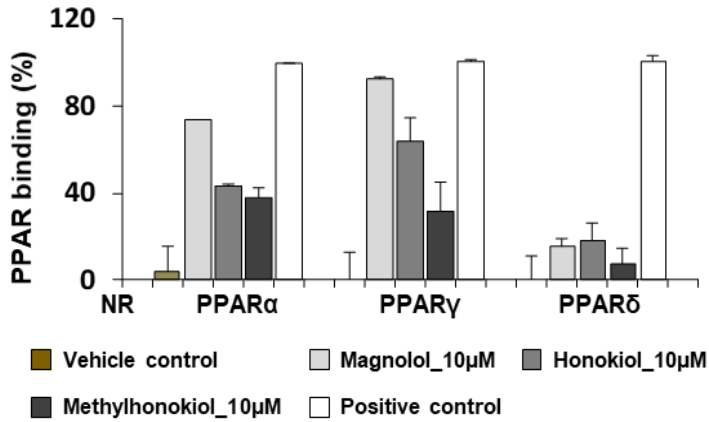


Figure 5. Nuclear binding activities screening of magnolol, honokiol and methylhonokiol.

TR-FRET competitive binding assays of PPAR subtypes were performed at 10 μM of magnolol, honokiol and methylhonokiol. The positive controls included GW7647 for PPARα, GW1929 for PPARγ, and GW0742 for PPARδ. DMSO in buffer was used as a blank control. Results are the mean ± standard deviation (SD) of three measurements (n=3).

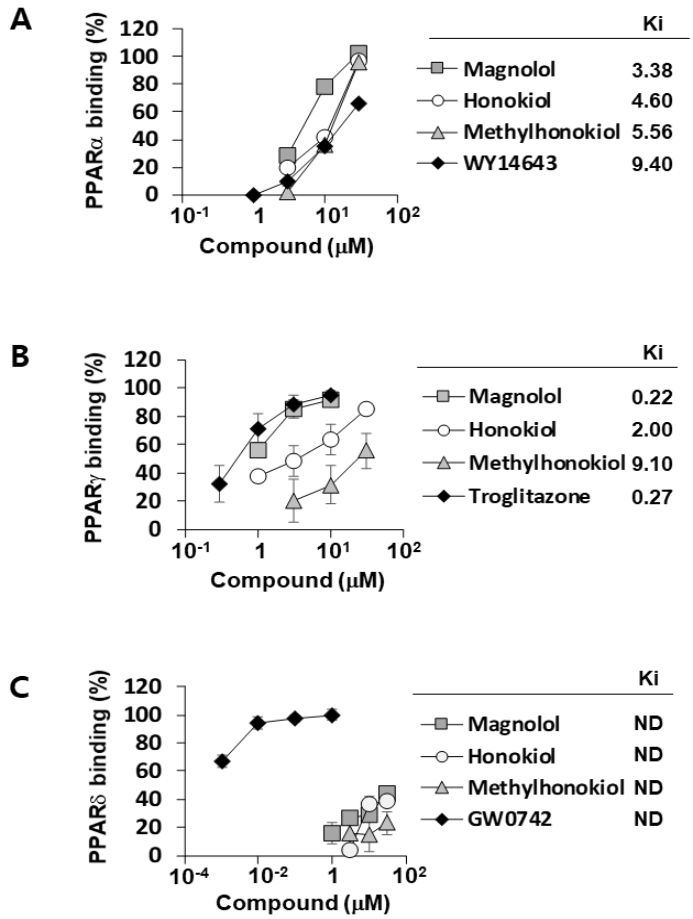


Figure 6. Binding activity of magnolol, honokiol and methylhonokiol on each three PPAR receptor subtypes

In order to determine the dose-dependent binding affinities of magnolol, honokiol and methylhonokiol to PPARα (A), PPARγ (B) and PPARδ (C), TR-FRET competitive binding assays were performed. The inhibition constant (K_i) was calculated by applying the Cheng-Prusoff equation. The positive controls included Wy-14643 for PPARα, troglitazone for PPARγ and GW0742 for PPARδ. DMSO in buffer was used as a blank control. Values were expressed in terms of percentage compared to each positive control. Percentage values represent mean ± SD (n = 3).

4. Magnolol, honokiol and methylhonokiol promote adiponectin secretion as PPAR full modulates.

Based on the results that magnolol, honokiol and methylhonokiol had high binding affinity with PPARα and PPARγ, the biochemical validation studies for each PPAR receptor partial agonistic activity were needed. Partial agonists usually have less efficacy compared to a full agonist at saturating concentration in all tissue influenced by the receptors, and have partial antagonistic action when co-treated with a full agonist. We treated the combination of PPARγ full agonist troglitazone at 0.1 μM and compounds at 3 μM in hBM-MSCs. Also mono-treatment of PPARγ full agonist troglitazone was also performed to evaluate adiponectin promoting potential at 0.1 μM. As the results showed the adiponectin secretion of co-treatment of compounds and troglitazone were all increased compared to the troglitazone mono-treatment. Next, we analyzed the binding mode of magnolol, honokiol and methylhonokiol in agonist co-crystalized human PPAR receptor ligand binding domains (LBDs) by using docking and compared with selective full agonists, WY14643 and troglitazone. The ligand binding pockets of all PPARs are Y-shaped structure consisted with three arms. The arm I, the hydrophilic pocket, is located between H3 and H12 including the activation factor-2(AF-2). The hydrophobic pocket which is mostly composed with hydrophobic amino acid residues, arm II, is the pocket between H3 and the β-sheets, while the entrance of the ligand binding pocket, arm III, is composed with hydrophilic and hydrophobic amino acid residues.

Full agonists occupy arms I and II and establish hydrogen bonds which stabilize H12. However, partial agonists mainly interact with arm III and arm II through

several hydrophobic interactions (23-27). In the binding mode analysis between PPARα receptor LBD (PDB ID: 4BCR) and magnolol, honokiol and methylhonokiol, they all stabilized the armI hydrophilic pocket with bonding on Helix 12 which indicated the full modulation of magnolol, honokiol and methylhonokiol for PPARα In the same way, we analyzed the binding mode between PPARγ receptor LBD (PDB ID: 1FM9) and magnolol, honokiol and methylhonokiol. They also stabilized the armI hydrophilic pocket with bonding on Helix 12 which indicated the full modulation of magnolol, honokiol and methylhonokiol for PPARγ. Based on these results, we suggested that magnolol, honokiol and methylhonokiol had adiponectin-promoting activity as PPAR full modulators.

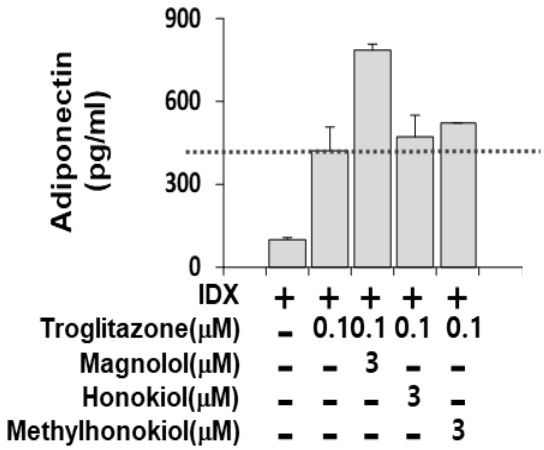


Figure 7. Synergistic adiponectin promoting effect of magnolol, honokiol and methylhonokiol co-treatment with PPAR full agonists in hBM-MSCs.

hBM-MSCs were grown in adipogenesis inducing medium (IDX). To confirm the synergistic effects of combination of PPAR full agonism at low concentrations, hBM-MSCs were treated with full agonist troglitazone only at 0.1 μM or co-treatment of troglitazone at 0.1 μM with each magnolol, honokiol and methylhonokiol at 3 μM for 7 days. Adiponectin production in the supernatant were evaluated by ELISA. All data represent mean value ± SD (n=3).

