Regular Article

Effects of KY-903, a Novel Tetrazole-Based Peroxisome Proliferator-Activated Receptor γ Modulator, in Male Diabetic Mice and Female Ovariectomized Rats

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Peroxisome proliferator-activated receptor γ (PPARγ) modulators are expected to exert anti-diabetic effects without PPARγ-related adverse effects, such as fluid retention, weight gain, and bone loss. The present study showed that the novel tetrazole derivative KY-903 exerted similar selective PPARγ partial agonist properties to INT-131, a known PPARgamma modulator, in transactivation assays, and decreased plasma glucose and triglyceride levels with increases in adiponectin levels in diabetic KK-Ay mice. These effects were similar to those of pioglitazone. Pioglitazone, but not KY-903, increased adipose tissue and heart weights. In pre-adipocytes (3T3-L1), KY-903, in contrast to pioglitazone, increased adiponectin mRNA levels without adipocyte differentiation, indicating anti-diabetic effects via adiponectin without adipogenesis. In ovariectomized rats fed a high-fat diet (OVX/HFD), KY-903 and pioglitazone decreased plasma triglyceride and non-esterified fatty acid levels and increased adiponectin levels, indicating insulin sensitization via adiponectin. KY-903 reduced body weight gain and adipose tissue weight, while pioglitazone increased heart weight and markedly reduced bone mineral density. In mesenchymal stem cell-like ST2 cells, KY-903 slightly reduced osteoblast differentiation without adipocyte differentiation, while pioglitazone markedly reduced it with adipocyte differentiation. In conclusion, KY-903 is a novel PPARγ modulator that exerts anti-diabetic effects without body weight gain or cardiac hypertrophy in diabetic mice and anti-obesity effects with minor bone loss in OVX/HFD, possibly due to increases in adiponectin levels without adipogenesis.

Key words  peroxisome proliferator-activated receptor γ (PPARγ); partial agonist; diabetes; obesity; adiponectin; adverse effect

INTRODUCTION

Peroxisome proliferator-activated receptor γ (PPARγ) is a master regulator of adipocyte differentiation in adipose tissues.1) Synthetic PPARγ agonists, such as pioglitazone (Fig. 1), have been clinically used as anti-diabetic drugs; they improve insulin resistance by promoting adipocyte differentiation and increase adiponcin release in type 2 diabetic patients. However, they exert various PPARγ-related adverse effects, such as fluid retention, body weight gain, cardiac hypertrophy, and reductions in bone mineral density (BMD).2,3) Therefore, a large number of PPARγ agonists with different properties have been synthesized with the aim of developing safer and more efficacious insulin sensitizers; however, none have been successful.4)

Three approaches have been used: PPARα/γ dual agonists, PPARγ agonists with inhibitory activity against protein tyrosine phosphatase-1B (PTP1B), and PPARγ partial agonists. PPARα/γ dual agonists, such as muraglitazar and tesaglitazar, were expected to exert the synergistic effects of PPARα and PPARγ activation on hyperglycemia and hyperlipidemia and to decrease body weight gain by PPARα activation; however, clinical studies were suspended due to unexpected adverse effects on cardiovascular and renal functions.5) PPARγ agonists that inhibit PTP1B were expected to be highly effective because PTP1B negatively regulates insulin signaling, and, thus, its suppression results in insulin sensitization.6) We previously reported that 3-carboxyl-tetrahydroisoquinoline-based PPARγ full agonists, PPARα/γ dual agonists, and PPARγ partial agonists, all with PTP1B inhibitory activities, exerted similar experimental anti-diabetic effects to and weaker adverse effects than rosiglitazone.7–10) However, their PPARγ-related and PTP1B-inhibition-related toxicities remain unclear. A larger number of PPARγ partial agonists have been synthesized and their therapeutic efficacies and safety have been reported in experimental animals.11–18) PPARγ partial agonists, such as INT-131 (Fig. 1), are considered to exert anti-diabetic effects through the partial activation of PPARγ-mediated transcription, which is not sufficient to induce adverse effects on adipogenesis and renal function. Alternatively, they may selectively activate the transcription of insulin-sensitizing genes or may act selectively in cells involved in insulin resistance. However, they have not yet been successfully developed for clinical use, possibly due to insufficient efficacy or PPARγ-mediated and/or structure-dependent adverse effects. PPARγ-related adverse effects on adipogenesis and BMD differ among PPARγ agonists.15–17) A structurally new PPARγ partial agonist that more safely and efficaciously improves insulin resistance is desired as therapy for type 2 diabetes.

