A Novel Partial Agonist of Peroxisome Proliferator-Activated Receptor γ with Excellent Effect on Insulin Resistance and Type 2 Diabetes

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ABSTRACT

Partial agonists of peroxisome proliferator–activated receptor γ (PPARγ) reportedly reverse insulin resistance in patients with type 2 diabetes mellitus. In this work, a novel non–thiazolidinedione–partial PPARγ ligand, MDCCCL1636 [N-(4-hydroxyphenethyl)-3-mercapto-2-methylpropanamide], was investigated. The compound displayed partial agonist activity in biochemical and cell-based transactivation assays and reversed insulin resistance. MDCCCL1636 showed a potential antidiabetic effect on an insulin-resistance model of human hepatocarcinoma cells (HepG2). High-fat diet–fed streptozotocin-induced diabetic rats treated with MDCCCL1636 for 56 days displayed reduced fasting serum glucose and reversed dyslipidemia and pancreatic damage without significant weight gain. Furthermore, MDCCCL1636 had lower toxicity in vivo and in vitro than pioglitazone. MDCCCL1636 also potentiated glucose consumption and inhibited the impairment in insulin signaling targets, such as Akt, glycogen synthase kinase 3β, and glycogen synthase, in HepG2 human hepatoma cells. Overall, our results suggest that MDCCCL1636 is a promising candidate for the prevention and treatment of type 2 diabetes mellitus.

Introduction

Peroxisome proliferator–activated receptor γ (PPARγ) is a ligand-dependent nuclear receptor that regulates gene expression associated with glucose homeostasis and insulin sensitization (Evans et al., 2004; Lehrke and Lazar, 2005; McKenna et al., 2009). PPARγ is expressed in the liver, fat, and skeletal muscle. PPARγ gene deletion leads to insulin resistance and diet-induced obesity in mice, indicating that PPARγ plays a regulatory role in lipid and glucose homeostasis and tissue inflammation. PPARγ is a valid molecular target for metabolic syndrome and type 2 diabetes (Walczak and Tontonoz, 2002; Waki et al., 2007). Several chemically diverse full agonists of PPARγ, such as thiazolidinediones (TZDs), have been used as insulin-sensitizing drugs in type 2 diabetes. Partial agonists have recently been developed to reduce the side effects (e.g., weight gain, risk of heart attack and edema) of TZDs (Berger et al., 2005). Full and partial PPARγ agonists are categorized by their transcriptional activities in the cell-based reporter assay (Reginato et al., 1998). Partial PPARγ agonists possess higher safety margins than full PPARγ agonists (Berger et al., 2005). Recent studies have exerted considerable efforts to design partial PPARγ agonists that retain their insulin-sensitizing efficacy without significant side effects.

Insulin resistance, which results from the defective utilization of metabolites in insulin-targeted tissues, is the major cause of type 2 diabetes and obesity (Leclercq et al., 2007). The insensitivity of the liver to the biologic effects of insulin leads to a decrease in insulin-induced glucose transport; hence, human hepatocarcinoma cells (HepG2) are a good experimental system to investigate insulin resistance and the metabolic disorder (Postic et al., 2004). Dexamethasone (DEX) induces insulin resistance (Møller et al., 2009) by accelerating hepatic glucose production and proteometabolism and suppressing peripheral glucose transport and utilization. In this work, an in vitro insulin resistance cell model was established to screen active compounds that can reverse insulin resistance (Sangeetha et al., 2010).