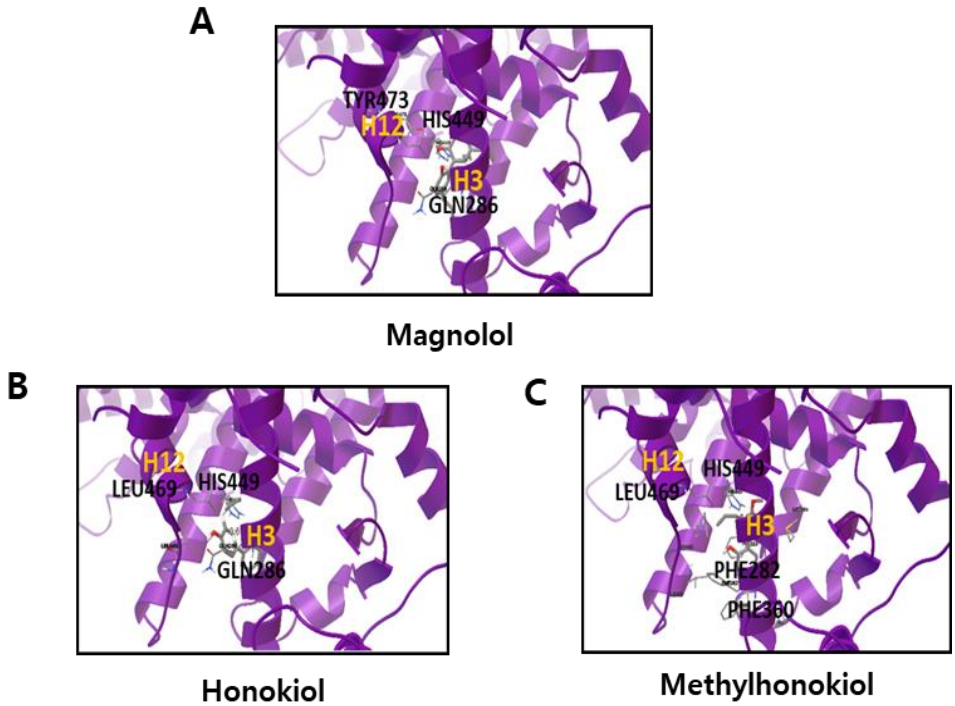


Figure 8. Binding modes of magnolol, honokiol and methylhonokiol with PPARγ LBD

We analyzed the protein-ligand interactions between the human PPARγ LBD (PDB ID: 1FM9) and Magnolol (A), honokiol (B), and methylhonokiol (C) were depicted and the interactions were visualized by using AutoDock Vina. Key amino acid residues involved in the PPARγ binding pocket have been labeled.

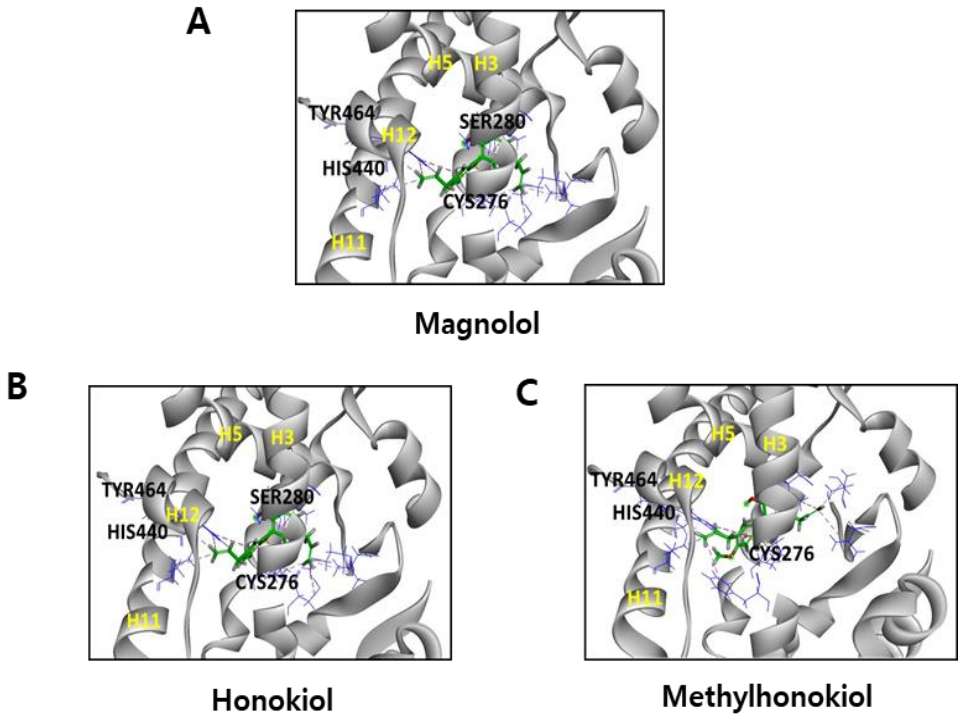


Figure 9. Binding modes of magnolol, honokiol and methylhonokiol with PPARα LBD

We analyzed the protein-ligand interactions between the human PPARα LBD (PDB ID: 4BCR) and Magnolol (A), honokiol (B), and methylhonokiol (C) were depicted and the interactions were visualized by using Discovery Studio software. Key amino acid residues involved in the PPARα binding pocket have been labeled.

5. Magnolol, honokiol and methylhonokiol modulate cholesterol and fatty acid synthesis in adipocyte-differentiated hBM-MSCs.

Next, we investigated the cell based gene expression profile with magnolol, honokiol and methylhonokiol in adipocyte-differentiated hBM-MSCs to elucidate the functional activity for PPARα and PPARγ. Ligands of PPARγ and PPARα are important modulators of lipogenesis and help promote adipogenic differentiation. . In this study, the expression of the PPAR target genes, adiponectin (ADIPOQ), and fatty acid binding protein 4 (FABP4), were measured as markers of adipogenesis. As anticipated, the PPARγ agonist, troglitazone significantly increased the transcription of adipogenic genes. However, the PPARα agonist Wy-14643 had no significant effect on the mRNA levels of ADIPOQ and FABP4 in hBM-MSCs. The combined treatment of the two mono-agonists increased the mRNA levels of ADIPOQ and FABP4 as similar level as troglitazone mono-treatment. Similarly, magnolol, honokiol and methylhonokiol increased both ADIPOQ and FABP4 gene transcription. Next, we evaluated acetyl-CoA carboxylase beta (ACACB), fatty acid desaturase 1 (FADS1) as the regulator gene of fatty acid synthesis, 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) as cholesterol synthesis related gene, and lipoprotein lipase (LPL) as lipoprotein metabolism related gene. We found thar magnolol, honokiol and methylhonokiol increased ACACB, FADS1, HMGCS1 and LPL expressions in adipocyte-differentiated hBM-MSCs. As the results showed, the mRNA levels of these genes increased by magnolol were more significant than honokiol and methylhonokiol. What's more, these four genes transcriptions all up-

regulated by PPARγ agonist, troglitazone. However, the mRNA levels had no increase by PPARα agonist, Wy-14643 at ACACB and LPL in adipocyte-differentiated hBM-MSCs. In contrast, we found up-regulation of gene expressions at HMGCR and FADS1 in WY14643 treated adipocytes. The results showed that the mRNA levels of HMGCR and FADS1 were increased by both PPARα and PPARγ agonists. Based on the results, we suggested that magnolol, honokiol and methylhonokiol regulated adipogenesis and adiponectin production as PPARα and PPARγ dual modulators.