Previously reported partial agonists were classified into natural products, fatty acid derivatives, thiazolidinediones, carboxylates, and sulfonamides. We found a 6-tetrazoyl-tetrahydroisoquinoline-based PPARγ partial agonist without PTP1B inhibition that exerted similar anti-diabetic effects to pioglitazone; however, its safety currently remains unclear.18)
We recently synthesized the novel tetrazole derivative KY-903 (Fig. 1). The present study investigated and compared the PPARγ agonist activity of KY-903 with those of pioglitazone and INT-131 as well as its efficacy and adverse effects in diabetic mice (KK-Ay mice) and ovariectomized rats fed a high-fat diet (OVX/HFD rats) with those of pioglitazone.

MATERIALS AND METHODS

Materials and Animals  KY-903, INT-131, pioglitazone, and farglitazar were synthesized in our laboratories. They were dissolved in dimethyl sulfoxide (DMSO; Nacalai Tesque, Kyoto, Japan) and diluted in buffer or medium for in vitro study. COS-1 and 3T3-L1 cells were purchased from the American Type Culture Collection (Manassas, VA, U.S.A.). ST2 cells were purchased from the RIKEN BioResource Center (Tsukuba, Japan). KK-Ay mice (KK-Ay/TaJcl) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Animals were housed under conditions with controlled temperature, humidity, and light exposure (12-h light–dark cycle) and were allowed ad libitum access to commercial standard rodent chow (CE2; CLEA Japan) and tap water. Animals were handled in accordance with the “Guidelines for Animal Experimentation” approved by The Japanese Pharmaceutical Society and all procedures were approved by the Animal Ethical Committee of Kyoto Pharmaceutical Industries.

PPARγ and PPARα Agonist Activities  COS-1 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% (v/v) fetal bovine serum (FBS, JRH Bioscience, Carlsbad, CA, U.S.A.), 0.3% NaHCO3, and 1% penicillin–streptomycin under 5% CO2 at 37 °C. The full-length human PPARγ1 or PPARα plasmid (Open Biosystems, Inc., Huntsville, AL, U.S.A.) was co-transfected with the human retinoid X receptor α (RXRα) plasmid (GeneCopoeia, Inc., Rockville, MD, U.S.A.) and reporter plasmid pGL3-PPREx4- tk-luc into COS-1 cells using Nucleofector II (AAD-1001S; Lonza, Basel, Switzerland). Cells were then seeded at a density of 1.5 × 103 cells/well on 96-well plates and incubated for 24 h in the presence or absence of test compounds without farglitazar to assess agonist activity and with farglitazar (100 nM) to evaluate antagonist activity. Luciferase activities were assessed using Picagen LT7.5 (TOYO B-Net Co., Ltd., Tokyo, Japan). Responses to test compounds were expressed as relative values when the maximal response to farglitazar (100 nM) was taken as 100%. The potencies of test compounds as agonists were evaluated using half maximal IC50 values, which were calculated from concentration–inhibition curves, while their efficacies were estimated using maximal inhibition.

PP1B Inhibitory Activity  PTP1B inhibitory activities were measured using recombinant human PTP1B (Enzo Life Sciences, Inc., Farmingdale, NY, U.S.A.) and p-nitrophenyl phosphate (pNPP) as substrates in the absence and presence of the test compounds in 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.2) containing the enzyme, 1 mM dithiothreitol (DTT), and 1 mM ethylenediaminetetraacetic acid (EDTA). The reaction was initiated by the addition of pNPP and stopped by the addition of 1 M NaOH after a 30-min incubation at 37 °C. The absorbance of p-nitrophenol produced was measured at 405 nm.