ABBREVIATIONS: DEX, dexamethasone; DMEM, Dulbecco’s minimum essential medium; FSG, fasting serum glucose; GS, glycogen synthase; GSK, glycogen synthase kinase; HDL-c, high-density lipoprotein cholesterol; HepG2, human hepatocarcinoma cells; HFD-STZ, high-fat diet–fed streptozotocin; LBD, ligand-binding domain; LDL-c, low-density lipoprotein cholesterol; MDCCCL1636, N-(4-hydroxyphenethyl)-3-mercapto-2-methylpropanamide; MDCCK, Madin-Darby canine kidney; MS, mass spectrometry; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI3K, phosphatidylinositol 3-kinase; PPARγ, peroxisome proliferator-activated receptor γ; TC, total cholesterol; TG, triglyceride; TZD, thiazolidinedione.
We searched for novel PPARγ agonists in a library of structurally diverse organic compounds and identified a novel non-thiazolidinedione-partial PPARγ ligand, MDCCCL1636 [N-(4-hydroxyphenethyl)-3-mercapto-2-methylpropanamide] (Fig. 1A). This ligand was synthesized in our laboratory (Li et al., 2014), and its partial activation of PPARγ and effect on insulin resistance reversal were assessed in vitro. The cytotoxicity and the developmental toxicity in zebrafish embryos treated with MDCCCL1636 were evaluated and compared with those treated with pioglitazone. MDCCCL1636 effectively improved glucose tolerance and total plasma cholesterol level in high-fat diet–fed streptozotocin (HFD-STZ)–induced diabetic rats. We also investigated the major markers involved in insulin signaling, such as phosphatidylinositol 3-kinase (PI3K) signaling, to elucidate the mechanism by which MDCCCL1636 reverses insulin resistance.

**Materials and Methods**

**Cells and Animals.** Human hepatocarcinoma HepG2, HEK-293T, HEK-293, NIH-3T3, and Madin-Darby canine kidney (MDCK) cells were obtained from KAIJI Company (Nanjing, China). These cells were maintained in Dulbecco’s minimum essential medium (DMEM)/high glucose with 10% fetal bovine serum at 37°C in a humidified 5% CO2 environment.

AB strain zebrafish (Danio rerio) was obtained from Tianjin International Joint Academy of Biomedicine (Tianjin, China). Normal-glycemic Wistar albino rats were obtained from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). Antibodies and Reagents. Antibodies to phosphoAkt (Ser473), phospho-glycogen synthase kinase 3β (GSK-3β), and phospho-glycogen synthase (Ser21/27) purchased from Affinity (Ancaster, ON, Canada). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and dimethyl sulfoxide were purchased from Sangon Biotech (Shanghai, China). Glucose assay kit was purchased from Leadman (Shanghai, China). TOF MS was purchased from Meilun Biotech Co., Ltd. (Dalian, Liaoning, China).

**Transcriptional Transactivation Assay.** HEK-293T cells cotransfected with pGal4-DBD (pBIND)-PPARγ-ligand-binding domain (LBD) fusion and pG5luc vector were used to measure the transcriptional transactivation activities of the compounds through luciferase reporter assay (Burgermeister et al., 2006). Approximately 204–505 residues of the human PPARγ-LBD were cloned into the pBIND to generate fusion proteins with the DNA-binding domain of GAL4. HEK-293T cells were grown in DMEM with 10% fetal bovine serum at 37°C in a humidified 5% CO2 environment.

**Mass Spectrometry Assay.** The purified PPARγ-LBD protein (methods for expression and purification of PPARγ-LBD are available in the Supplemental Material) was diluted to 50 μM in 10 mM ammonium acetate and incubated with MDCCCL1636 in a 1:5 molar excess for 15 minutes. The PPARγ-LBD-MDCCCL1636 complex was analyzed with a Waters (Milford, MA) SYNAPT G1 high-definition mass spectrometry (MS).

**Development of Insulin-Resistant Model by Using HepG2 Cells.** HepG2 cells were seeded in 96-well plates at a concentration of 1 × 104 cells per well and then treated in serum-free DMEM/high glucose overnight. Subsequently, the cells were induced with 100 nM, 1 μM, 5 μM, and 10 μM DEX for 24, 48, and 72 hours. At selected time points, the cells were washed three times with phosphate-buffered saline and stimulated for 24 hours with 1 nM insulin. Glucose uptake was detected by using a glucose assay kit. The kit was used to detect the glucose level in the medium via the glucose oxidase-peroxidase method. Subsequently, we calculated the consumption of glucose. The best time point for the insulin-resistant model was determined by measuring the maximum inhibition in glucose uptake (Yuan et al., 2003). The insulin-resistant model was validated using pioglitazone. The HepG2 cells were induced with 100 nM DEX for 24 hours and then treated with varying concentrations of pioglitazone for 24 hours.
with 1 nM insulin. Glucose uptake was detected using a glucose assay kit.