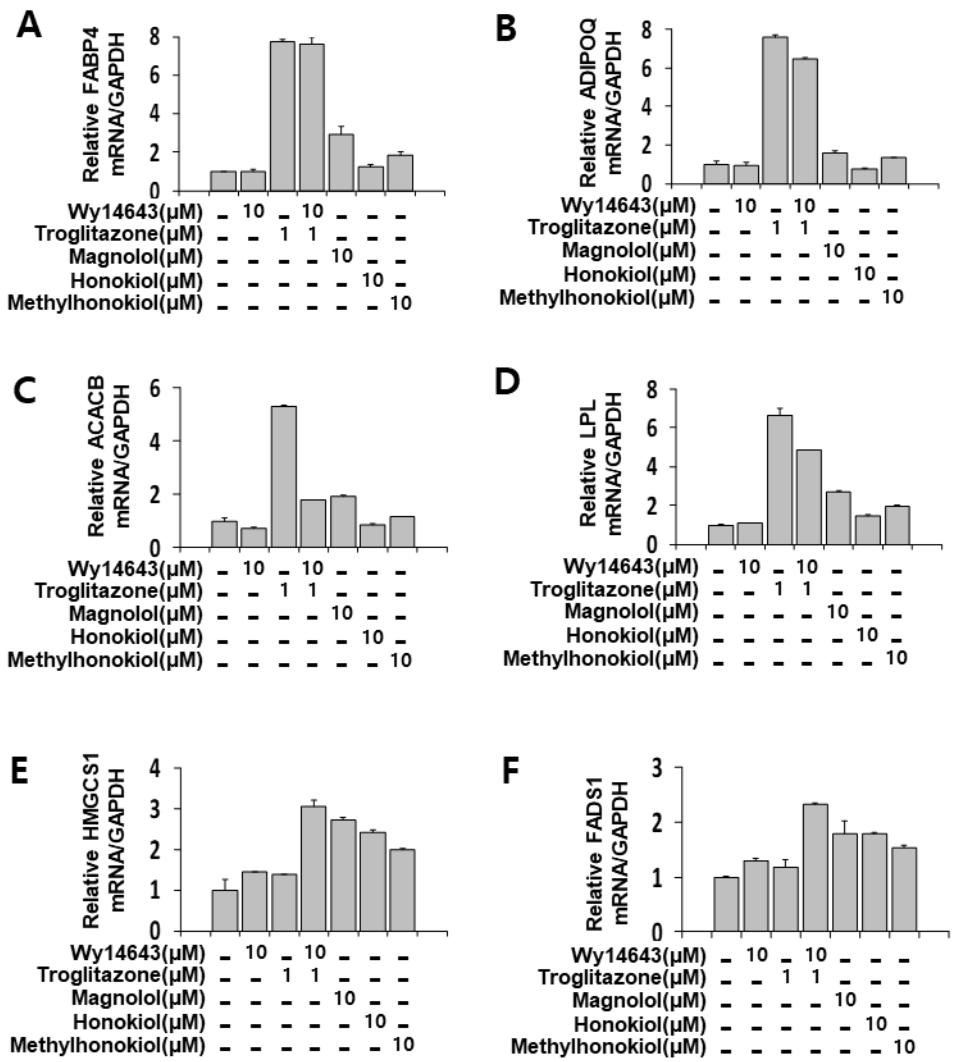


Figure 10. Transcriptional expression profile of magnolol, honokiol and methylhonokiol in hBM-MSCs.

Differentiated hBM-MSCs were treated with wy14643 at 10 μM, troglitazone at 1 μM , co-treatment of wy14643 and troglitazone, magnolol at 10 μM, honokiol at 10 μM and methylhonokiol at 10 μM. 24h after treatment, total RNA was extracted and Q-RT-PCR was performed for FABP4 (A), ADIPOQ (B), ACACB (C), LPL (D),

HMGCS1 (E), FADS1 (F). GAPDH was used as an internal control for Q-RT-PCR standardization. The positive controls included wy14643 for PPARα, troglitazone for PPARγ, and the co-treatment of Wy14643 and Troglitazone for PPARα/γ. Values from the present study are expressed as means ± SD (n = 3).

IV. Discussion

The aim of this study was to discover potential compounds that promote adiponectin secretion in hBM-MSCs by regulating adipocyte differentiation. And PPARs mainly control the expression of gene networks involved in adipogenesis, lipid metabolism, and the maintenance of metabolic homeostasis (16, 17). Therefore, we hypothesized that the PPAR signaling pathway may be involved in the adiponectin-secreting activity of our natural plant extracts in hBM-MSCs during adipogenesis. In our study, we found that major compounds of *Magnolia officinalis*, magnolol honokiol and methylhonokiol promoted adiponetcin secretion by modulating both PPARα and PPARγ which also named PPAR dual modulator. Recently, several pharmaceutical agents targeting PPAR dual modulator have been used in the management of type 2 diabetes (32). The separate metabolic effects of PPARα and PPARγ modulators on insulin sensitivity and lipid metabolism have encouraged the development of novel drugs targeting both PPARα and PPARγ (33). PPARα and PPARγ dual modulators combine the therapeutic advantage of 2 agents in a single pill. However, the majority of these drugs have been shown to have unexpected side effects, a limitation of the dual PPAR modulators has been that they seem to have similar side effects as the TZDs, PPAR full agonists (32-34). This has limited the further clinical development of PPAR dual modulators drugs. If these side effects can be improved, having 2 potent PPAR agonists in one pharmaceutical agent will develop a further way towards simplifying the complex treatment and improving the therapeutics in a safe and effective manner (35). Therefore, the

development of novel PPAR dual modulators with fewer side effects is urgently required.

In a further mechanism-of-action study, we found that magnolol honokiol and methylhonokiol up-regulated the transcription of enzymes involved in cholesterol and fatty acid synthesis. What's more, we noticed that there was a synergistic effect on lipid synthesis when both PPARα and PPARγ were simultaneously activated. Relative to the PPARα and PPARγ treatments alone, the co-treatment of the agonist wy-14643 and troglitazone enhanced the expressions of cholesterol synthesizing gene (HMGCR) and fatty acid synthesizing gene (FADS1). Cholesterol plays an important role in dermal extracellular matrix remodeling and is known to mediate collagen degradation, which may exhibit anti-aging potential in the skin by promoting extracellular matrix synthesis (47, 48). Based on clinical studies of fatty acid, FADS are known to decrease in activity over age, it has been reported that reduced levels of FADS are associated with abnormal barrier function that results as of decreased levels of essential long-chain fatty acid derivatives in the permeability barrier (49). Aged skin is characterized by the reduced ability of subcutaneous adipocytes to produce and accumulate essential lipids, and the inability to maintain adequate levels of lipids may contribute to the overall weakening and thinning of the skin (36-39). So, we suggest that PPARα/γ dual agonists such as magnolol honokiol and methylhonokiol may exhibit skin anti-aging through maintaining subcutaneous fat volume. Based on these effects, we suggest that major chemical components of *Magnoliae* extracts: magnolol, honokiol and methylhonokiol may be used as potential treatments in type 2 diabetes and related metabolic disorders by increasing

glucose and lipid levels as PPARα/γ dual modulators, and also an alternative approach in cosmetic industry.