Effects on KK-Ay Mice  Male KK-Ay mice (18 weeks old) were randomly allocated to five groups. KY-903 and pioglitazone were administered as a food admixture at 10 and 30 mg/kg for 4 weeks, during which time body weight was measured daily. After repeated administration, non-fasting blood was taken from the tail vein, and hematocrit (Ht) values and plasma glucose, triglyceride (TG), and adiponectin levels were assessed. Whole subcutaneous adipose tissue, visceral adipose tissues (epididymal, mesenteric, and perirenal adipose tissues), and hearts were isolated and weighed. BMD in distal femurs was measured by Dual energy X-ray absorptiometry (DEXA, DCS-600 EX-III, ALOKA Co., Ltd., Tokyo, Japan).

Effects on in Vitro Adipocyte Differentiation  Preadipocytes (3T3-L1) were cultured in DMEM supplemented with 4.0 g/L d-glucose, 10% (v/v) calf serum, 0.3% NaHCO3, and 1% penicillin–streptomycin. Cells were seeded at a density of 1 × 103 cells/well on 24-well plates and cultured in differentiation medium (DMEM supplemented with 4.0 g/L d-glucose, 5% FBS, 0.3% NaHCO3, and 1% penicillin–streptomycin). Two days later, cells were differentiated into adipocytes by culturing with differentiation medium supplemented with 1 μM dexamethasone (Wako Pure Chemical Ind., Ltd., Osaka, Japan) and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich, St. Louis, MO, U.S.A.) for 2 d, followed by a treatment with 1 ng/mL insulin and test compounds. Glycerol-3-phosphate dehydrogenase (GPDH) activities after a 2-d culture with drugs were assessed using a commercial GPDH assay kit (Primary Cell Co., Ltd., Sapporo, Japan). Total RNA was prepared after a 4-d culture with drugs using Trizol (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer’s instructions. GPDH and adiponectin mRNAs were assessed by a real-time PCR.

Effects on OVX/HFD Rats  Nine-week-old female F344/NSlc rats were randomly allocated to OVX and sham-operated (Sham) groups after being fed an HFD (Research Diets, New Brunswick, NJ, U.S.A.; 20% protein, 60% fat, and 20% carbohydrate) ad libitum for 2 weeks. Rats were anesthetized using ketamine (37.5 mg/kg, intraperitoneally (i.p.)) and xylazine (7.5 mg/kg, i.p.), and bilateral ovariectomy or a sham

Fig. 1. Chemical Structures of Pioglitazone, INT-131, and KY-903
operation was performed. Two days after surgery, OVX/HFD rats were randomized into 5 groups and orally administered vehicle (0.5% methylcellulose) or KY-903 or pioglitazone (30 and 100 mg/kg/d) for 8 weeks. Sham/HFD rats were orally administered vehicle for 8 weeks. Body weight was monitored during the experiment period, and BMD in the distal femurs was measured by micro-computed tomography (micro-CT, R_mCT; Rigaku, Tokyo, Japan) under anesthesia with isoflurane after the 8-week treatment. Body adipose tissue volumes were also measured by micro-CT under anesthesia. After repeated administration, non-fasting blood was taken from the jugular vein, Ht values and plasma glucose, TG, non-esterified fatty acid (NEFA), adiponectin, and leptin levels were assessed, and periumbilical white adipose tissues, interscapular brown adipose tissues, and hearts were isolated and weighed. Femurs were isolated and embedded in paraffin, and 5-μm-thick sections were cut on a rotary microtome. Sections were stained with hematoxylin and eosin, the adipocyte area in the medullary cavity was measured using microscopy (BZ-8100; Keyence Corporation, Osaka, Japan), and the adipocyte ratio was calculated.

Effects on in Vitro Osteoblast Differentiation  ST2 cells were seeded on 96-well plates at a density of 4×10³ cells/well. Test compounds were added 24 h later and cultured for 4 d. Cells were then washed with phosphate buffered saline (PBS) (pH 7.4) and lysed in 50 μL saline containing 1% NP-40 for the measurement of alkaline phosphatase (ALP) activity. The reaction was started by the addition of 50 μL of 10 mM pNPP in 50 mM ethanolamine and stopped by the addition of 50 μL of 1 M NaOH after a 30-min incubation at 37 °C. ALP activities were assessed by measuring absorbance at 405 nm.