Effect of MDCCCL1636 on Glucose Uptake in Insulin-Resistant HepG2 Cells. MDCCCL1636 and pioglitazone were used to treat the normal control cells and insulin-resistant cells for 24 hours to assess the effect of MDCCCL1636 and pioglitazone on glucose uptake. A glucose assay kit was used for the glucose uptake experiments.

Cytotoxicity Assessment. MDCCCL1636 and pioglitazone were assessed for their cytotoxic effects on HEK-293, HepG2, NIH-3T3, and MDCK cells by using an MTT reagent. The assay was performed 24 hours after treatment with varying concentrations of MDCCCL1636 and pioglitazone. Formazan concentration, which is directly proportional to cell viability, was measured at 492 nm.

Developmental Toxicity Assay in Zebrafish Embryos. AB line zebrafish were maintained in accordance with the standard procedures (Westerfield, 1994). The night before breeding, adult male and female zebrafish were placed in a breeding tank and then separated from each other with a mesh screen. The embryos were generated through natural mating the next morning, after turning on the light and withdrawing the mesh screen. The embryos were collected within 30 minutes after spawning, rinsed three times, transferred into Petri dishes containing the embryo medium, and then cultured at 28.5°C.

The protocol described by Reimers et al. (2004) was adopted in this experiment. The normal embryos were selected and transferred into a multiwell microplate with one embryo per well in 300 μl of treatment solution. A total of 30 embryos at 4 hours after fertilization were treated with varying concentrations of MDCCCL1636 and pioglitazone, and the control group was treated with 0.1% dimethyl sulfoxide. All experiments were repeated three times. The mortality and malformation rates of embryos were calculated at the end of the experiment. The study was performed in accordance with the national and institutional guidelines for animal welfare.

In Vivo Efficacy of MDCCCL1636 in HFD-STZ-Induced Type 2 Diabetic Rats. Normoglycemic male Wistar albino rats weighing 180–200 g were used. All rats, except those in the normal control group, were fed with HFD (20% glucose, 10% egg yolk powder, 10% lard, 0.2% bile salts, 1.5% cholesterol, and 58.3% normal commercial pellet diet). After 10 days of HFD, the rats were fasted overnight for 12 hours and then given a single injection of freshly prepared solution of STZ (40 mg/kg) in citrate-phosphate buffer (0.1 M, pH 4.2) (Sharma et al., 2011). Hyperglycemia in the rats was assessed by measuring fasting serum glucose (FSG) levels after 72 hours of STZ administration. Rats with an FSG levels higher than 13.89 mmol/liter were selected for the subsequent experiments (Sharma et al., 2011). The rats were randomly divided into six groups of six animals each: (1) normal control group (rats without any treatment); (2) diabetic control group (diabetic rats treated with vehicle); (3) positive control group (diabetic rats treated with pioglitazone at 30 mg/kg per day); (4) high-dose group of MDCCCL1636 (diabetic rats treated with MDCCCL1636 at 15 mg/kg per day); (5) medium-dose group of MDCCCL1636 (diabetic rats treated with MDCCCL1636 at 7.5 mg/kg per day); and (6) lower-dose group of MDCCCL1636 (diabetic rats treated with MDCCCL1636 at 3.75 mg/kg per day). Treatment was continued for 56 days.