Part II.

Adiponectin-promoting activity of benzoimidazole derivatives in human mesenchymal stem cells as peroxisome proliferator-activated receptor gamma modulators

Ⅰ. Introduction

Metabolic syndrome includes abdominal obesity, disorders of adipose tissue, insulin resistance and a constellation of independent factors (molecules of hepatic, vascular and immunologic origin), which characterized by abnormal adipokine production, and the activation of certain pro-inflammatory signaling pathways (44). Specially, type 2 diabetes which is main form of diabetes has become a principal threat to human health all over the world. And the number of people affected by diabetes is rapidly increasing nowadays (2). Several characteristics of type 2 diabetes including resistant insulin and incomplete metabolic profiles, and impaired glucose tolerance mostly in skeletal muscle, adipose tissue, and liver has made it become increasingly important to discover potent therapeutic agents to control lipid and glucose levels (3). Adiponectin is a protein hormone that modulates a number of metabolic processes and exhibits close relationships with obesity, inflammation, and diabetes (45). Owing to its high abundance, small variation and high stability, adiponectin has been a popular epidemiologic target and biomarker of diabetes and cardiovascular disease risk (4, 45). In our study, new benzoimidazol derivatives were synthesized and identified. We found that new benzoimidazol derivatives significantly promoted adipogenesis and increased adiponectin production in hBM-MSCs. 7 compounds all promoted adiponectin secretion but at different levels. Three of these derivatives which the substituents of benzoimidazole were displaced to phenlycarbamolyl had weaker adiponectin-promoting activity than other derivatives.

In previous study, the changes presented by the adipocytes in metabolic syndrome favors the production of several molecular mediators capable of modulating a number of transcription factors, such as the peroxisome proliferator-activated receptors (PPARs), a group of nuclear receptor proteins that belong to a subfamily of the nuclear receptor superfamily of ligand-inducible transcription factors (44). During identification on molecular targets, we found they only had high binding affinity with PPARγ. PPARγ is shown to be a major regulatory factor for the regulation of adiponectin secretion (46). In the human body, PPARγ is the master modulator of adipocyte differentiation, and plays an important role in lipid metabolism and glucose homeostasis, as well as controls cell proliferation (16). This result also authenticates the close relationships between adiponectin secretion promoting activity and PPARγ modulator. For further research of PPARγ agonism, we identified the types of PPARγ modulator. There are full PPARγ agonists and selective/partial PPARγ agonists. Partial agonists have fewer side effects than pure full agonists but weaker adiponectin promoting activity. In our study, three of these derivatives, which the substituents of benzoimidazole were displaced to phenlycarbamolyl showed PPARγ partial agonism effect, while others showed PPARγ full agonism effect.

II. Materials and Methods

1. Cell culture and differentiation

hBM-MSCs were purchased from Lonza (Walkersville, MD, USA) and cultured as previously described (18, 19). hBM-MSCs were cultured and maintained by DMEM with low glucose (1g/L glucose) supplemented with 10% FBS, supplemented with antibiotics and GlutamaxTM (Invitrogen, Carlsbad, CA, USA). When hBM-MSCs were in 100% confluence, adipocyte differentiation was induced, the medium was exchanged with DMEM with high glucose (4.5 g/L glucose) with 10% FBS, 10 μg/mL insulin, 0.5 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) (IDX condition). Dexamethasone, insulin, IBMX, glibenclamide, pioglitazone were purchased from Sigma–Aldrich (St. Louis, MO, USA). All the compounds were synthesized by Prof.Yongnan Xu's Lab (Shenyang Pharmaceutical University, China).

2. PDK1 Human AGC Kinase Enzymatic Radiometric Assay

PDK1 Human AGC Kinase Enzymatic Radiometric Assay was performed by Eurofins Pharma Discovery Services. PDK1 (h) is incubated with 50 mM Tris pH 7.5, 100 uM PDKtide, 0.1% 6-mercaptoethanol, 10 mM MgAcetate and [gamma-33P]-ATP. The reaction is initiated by the addition of the Mg/ATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of phosphoric acid to a concentration of 0.5%. 10 uL of the reaction is then

spotted onto a P30 filtermat and washed four times for 4 minutes in 0.425% phosphoric acid and once in methanol prior to drying and scintillation counting.

3. Cell counting kit-8 (CCK-8) assay

Cell counting kit-8 (CCK-8) assay (Dojindo laboratories, Kumamoto, Japan) was used to assess the cytotoxicity of compounds on hBM-MSCs. Cells were inoculated in 48-well plates and incubated at 37 ℃ in 5 % CO2. When cells in suspension reach 100% confluence, cells were washed three times and treated with compounds in concentration-dependent manner. After incubation, the supernatant of each well was removed and CCK-8 solution (200 μl/well), diluted 20 fold with media, were added to each well and further incubated for 20 min at 37 ℃. The absorbance was measured at 450 nm using Epoch microplate spectrophotometer (BioTek Instruments, Inc). The cell viability was calculated by comparison with percentage of control.

4. Oil Red O and Hematoxylin staining

The level of adipocyte differentiation was assessed using Oil Red O stain from Sigma–Aldrich (St. Louis, MO, USA) as an indicator of intracellular lipid accumulation. Differentiated adipocytes were rinsed twice with phosphate-buffered saline (PBS), fixed with 10% formalin in PBS (pH 7.4) for 1 h, and then washed once with 60% isopropanol. Cultures were stained with 0.2% Oil Red O reagent for

10 min at room temperature and washed four times with H2O. To visualize the nucleus, the differentiated hBM-MSCs were counterstained with hematoxylin reagent from Sigma–Aldrich (St. Louis, MO, USA) for 1 min and then washed four times with H2O. The level of adipocyte differentiated was observed using an inverted phase-microscope.

5. Enzyme-linked immunosorbent assay (ELISA)

For quantitative measurement of adiponectin in cell culture supernatants, a Quantikine^{TM} immunoassay kit (R&D Systems, Minneapolis, MN, USA) was used, and adiponectin concentrations were determined according to the manufacturer's instructions.

6. Nuclear receptor (NR) assays

LanthascreenTM's time resolved fluorescence resonance energy transfer (TR-FRET) competitive binding assay kits (Invitogen, Carlsbad, California, United States) were used to evaluate the PPARα, PPARδ, and PPARγ binding activities of chemical ligands. All assay measurements were performed according to the manufacturer's instructions using CLARIOstar (BMG LABTECH, Ortenberg, Germany).