Total RNA was harvested using Trizol and ALP, GPDH, and PPARγ2 mRNA levels were assessed by real-time PCR.

Effects on Gene Expression (Real-Time PCR)  To synthesize cDNAs, 0.5 μg total RNAs were reverse-transcribed using a commercial kit (TaKaRa Bio Inc., Shiga, Japan). The reaction was performed under the following conditions: synthesis at 37 °C for 15 min and the heat inactivation of reverse transcriptase at 85 °C for 1 min. Real-time PCR was performed in 10 μL of the total reaction volume containing 5 μL of cDNAs, individual primer pairs, a fluorescently-labeled probe, and the commercial reagent (Light Cycler 480 Probes Master; Roche Diagnostics, Basel, Switzerland) according to the manufacturer’s protocol. Reactions were initiated by incubating at 95 °C for 10 min and PCR (denaturation at 95°C for 10s and annealing and extension at 60°C for 30s) was performed for 45 cycles. The expression of each mRNA was standardized with 18S ribosomal RNA (rRNA). The sequences of primers were designed using the Universal ProbeLibrary (Roche Diagnostics): adiponectin, 5'-TGGTCAAAATGGGATACCG-3' and 5'-CCC TTAGGAGCACACCCT-3'; GPDH, 5'-AGGGTCTCTGGACAAGT-3' and 5'-CAGGGCAGCAGATGAACTC-3'; ALP, 5'-CGGATCTCCACAAAAACC-3' and 5'-TCAAGTGTCGTTGCAAT-3'; PPARγ2, 5'-GAAAGCCTGT GAAACACAA-3' and 5'-GAATTGAGCTCATTGGAACAA-3'; 18S rRNA, 5'-GTTGCATGGCCTTCAATAC-3' and 5'-TGCTT CGTATCCGATATACC-3'.

Statistical Analysis  All data are expressed as the mean ± standard error of the mean (S.E.M.). The significance of differences was assessed by a two-way ANOVA followed by Duncan’s multiple comparison tests for in vitro assays and by a one-way ANOVA followed by Dunnett’s multiple com-

![Fig. 2. Transactivation Activities of Human PPARγ and PPARα by KY-903, INT-131, and Pioglitazone (a, b), PPARγ Agonist and Antagonist Activities of KY-903 and INT-131 (c, d)](image)

The maximal activation level by farglitazar (100 nM) was taken as 100%.
parison tests for in vivo experiments.

RESULTS

PPARγ Agonist Activity  KY-903 exhibited PPARγ partial agonist activity (Fig. 2). The PPARγ agonist potency of KY-903 based on its EC50 value was 68-fold stronger than that of pioglitazone, a PPARγ full agonist, and 4-fold stronger than that of INT-131, a reported PPARγ modulator (EC50 = 7.6 nM for KY-903, EC50 = 514 nM for pioglitazone, EC50 = 30 nM for INT-131). The maximal activation levels (efficacy) of KY-903, pioglitazone, and INT-131 were 22.5, 104.8, and 22.7%, respectively, of that of 100 nM farglitazar, a PPARγ full agonist. KY-903 did not activate PPARα. KY-903 and INT-131 partially antagonized farglitazar (100 nM) with IC50 values of 160 and 501 nM, respectively. The maximal inhibitory levels by KY-903 and INT-131 were 74.8 and 78.5%, respectively.

PTP1B Inhibitory Activity  KY-903 did not exhibit PTP1B inhibitory activity (IC50 > 10 µM).

Anti-Diabetic Effects in Male KK-Ay Mice  The administration for 4 weeks of KY-903 and pioglitazone at 10 and 30 mg/kg resulted in similar dose-dependent decreases in plasma glucose levels and increases in adiponectin levels (Fig. 3). KY-903 at 30 mg/kg reduced plasma TG levels, but did not affect Ht values (data not shown).