In the oral glucose tolerance test assay, rats fasted for 12 hours were administered with vehicle or drugs after FSG measurement and then with an oral bolus of glucose (2 g/kg). Subsequently, the blood glucose level was measured at 0, 0.5, 1, and 2 hours. On the last day, FSG levels were measured and then blood was withdrawn from the heart. Serum was analyzed for triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) after being separated by centrifugation at 3000g for 10 minutes. The animals were sacrificed, and the pancreas was subjected to histopathological studies (Kamalakkannan and Prince, 2004). The lipid profiles were assessed using commercial kits. Formalin-fixed pancreatic tissue was cut in 4-μm-thick sections and then stained with H&E (Sharma et al.,
The stained sections were blindly examined using a light microscope (Nikon, Tokyo, Japan). The study was performed in accordance with the national and institutional guidelines for animal welfare.

Effect of MDCCCL1636 on the Expression of Insulin Signaling Markers in PI3K Signaling. Insulin-sensitive cells, nontreated insulin-resistant cells, and MDCCCL1636 or pioglitazone-treated insulin-resistant cells were lysed at 4°C with radioimmunoprecipitation assay lysis buffer. The cell lysates were subjected to SDS-PAGE and then Western blot analysis using anti-phospho antibodies for AKT, GSK-3β, and glycogen synthase (GS).

Statistical Analysis. Data were reported as mean ± SD. All data were analyzed using one-way analysis of variance followed by Bonferroni post hoc test (SPSS software package version 17.0; SPSS Inc., Chicago, IL). Statistical significance was considered at P < 0.05.

Results

Identification of the Partial PPARγ Agonist MDCCCL1636

MDCCCL1636 (molecular mass: 239.1 Da) was identified as a lead hit among a class of partial PPARγ ligands through transcriptional transactivation assay of a PPARγ agonist library. In HEK-293T cells, MDCCCL1636 (EC50 = 7.092 μM) was a partial agonist with 40% efficacy compared with pioglitazone (EC50 = 3.36 μM) at 100 μM (Fig. 1B).

Verifying the Interaction of MDCCCL1636 and PPARγ-LBD

MS data revealed peaks corresponding to the molecular weight of the protein PPARγ-LBD and the complex PPARγ-LBD-MDCCCL1636. The molecular masses of the PPARγ-LBD protein the complex were 32910 Da (Fig. 2A) and 33146 Da (Fig. 2B), respectively. The difference in molecular mass between PPARγ-LBD protein and the PPARγ-LBD-MDCCCL1636 complex was 236 Da, which was approximately equal to the molecular mass of MDCCCL1636. This result demonstrated that the MDCCCL1636 strongly interacted with the ligand binding domain of human PPARγ.

Effect of MDCCCL1636 on the Insulin-Resistant Model of HepG2 Cells

Time and Concentration Course Analyses of DEX Induction on HepG2 Cells. Time and concentration course analyses of DEX treatment at 100 nM, 1 μM, 5 μM, and 10 μM concentrations in 24, 48, and 72 hours were performed. Insulin resistance of DEX was also assessed based on the inhibition of glucose uptake. Insulin-stimulated glucose uptake was reduced maximally to approximately 26.83% at 24 hours of 100 nM DEX treatment compared with controls (Fig. 3A). Thus, treatment with 100 nM DEX for 24 hours was significantly different from the control group in all three time points (Fig. 3B).

MS data revealed peaks corresponding to the molecular weight of the protein PPARγ-LBD and the complex PPARγ-LBD-MDCCCL1636. The molecular masses of the PPARγ-LBD protein the complex were 32910 Da (Fig. 2A) and 33146 Da (Fig. 2B), respectively. The difference in molecular mass between PPARγ-LBD protein and the PPARγ-LBD-MDCCCL1636 complex was 236 Da, which was approximately equal to the molecular mass of MDCCCL1636. This result demonstrated that the MDCCCL1636 strongly interacted with the ligand binding domain of human PPARγ.
the optimal conditions for the establishment of insulin-resistant model.

Cell viability was assayed at different time points (24, 48, and 72 hours) of DEX induction to eliminate the influence of DEX toxicity on the inhibition of glucose uptake. DEX exhibited no significant cytotoxicity to the cells at 24 hours, which confirmed the insulin-resistant inducing effect of DEX on HepG2 cells (Fig. 3B).

Validation of DEX-Induced Insulin-Resistant Model.