7. Molecular docking simulation

Docking simulations were performed using AutoDock Vina 1.1.2 software (The Scripps Research Institute, La Jolla, CA, USA) against crystal structures of PPARs (20, 21). The protein coordinate of PPAR was downloaded from the Protein Data Bank. The protein crystal structure was prepared for docking by removing the native ligand from the ligand-binding domain, followed by adding polar hydrogens using MGLTools 1.5.6 (The Scripps Research Institute). The Center of Grid box was calculated and the size of the grid box (docking space) was confined by the native ligand. We focused on key amino acid residues of the hydrophilic and hydrophobic regions of the ligand-binding domain for successful docking. Docking success was evaluated based on the lowest affinity value.

8. Statistical analysis

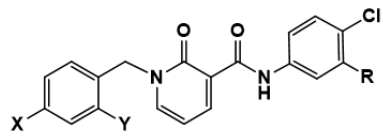
Experimental values are expressed as the mean ± standard deviation. Statistical analysis was performed using one-way ANOVA. *P*-values of less than 0.05 and 0.01 were regarded as statistically significant.

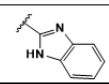
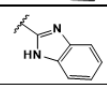
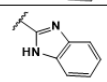
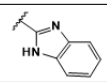
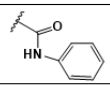
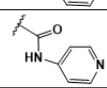
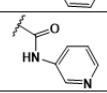
III. Results

1. PDK1 activity of benzoimidazole derivatives.

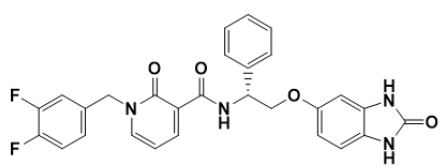
In previous study, 3-Phosphoinositide-dependent protein kinase-1 (PDK1) serves as an important regulatory point in insulin signaling through its ability to phosphorylate several protein kinase families (28). PDK1 is important in maintenance of glucose homeostasis. And the inhibitors of PDK1 inhibitors reportedly inhibit insulin-stimulated glucose transport (29, 30). As the structures showed, these new synthetic benzoimidazol derivatives have similar group with PDK1 inhibitor, MP7 (31). So we assumed that these compounds also have PDK1 inhibiting activity. We performed the PDK1 Human AGC Kinase Enzymatic Radiometric Assay to test PDK1 activity at 30 μM and 10 μM. However, as the results showed, all the 7 compounds had significant PDK1 activity that over 50% which suggested that they all had no PDK1 inhibiting activity.

A



Compound	X	Y	R	PDK1 activity(%) at 30μM	PDK1 activity(%) at 10μM
Cpd1	F	H		75 ± 0	80 ± 5
Cpd2	F	F		85 ± 4	79 ± 3
Cpd3	CH_3O	H		76 ± 2	83 ± 4
Cpd4	Cl	Cl		90 ± 0	84 ± 5
Cpd5	F	F		72 ± 0	76 ± 4
Cpd6	F	F		84 ± 4	83 ± 2
Cpd7	F	F		78 ± 0	72 ± 5

B



MP7

phosphoinositide-dependent kinase-1 (PDK1) inhibitor

Figure 11. PDK1 activity of benzoimidazole derivatives

The structures of new synthesized benzoimidazole derivatives (A) have similar groups with PDK1 inhibitor, MP7 (B). Then we tested the PDK 1 inhibiting activity of compound 1 to compound 7 at 30 μM and 10 μM by PDK1 Human AGC Kinase Enzymatic Radiometric Assay (A). Percentage values represent mean ± SD (n = 3).

2. Benzoimidazole derivatives promote adiponectin secretion during adipogenesis in hBM-MSCs.

Then, we focused on the adipogenesis activity of these compounds. CCK-8 assay was firstly performed to evaluate the cell viability in hBM-MSCs. According to the result of CCK-8 assay, we found that all the compounds had serious cytotoxicity at 60 μM and 30 μM compared to the 100 vehicle. What's more, compound 1, 2 and 3 had cytotoxicity at 10 μM with the cell viability of 88%, 85% and 83%. Due to the cytotoxicity of this three compounds at 10 μM, all the compounds were treated at 3 μM to test the activity of adipogenesis in hBM-MSCs. In quantitative analysis of adiponectin production, adiponectin ELISA results, 7 compounds all promoted adiponectin secretion compared to IDX control but at different levels. As the results showed, compounds 1, 2, 3 and 4 significantly promoted adiponectin levels during adipogenesis in hBM-MSCs by 624%, 568%, 407% and 569% compared to 100% IDX control, but less potent than the anti-diabetic drug glibenclamide at 10 μM (857%), and pioglitazone at 10 μM (1468%). We notably found that compound 5, 6 and 7 which the substituents of benzoimidazole were displaced to phenlycarbamoly had weaker adiponectin-promoting activity than compound 1 to 4 with the relative adiponectin production of 161%, 273% and 245% compared to 100% IDX control.

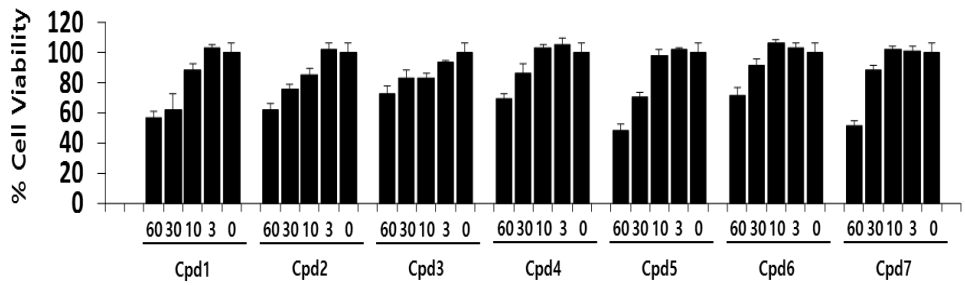


Figure 12. Cell viability of benzoimidazole derivatives in hBM-MSCs.

hBM-MSCs were cultured and then treated with benzoimidazole derivatives in a dose-dependent manner (60 μM, 30 μM, 10 μM, 3 μM) for 5days, the period is same as adipogenesis process . Cell viability was measured by CCK-8 assay. After 20min incubation with CCK-8 solution, the O.D. value was quantified at 450nm. The ratio of cells surviving in each concentration and compounds was calculated relative to the vehicle. Values are expressed as mean ± S.D. (n=3).