KY-903 at 10 and 30 mg/kg and pioglitazone at 10 mg/kg significantly increased body weight gain by approximately 4-fold, while pioglitazone at 30 mg/kg increased it by approximately 10-fold (Fig. 4). Pioglitazone at 30 mg/kg significantly increased subcutaneous adipose, visceral adipose tissue, and heart weights, whereas KY-903 did not. In DEXA analyses, the BMD values of the distal femur did not significantly differ among the control, KY-903 (10 mg/kg), KY-903 (30 mg/kg), pioglitazone (10 mg/kg), and pioglitazone (30 mg/kg) groups (41.1 ± 0.70, 39.5 ± 0.45, 39.9 ± 0.77, 40.5 ± 1.36, and 40.7 ± 0.67 mg/cm2, respectively).

Effects on Adipocyte Differentiation  In 3T3-L1 cells pretreated with dexamethasone, IBMX, and insulin, KY-903 and INT-131 increased adiponectin mRNA levels from 10 nM

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**Fig. 3.** Effects of KY-903 and Pioglitazone on Plasma Glucose, TG, and Adiponectin Levels in Male KK-Ay Mice

Pio: Pioglitazone. Values are the mean ± standard error of the mean (S.E.M.) (n = 6). *p < 0.05, **p < 0.01 vs. Cont (Control), a one-way ANOVA followed by Dunnett’s multiple comparison test.

**Fig. 4.** Effects of KY-903 and Pioglitazone on Body Weight Gain and Heart, Subcutaneous Adipose, and Visceral Adipose Tissue Weights in Male KK-Ay Mice

Pio: Pioglitazone. Values are the mean ± S.E.M. (n = 6). *p < 0.05, **p < 0.01 vs. Cont (Control), a one-way ANOVA followed by Dunnett’s multiple comparison test.
Fig. 5. Effects of KY-903, INT-131, and Pioglitazone on Adiponectin and GPDH mRNA Expression (a, b), and Their Agonist and Antagonist Activities (c) in 3T3-L1 Cells

C: Control (dexamethasone, IBMX and insulin-treated cells). GPDH: glycerol-3-phosphate dehydrogenase, IBMX: 3-isobutyl-1-methylxanthine. Values are the mean ± S.E.M. a, b: n = 4, c: n = 3. *p < 0.05, **p < 0.01 vs. C (Control), a two-way ANOVA followed by Duncan’s multiple comparison test.

Fig. 6. Effects of KY-903 and Pioglitazone on Plasma TG, NEFA, Adiponectin, and Leptin Levels in OVX/HFD Rats
Pio: Pioglitazone. Values are the mean ± S.E.M. (n = 7–8). *p < 0.05, **p < 0.01 vs. Cont (Control), a one-way ANOVA followed by Dunnett’s multiple comparison test.
and pioglitazone from 100 nM (Fig. 5). On the other hand, KY-903 and INT-131 significantly increased GPDH mRNA levels only by approximately 2-fold from 10 nM, while pioglitazone markedly increased them by 3–5-fold from 100 nM. KY-903 and INT-131 did not enhance GPDH activity, and attenuated farglitazar-induced increases in GPDH activity. Pioglitazone markedly increased GPDH activity.

Effects on OVX/HFD Rats: OVX/HFD did not affect plasma glucose levels or Ht values (data not shown), but resulted in slightly higher plasma TG, NEFA, and adiponectin levels than Sham/HFD (Fig. 6). The repeated administration of KY-903 and pioglitazone did not affect Ht values (data not shown), but decreased plasma TG, NEFA, and leptin levels and increased plasma adiponectin levels (Fig. 6). KY-903 appeared to exert slightly weaker effects than pioglitazone on TG and adiponectin, but slightly stronger effects than pioglitazone on leptin.