Pioglitazone was used as a positive control to determine the validity and the reversal properties of the DEX-induced insulin-resistant model. The induced HepG2 cells were treated with varying concentrations of pioglitazone for 24 hours. Glucose uptake analysis showed that pioglitazone reversed the DEX-induced insulin resistance in a dose-dependent manner (Fig. 3C).

Glucose Uptake Potential of MDCCCL1636 on the DEX-Induced Insulin-Resistant Model. The effects of MDCCCL1636 and pioglitazone on insulin resistance were assessed and compared using the DEX-induced insulin-resistant model. MDCCCL1636 effectively restored the DEX-induced glucose uptake inhibition in a dose-dependent manner (Fig. 3D). MDCCCL1636 maximally augmented glucose uptake to approximately 45.13% at 150 μM in the DEX-induced insulin-resistant model. The response of MDCCCL1636 at 75 μM (approximately 41.45%) to insulin resistance reversal was higher than that of pioglitazone (34.82%).

Cytotoxicity of MDCCCL1636 and Pioglitazone on HEK-293, HepG2, NIH-3T3, and MDCK Cells

The cytotoxic effects of MDCCCL1636 and pioglitazone on HEK-293, HepG2, NIH-3T3, and MDCK cells were assessed via a cytotoxicity assay. MDCCCL1636 showed negligible effect on cell viability, whereas pioglitazone exhibited about 25% (Fig. 4, A–C) and 50% (Fig. 4D) toxic effects at its highest concentration. This result confirmed that MDCCCL1636 had lower cytotoxicity than pioglitazone.

Toxic Effects of MDCCCL1636 and Pioglitazone on Embryonic Development of Zebrafish

The mortality rate of zebrafish embryos treated with various concentrations of MDCCCL1636 and pioglitazone for 96 hours is presented in Fig. 5A. Treatment with 1 mM of pioglitazone for 96 hours resulted in 95% embryo death compared with 65% embryo death after MDCCCL1636 treatment. MDCCCL1636 and pioglitazone showed dose-dependent lethal effects. The lethal concentration of

| TABLE 1 |
| Effects of MDCCCL1636 on body weight and biochemical parameters of normal and diabetic rats |
| All values are expressed as mean ± S.D. Significance level was determined by one-way analysis of variance followed by Bonferroni post hoc test. |

<table>
<thead>
<tr>
<th>Biochemical Parameter</th>
<th>Normal Control</th>
<th>Diabetic Control</th>
<th>Pioglitazone 30 mg/kg</th>
<th>MDCCCL1636</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3.75 mg/kg</td>
<td>7.5 mg/kg</td>
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<tr>
<td>FSG (mmol/l)</td>
<td>5.6 ± 0.9</td>
<td>25.1 ± 2.9abc</td>
<td>20.4 ± 2.8ab</td>
<td>21.8 ± 2.3c</td>
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<tr>
<td>TG (mmol/l)</td>
<td>0.81 ± 0.10</td>
<td>1.01 ± 0.27abc</td>
<td>0.69 ± 0.19ab</td>
<td>0.74 ± 0.26bc</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>1.47 ± 0.18</td>
<td>8.88 ± 3.64abc</td>
<td>5.03 ± 1.27ab</td>
<td>4.80 ± 0.76bc</td>
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<tr>
<td>HDL-c (mmol/l)</td>
<td>0.78 ± 0.01</td>
<td>0.62 ± 0.12abc</td>
<td>0.68 ± 0.13ab</td>
<td>0.67 ± 0.13c</td>
</tr>
<tr>
<td>LDL-c (mmol/l)</td>
<td>0.26 ± 0.03</td>
<td>2.98 ± 1.44abc</td>
<td>1.62 ± 1.27abc</td>
<td>1.46 ± 0.56bc</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>553.85 ± 22.71</td>
<td>365.09 ± 12.53a</td>
<td>409.37 ± 16.39a</td>
<td>379.94 ± 22.46</td>
</tr>
</tbody>
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|          | P < 0.001 versus normal control. |
|          | P < 0.001 versus diabetic control. |
|          | P < 0.05 versus normal control. |

Fig. 6. Concentration-dependent developmental toxicity endpoints in zebrafish. Embryos were exposed to either water or to the indicated MDCCCL1636 or pioglitazone concentrations. Lateral view of 96 hours postfertilization embryos revealing numerous malformations. AM, axial malformation; PE, pericardial edema.
MDCCCL1636 that caused 50% mortality (LC$_{50}$) in embryos was 742.569 μM, whereas that of pioglitazone was 390.226 μM.