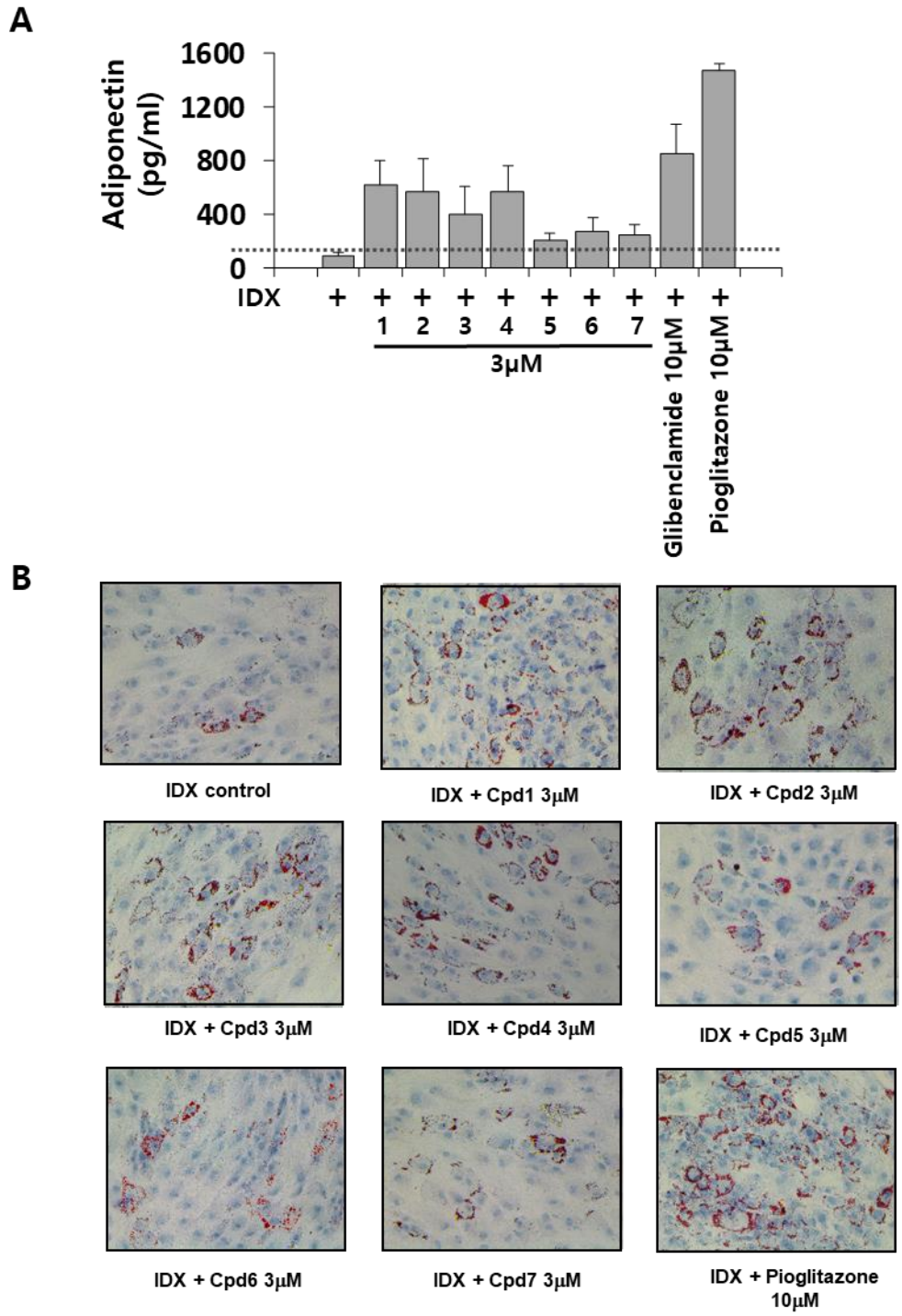


Figure 13. Adiponectin-promoting effects of benzoimidazole derivatives during adipogenesis in hBM-MSCs.

hBM-MSCs were treated with benzoimidazole derivatives at 3 μM in IDX supplemented medium. The cell culture supernatants were harvested on the 7th day in culture and used to measure the amount of adiponectin secretion. Pioglitazone and glibenclamide were used as positive controls (A). Relative adiponectin levels were normalized to IDX control as 100.Values represent mean ± SD (n=3). The levels of lipid accumulation of differentiated hBM-MSCs were qualitatively measured using Oil Red O staining. The extent of lipid accumulation was assessed relative to IDX control and positive control, pioglitazone and glibenclamide (B).

3. Ligand binding affinity of benzoimidazole derivatives to PPARγ

It was reported that PPARs are involved in the process of adipocyte differentiation, so we speculated that the adiponectin-promoting potential of benzoimidazole derivatives also had close correlations with PPAR signaling pathway. In order to identify the molecular target, TR-FRET NR competitive binding assays were performed to do screening with 7 compounds first. Unfortunately, these benzoimidazole derivatives had low binding affinity with PPARα and PPARδ. We found that all the benzoimidazole derivatives had high binding affinity with PPARγ. Next, we determined the concentration–response relationship of benzoimidazole derivatives in terms of their binding activity to PPARγ. Compared to positive control, pioglitazone, as anticipated, benzoimidazole derivatives displayed significant competitive binding activities in a concentration-dependent manner. The K_i values of compound 1 to 7 were 0.11, 0.09, 0.22, 0.45, 0.23, 0.53, and 0.41 for PPARγ respectively. Therefore, we speculated that the competitive and selective activation suggests that the binding activities of PPARγ are positively correlated with the adipogenic potential of benzoimidazole derivatives during adipogenesis in hBM-MSCs.

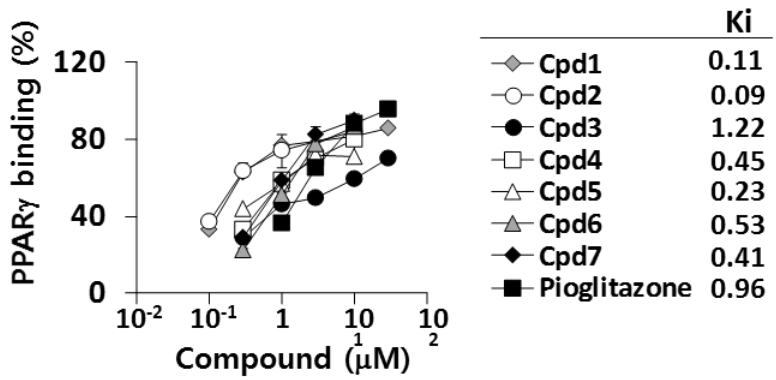


Figure 14. Binding activity of benzoimidazole derivatives on PPARγ

In order to determine the dose-dependent binding affinities of benzoimidazole derivatives to PPARγ, TR-FRET competitive binding assays were performed. The inhibition constant (K_i) was calculated by applying the Cheng-Prusoff equation. The positive control was pioglitazone for PPARγ. DMSO in buffer was used as a blank control. Values were expressed in terms of percentage compared to each positive control. Percentage values represent mean ± SD (n = 3).

4. Partial agonism effect and binding mode of benzoimidazole derivatives

Based on the results that benzoimidazole derivatives had high binding affinity with PPARγ, the biochemical validation studies for PPAR partial agonistic activity were needed. Partial agonists usually have less efficacy compared to a full agonist at saturating concentration in all tissue influenced by the receptors, and have partial antagonistic action when co-treated with a full agonist. Compounds were treated together with 1 μM PPARγ full agonist, pioglitazone at 3 μM in hBM-MSCs. Mono-treatment of PPARγ full agonist pioglitazone was also performed to evaluate adiponectin promoting potential at 1 μM. As the result showed, the adiponectin secretion of co-treatment of pioglitazone and compound 1, 2, 3 and 4 were increased when compared to the full agonist pioglitazone treatment only. While compound 5, 6 and 7 decreased the adiponectin levels when co-treatment with pioglitazone compared to pioglitazone mono-treatment. In order to further prove the partial agonism effect of compound 5, 6 and 7, TR-FRET NR competitive binding assays were performed to analyze binding affinity in a concentration-dependent manner. We determined the concentration–response relationship of PPARγ full agonist pioglitazone, or various concentration of pioglitazone with compound 5, 6 and 7 at 3 μM in terms of their binding activity to PPARγ. As anticipated, compound 5, 6 and 7 all showed significant partial agonism effect compared to binding affinity of pioglitazone. Next, we analyzed the binding mode of benzoimidazole derivatives in agonist co-crystalized human PPAR receptor ligand binding domains (LBDs) by using docking analysis. Full agonists occupy arms I and II and establish hydrogen

bonds which stabilize H12. However, partial agonists mainly interact with arm III and arm II through several hydrophobic interactions (23-27). In the binding mode analysis between PPARγ receptor LBD (PDB ID: 3R5N) and benzoimidazole derivatives, compound 1, 2, 3 and 4 all stabilized the arm I hydrophilic pocket with bonding on Helix 12 which indicated the full modulation for PPARγ., while compound 5, 6 and 7 had binding interaction residues mostly in the arm II which indicated the partial modulation for PPARγ. Based on these results, we suggested that compound 1, 2, 3 and 4 with group of benzaimidazol had adiponectin-promoting activity as PPARγ full modulators. And compound 5, 6 and 7 with group of phenylcarbamoyl promoted adipogenesis and adiponectin secretion as PPARγ partial modulators.

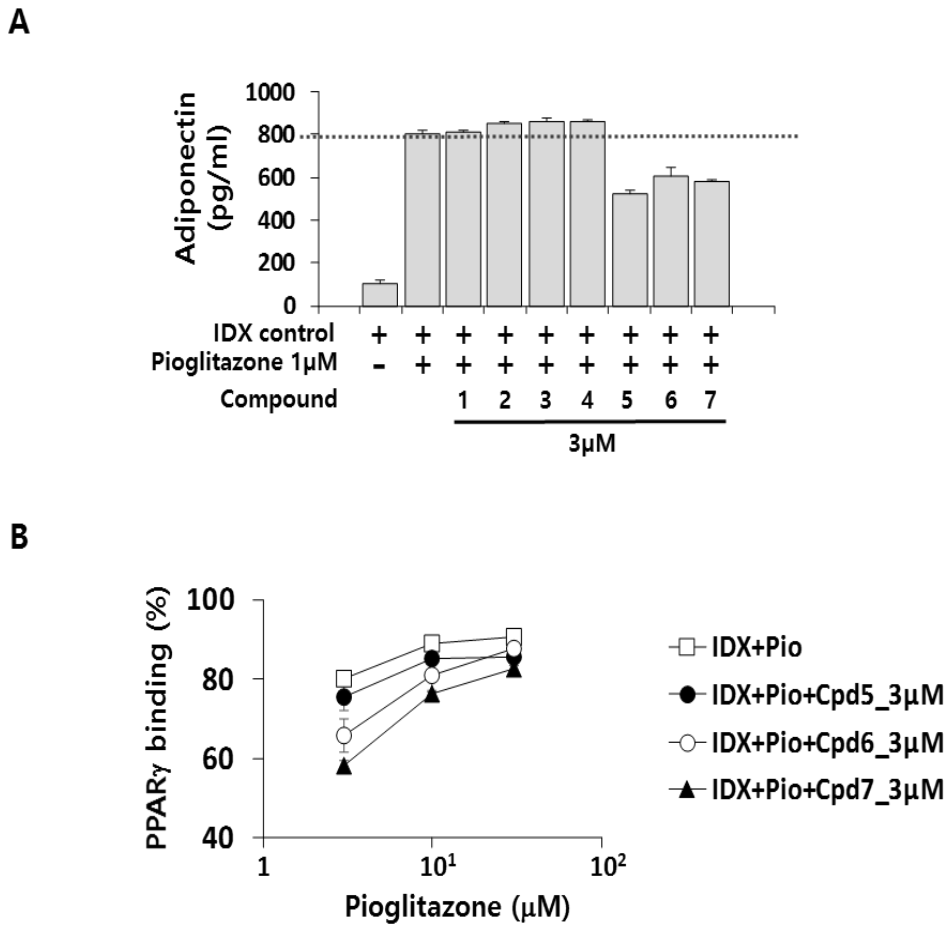


Figure 15. Synergistic adiponectin promoting effect of benzoimidazole derivatives co-treatment with PPARγ full agonist in hBM-MSCs.

hBM-MSCs were grown in adipogenesis inducing medium (IDX). To confirm the synergistic effects of combination of PPAR full agonist at low concentrations, hBM-MSCs were treated with PPARγ full agonist pioglitazone only at 1 μM or co-treatment of pioglitazone at 1 μM with each benzoimidazole derivatives at 3 μM for 7 days. Adiponectin production in the supernatant were evaluated by ELISA (A).

Then, TR-FRET competitive binding assay was performed to confirm partial agonism effects of PPARγ full agonists and each compound 5, 6 and 7. The positive control was pioglitazone and DMSO in buffer was used as a blank control (B). All data represent mean ± SD (n = 3).

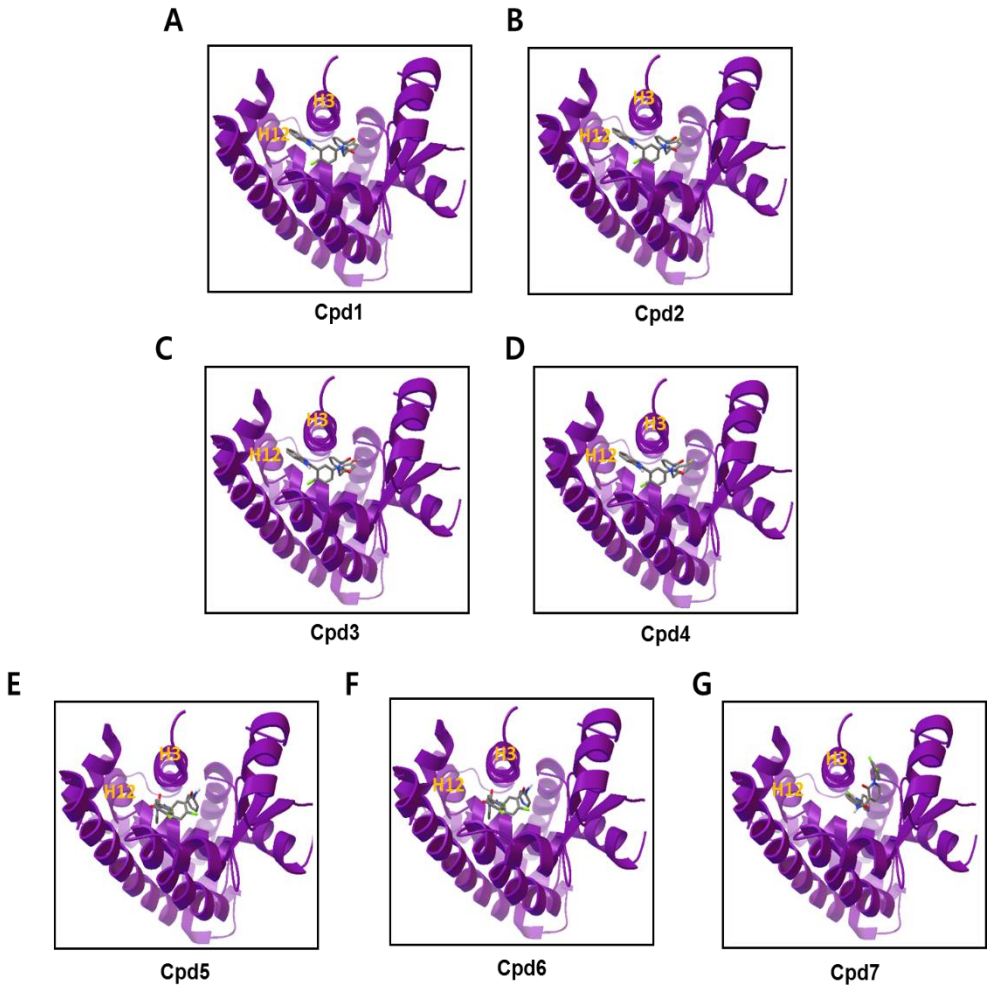


Figure 16. Binding modes of benzoimidazole derivatives with PPARγ LBD

We analyzed the protein-ligand interactions between the human PPARγ LBD (PDB ID: 3R5N and compound 1 (A), compound 2 (B), compound 3 (C), compound 4 (D), compound 5 (E), compound 6 (F) and compound 7 (G) were depicted and the interactions were visualized by using AutoDock Vina. Key amino acid residues involved in the PPARγ binding pocket have been labeled.