Exposure experiments revealed that the effective concentrations of MDCCCL1636 and pioglitazone to induce 50% malformations (EC$_{50}$) of the embryos was 916.044 μM and 420.741 μM (Fig. 5B), respectively.

Pericardial edema and axial malformation were observed in embryos exposed to 50 mM pioglitazone, whereas no obvious malformation was observed in embryos exposed to less than 250 mM MDCCCL1636. Embryos subjected only to more than 500 mM MDCCCL1636, would showed pericardial edema, and those exposed to more than 750 mM MDCCCL1636 would have axial malformations (Fig. 6). These results indicated that MDCCCL1636 exerted lower toxicity on zebrafish embryos than did pioglitazone.

**Hypoglycemic Effect of MDCCCL1636 on HFD-STZ–Induced Type 2 Diabetic Rats**

Administration of MDCCCL1636 at 15 mg/kg daily and pioglitazone at 30 mg/kg per day for 56 days resulted in a significant decrease in FSG compared with the diabetic control rats with a reduction of 20.72% and 18.73%, respectively.

As shown in Table 1, the HFD-STZ–induced diabetic rats had higher serum TG, TC, and LDL-c and significantly lower HDL-c than did the normal control rats. Treatment with 7.5 mg/kg MDCCCL1636 and 30 mg/kg pioglitazone significantly reduced serum TG (53.5% and 31.7%, respectively) and TC (56.86% and 43.4%, respectively) compared with diabetic control rats. In addition, rats treated with MDCCCL1636 and pioglitazone had significantly lower LDL-c (65.4% and 45.6%, respectively, $P < 0.001$) but significantly higher HDL-c (16.1% and 9.7%, respectively) than did the diabetic controls. Administration of pioglitazone at 30 mg/kg for 56 days resulted in a significant increase in body weight (12.13%), whereas MDCCCL1636-treated groups did not show significant changes in body weight in comparison with the diabetic control rats.

The oral glucose tolerance test was performed in HFD-STZ–induced type 2 diabetic rats on day 42 of the treatment period. After being challenged with an oral bolus of glucose, the MDCCCL1636-treated animals showed lower glucose excursion compared with the vehicle-treated diabetic animals. The results indicated that MDCCCL1636 improved the impaired glucose tolerance of type 2 diabetic rats (Fig. 7).

Figure 8A showed the histopathological changes in the pancreatic islet of the experimental groups (histopathological changes in the pancreas islets of rats in experimental groups [40×] were shown in Supplemental Fig. 1). The number and area of islets in the pancreas (Fig. 8, B and C) were also...
analyzed. Neither β-cell damage nor inflammatory changes were observed in the normal architecture of pancreatic islet (Fig. 8A 1). However, the β-cells were obviously damaged, and the area of pancreatic islets was significantly reduced in the HFD-STZ-induced diabetic rats (Fig. 8A 2). Damage to the pancreatic islets and β-cells in the diabetic rats was restored by MDCCCL1636 and pioglitazone, as evidenced by their protective effects on β-cell damage (Fig. 8A 3 and 4). Compared with pioglitazone, MDCCCL1636 also more effectively improved the fatty degeneration of renal tubular epithelial cells of the diabetic rats (supporting data are provided in the Supplemental Material and Supplemental Fig. 2).