IV. Discussion

In our study, we found that the new synthesized benzoimidazole derivatives promoted adiponetcin secretion and adipogenesis in hBM-MSCs. In new synthesized benzoimidazole derivatives, the substituent of 2-oxo-1, 2-dihydropyridine-3-carboxamide which is showed to have similar substituents with phosphoinositide-dependent kinase-1 (PDK1) inhibitor, MP7. However, all the 7 compounds had no PDK1 inhibiting activity. It has been reported that the inhibitors of PDK1 inhibit insulin-stimulated glucose transport (29, 30). So, it also authenticated the in vitro bioactivity results that new synthesized benzoimidazole derivatives promoted adipogenesis and adiponectin secretion significantly.

In previous study, PPARs mainly control the expression of gene networks involved in adipogenesis, lipid metabolism, and the maintenance of metabolic homeostasis (16, 17). The antidiabetic thiazolidinediones, which are known as ligands of the PPARγ, were shown to significantly increase adiponectin expression during adipocyte differentiation (3). Therefore, we hypothesized that the PPAR signaling pathway may be involved in the adiponectin-secreting activity of our synthetic compounds in hBM-MSCs during adipogenesis. In our study, all the benzoimidazole derivatives had high binding affinity with PPARγ. We suggest that new synthesized benzoimidazole derivatives may be used as potential treatments in type 2 diabetes and related metabolic disorders by increasing glucose and lipid levels as PPARγ modulators.

In the structures of 7 compounds, compound 1, 2, 3 and 4 which have the substituent of benzoimidazole showed PPARγ full modulating effect. While compound 5, 6 and 7 showed PPARγ partial modulating effect that the substituent of these compounds were displaced by phenylcarbamoyl. Based on the results, we suggested that it may have close correlation between substituents and PPARγ binding mode. We also found that the phenylcarbamolyl substituent in compound 5, 6 and 7 are similar to PPARγ antagonist, GW9662. Therefore, we supposed that owing to the displacement of benzoimidazole to phenlycarbamolyl, compound 5, 6 and 7 possessed both PPARγ agonist and antagonist effect, which showed PPARγ partial agonism effect during adipogenesis in hBM-MSCs. As reported, Thiazolidinediones (TZDs) are one important class of full agonists of PPARγ and are currently being used in the treatment of type 2 diabetes (40). Although they are potent insulin sensitizers and have many clinical benefit, PPARγ full agonists have been associated with many unexpected adverse side effects, including weight gain, renal fluid retention and a possible increased incidence of cardiovascular events (40). PPARγ partial agonists have been reported to retain their anti-diabetic potential while having less unexpected side effects. Recent studies have exerted considerable efforts to design partial PPARg agonists that retain their insulin-sensitizing activity without significant side effects (41-43). In this regard, compound 5, 6 and 7 that substituents were displaced by phenylcarbamoyl may provide a therapeutic advantage as partial PPARγ agonists over full PPARγ agonists.

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요약 (국문초록)

중간엽줄기세포 지방분화 아디포넥틴 분비 촉진 화합물의 페록시솜증식체활성화수용체 조절 기능 연구

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제약학과 천연물과학 전공

아디포넥틴은 지방세포에서 분비되는 단백질로 염증, 세포사멸, 포도당 및 지방대사 등 다양한 생리적 기능을 조절한다. 아디포넥틴은 제 2 형 당뇨병, 비만 그리고 미세 혈관 합병증 등의 대사성 질환을 개선할 수 있는 치료제로 주목받고 있다. 아디포넥틴 분비를 촉진시키는 천연물이나 합성 화합물을 평가하기 위하여 인간 골수에서 유래한 중간엽줄기세포(hBM-MSCs)의 지방분화 모델을 활용할 수 있다. 본 연구에서는 중간엽줄기세포 지방분화 과정에서 아디포넥틴 분비를 촉진하는 천연물유래화합물과 합성화합물에 대한 분자약물학적 타겟을

규명하였다. 아디포넥틴 분비를 촉진하는 천연물 유래 화합물 탐색 과정에서, 후박(*Magnolia Officinalis*)의 주성분인 magnolol, honokiol 및 methylhonokiol 이 중간엽줄기세포의 지방 분화를 촉진하고 아디포넥틴을 증가시켰다는 것을 확인하였다. 아디포넥틴 분비 촉진과 관련한 표적 탐색 결과 magnolol, honokiol, methylhonokiol 은 페록시솜증식체활성화수용체(peroxisome proliferator-activated receptor, PPAR) 알파형과 감마형에 결합하는 다중 PPAR 수용체 조절제임을 확인하였다. PPARα 또는 PPARγ 에 대한 단독 수용체 조절제와 비교하였을 때, magnolol, honokiol 및 methylhonkinol 은 PPARα 와 PPARγ 에 대한 다중 PPAR 수용체 조절제로 작용함으로써 지질 대사를 조절하는 효소들 중 HMGCS1, FADS1 의 발현을 유도함을 확인하였다. 중간엽줄기세포 지방분화 아디포넥틴 촉진 물질 검색 과정에서 합성화합물질인 벤조이미다졸 (benzoimidazole) 유도체가 아디포넥틴 촉진 효능이 있음을 발견하였고, 이에 대한 분자 표적을 연구하였다. 본 유도체는 PDK1 효소 저해제 약리단에 벤조이미다졸 그룹을 도입한 합성화합물이지만, PDK1 효소에 대한 억제 활성은 없었다. 분자약물 표적 탐색 결과 벤조이미다졸 유도체는 PPARγ 수용체에는 농도의존적인 결합 활성을 나타냈으며, 완전효능약으로 작용하였다. 벤조이미다졸 그룹을 페닐카바모일 유도체로 치환한 화합물도 아디포넥틴 분비 촉진 효과를 보였으며, PPARγ 수용체에 농도의존적으로 결합하였다. 그러나, 페닐카바모일 유도체는 PPARγ 수용체에 부분효능약으로 작용하였다. 본 연구에서 발견한 다양한 아디포넥틴 분비 촉진 화합물은 퍼옥시좀 증식체 활성화

수용체 조절제로서 분자 약물학적 연구와 치료제 개발에 대한 통찰력을 제공할 것으로 기대한다.

주요어: 아디포넥틴, 지방본화, 중간엽줄기세포, 페록시솜 증식체-활성화수용체

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