**Effect of MDCCCL1636 and Pioglitazone on Insulin Signaling in Insulin-Resistant Cells**

AKT ser^473^ phosphorylation was reduced in insulin-resistant cells compared with control cells. The insulin-resistant cells showed 73.9% lower AKT Ser^473^ phosphorylation than control cells. Both pioglitazone and MDCCCL1636 significantly increased AKT Ser^473^ phosphorylation by approximately 1.86- and 3.68-fold, respectively (Fig. 9A). AKT expression was similar in the normal control and insulin-resistant cells. Both pioglitazone and MDCCCL1636 increased AKT Ser^473^ phosphorylation, although MDCCCL1636 showed better effects than pioglitazone showed.

GSK-3β Ser^9^ phosphorylation in the insulin-resistant cells was reduced by approximately 19.1% compared with that in the normal control cells. Pioglitazone and MDCCCL1636 increased GSK-3β phosphorylation in the insulin-resistant cells (Fig. 9B). GSK-3β expression was similar in the normal control and insulin-resistant cells. MDCCCL1636 and pioglitazone upregulated GSK-3β Ser^9^ phosphorylation by 0.53- and 0.21-fold, respectively.

Glycogen synthase Ser^645^ phosphorylation was higher in the insulin-resistant cells than in the normal control cells. DEX-treated insulin-resistant cells showed lower activity than the insulin-sensitive cells showed. Pioglitazone and MDCCCL1636 increased glycogen synthase activity by 62% and 70%, respectively, in the insulin-resistant cells compared

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**Fig. 9.** Effect of MDCCCL1636 and pioglitazone on insulin signaling in insulin-resistant cells. HepG2 cells were incubated with or without 100 nM DEX for 24 hours and in 1 nM insulin with or without the drugs (pioglitazone or MDCCCL1636) for another 24 hours. Cell lysates were separated by SDS-PAGE and subjected to Western blot analysis with (A) anti-phosphoAkt (ser473) antibody, (B) anti-phospho-GSK-3β (Ser9) antibody, and (C) anti-phosphoglycogen synthase (Ser645) antibody. Data are expressed as a percentage of the normal controls. **P < 0.05, ***P < 0.001 versus insulin resistance control; #P < 0.05, ###P < 0.001 versus normal control.
with the normal control cells (Fig. 9C). GS expression was similar in the normal controls and the insulin-resistant cells. The insulin-resistant cells treated with MDCCCL1636 had higher GS activity than those treated with pioglitazone.

**Discussion**

This study introduced the partial PPARγ activator MDCCCL1636. Transactivation assay data confirmed that the propanamide derivative functions as a partial PPARγ agonist that strongly combines with PPARγ-LBD, as determined by MS. Insulin-sensitizing agents could function as drugs to treat various metabolic disorders caused by insulin resistance (Kahn and Flier, 2000). An in vitro model that mimics insulin resistance was established to screen insulin-sensitive agents (Sakoda et al., 2000; Olivares-Reyes et al., 2009). The model mimicked the exact in vivo clinical insulin-resistant conditions, and it can be reversed by insulin-sensitizing drug treatment (Sakoda et al., 2000). In this work, a model of HepG2 cells was developed using DEX, and the model was used to assess the potential of MDCCCL1636 in insulin resistance reversal. The extent of insulin resistance of the model was measured through the inhibition of glucose uptake. In this work, the maximum desensitization (approximately 26.83%) of insulin-stimulated glucose uptake was achieved after 24 hours of 0.1 μM DEX treatment.

In this study, the TZDs pioglitazone was used to validate the developed resistance model. TZDs as insulin-sensitizing drugs reportedly reverse DEX-induced insulin resistance by suppressing the adverse effects of DEX on insulin sensitivity and glucose tolerance (Willi et al., 2002; Samuel, 2011). Concentration course analysis of the effect of pioglitazone on the insulin-resistant model revealed that TZD reversed DEX-induced insulin resistance in a dose-dependent manner. This study determined whether MDCCCL1636 could also reverse the DEX-induced impairment in glucose uptake. Similar to pioglitazone, MDCCCL1636 showed potential reversal of DEX-induced insulin resistance as evidenced by the restoration of glucose uptake. MDCCCL1636 and pioglitazone showed similar effects on insulin resistance; however, MDCCCL1636 had lower toxicity on cells and zebrafish embryos than pioglitazone. This result may be ascribed to the partial PPARγ agonist activity of MDCCCL1636.

Rats fed with HFD may develop insulin resistance (Kraegen et al., 1991). In addition, low-dose STZ STZ leads to a mild β-cell dysfunction and impairment of insulin secretion, which result in hyperglycemia (Mythili et al., 2004). Thus, feeding rats with HFD and treating them with low-dose STZ (40 mg/kg) can mimic type 2 diabetes (Reed et al., 2000; Parveen et al., 2010). In the present work, the HFD-STZ–treated type 2 diabetic rats showed significantly increased serum glucose and β-cell dysfunction, as well as decreased body weight and dyslipidemia. Our results showed that MDCCCL1636 reduced the serum glucose level and improved the impaired glucose tolerance in type 2 diabetes.

In the HFD-STZ–induced diabetic rats, dyslipidemia was manifested in the increased levels of LDL-c, TG, and TC and in reduced HDL-c levels. The hypercholesterolemia and hypertriglycerideremia observed in the model resulted from the increased absorption of TGs and TC from the HFD and from the elevated concentrations of very-low-density lipoproteins, which, consequently, increased LDL-c and reduced HDL-c levels (Zammit, 2001; Taskinen et al., 2003). In the present work, MDCCCL1636 treatment significantly reduced the TG, TC, and LDL-c levels, whereas increased the HDL-c level in the HFD-STZ–induced diabetic rats. MDCCCL1636 improves dyslipidemia, possibly by inhibiting hepatic TG secretion or increasing peripheral TG clearance (Kamalakkannan and Prince, 2005). MDCCCL1636 reverses insulin resistance, which consequently improves lipid metabolism.

In the present study, MDCCCL1636 upregulated PI3K signaling. Our data suggested that DEX induced HepG2 cells insulin resistance by impairing the phosphorylation of insulin signaling proteins of AKT, GSK-3β, or GS. DEX treatment reduced insulin-stimulated AKT and GSK-3β phosphorylation and blocked the dephosphorylation and activation of GS.

In this study, DEX treatment reduced insulin-stimulated AKT Ser473 phosphorylation by approximately 73.9%. Both MDCCCL1636 and pioglitazone increased AKT Ser473 phosphorylation in the insulin-resistant cells, but the effect of MDCCCL1636 was 0.64-fold higher than that of pioglitazone. The insulin-resistant cells also exhibited 19.1% lower GSK-3β Ser9 phosphorylation than the normal control cells. MDCCCL1636 can reverse such a reduction. DEX inhibited the glycogen synthase activity. MDCCCL1636 and pioglitazone reduced GS phosphorylation by 70% and 62%, respectively. DEX induction significantly desensitized HepG2 cells toward insulin-stimulated glucose uptake (Pereira et al., 2008). The possibility of this desensitization can also be attributed to impairment in the insulin signaling targets, including AKT, GSK-3β, and GS. Our results showed that MDCCCL1636 effectively restored DEX-induced desensitization by restoring AKT and GSK-3β phosphorylation and GS activity.

In conclusion, the novel compound MDCCCL1636 can partially activate PPARγ. MDCCCL1636 can also reduce FSG and reverse the dyslipidemia and damage of the pancreas without significantly increasing body weight. Moreover, MDCCCL1636 was less toxic in vivo and in vitro than was pioglitazone. Therefore, MDCCCL1636 is a potential treatment of type 2 diabetes.

**Authorship Contributions**

**Participated in research design:** Sun, Zhou.
**Conducted experiments:** Song, H. Liu, C. Zhang, Xiao, Meng, Liang, Q. Zhang.
**Performed data analysis:** Song, H. Liu, C. Zhang, Li, and Wang.
**Wrote or contributed to the writing of the manuscript:** Song, H. Liu, C. Zhang, B. Zhang, Y. Liu.

**References**