OVX/HFD resulted in body weight gain, increased periumarine white adipose tissue and heart weights, and slightly increased interscapular brown adipose tissue weight (Fig. 7). In OVX/HFD rats, KY-903 dose-dependently reduced body weight gain, slightly decreased periumarine white adipose tissue weight, and slightly increased interscapular brown adipose tissue weight, but did not affect heart weight (Fig. 7). Body weight gain in the control and KY-903-treated groups correlated with white adipose tissue weight ($r^2 = 0.59$, $p < 0.01$). Pioglitazone did not affect periumarine white adipose tissue weight, but markedly increased interscapular brown adipose tissue, slightly increased body weight gain, and significantly and dose-dependently increased heart weight (Fig. 7). CT analyses revealed that KY-903 significantly decreased visceral adipose tissue volume and slightly decreased subcutaneous adipose tissue volume, whereas pioglitazone did not significantly affect subcutaneous or visceral adipose tissue volume (Fig. 8).

OVX/HFD markedly decreased BMD in the distal femur trabecular bone. In OVX/HFD rats, KY-903 at 100 mg/kg/d

![Fig. 7. Effects of KY-903 and Pioglitazone on Body Weight Gain and Periuterine White Adipose Tissue, Interscapular Brown Adipose Tissue, and Heart Weights in OVX/HFD Rats](image1)

| Pio: Pioglitazone. Values are the mean ± S.E.M. ($n = 7–8$). *$p < 0.05$, **$p < 0.01$ vs. Cont (Control), a one-way ANOVA followed by Dunnett’s multiple comparison test. |

![Fig. 8. Effects of KY-903 and Pioglitazone on Body Adipose Tissue Volume in Micro-CT Analyses of OVX/HFD Rats](image2)

| Pio: Pioglitazone. Values are the mean ± S.E.M. ($n = 7–8$). *$p < 0.05$, **$p < 0.01$ vs. Cont (Control), a one-way ANOVA followed by Dunnett’s multiple comparison test. |
and pioglitazone at 30 and 100 mg/kg/d significantly decreased BMD in the femur trabecular bone. Pioglitazone also markedly decreased BMD in the femur cortical bone (Fig. 9).

**Fig. 9. Effects of KY-903 and Pioglitazone on BMD in Femur Trabecular and Cortical Bones in OVX/HFD Rats**

*Pio: Pioglitazone. Values are the mean ± S.E.M. (n = 7–8). *p < 0.05, **p < 0.01 vs. Cont (Control), a one-way ANOVA followed by Dunnett’s multiple comparison test.

**Adipocytes in medullary cavity**

**Fig. 10. Effects of KY-903 and Pioglitazone on Adipocytes in the Medullary Cavity in OVX/HFD Rats**

and pioglitazone at 30 and 100 mg/kg/d significantly decreased BMD in the femur trabecular bone. Pioglitazone also markedly decreased BMD in the femur cortical bone (Fig. 9). Pioglitazone at 100 mg/kg/d significantly increased the adipocyte ratio in the medullary cavity of the distal femur (Fig. 10): Control 75.5 ± 1.56%, KY-903 (30 mg/kg) 78.1 ± 1.19%, KY-903 (100 mg/kg) 78.8 ± 0.75%, pioglitazone (30 mg/kg) 76.6 ± 1.62%, pioglitazone (100 mg/kg) 82.5 ± 1.21%, p < 0.05, vs. Control, Dunnett’s multiple comparison test.

**Effects on Osteoblast Differentiation** In ST2 cells, KY-903 and INT-131 decreased ALP mRNA and ALP activity less than pioglitazone. Pioglitazone, but not KY-903 or INT-131, markedly increased GPDH and PPARγ2 mRNA levels (Fig. 11).

**DISCUSSION**

In the present study, we examined the PPARγ agonist activity of KY-903, a novel tetrazole derivative, and investigated its efficacy and PPARγ-related adverse effects in diabetic mice and OVX/HFD rats.

The PPARγ agonist activity of KY-903 was compared with those of INT-131, a well-known sulfonamide-based PPARγ modulator, and pioglitazone, a PPARγ full agonist, in
transactivation assays using COS-1 cells. KY-903 and INT-131 showed the typical characteristics of PPAR\(\gamma\) partial agonists: agonist activity with a low maximal activation level, and antagonist activity against a PPAR\(\gamma\) full agonist. Neither KY-903 nor INT-131 exhibited PPAR\(\alpha\) agonist activity. KY-903 did not affect human PTP1B activity. These results demonstrated that KY-903 is a selective PPAR\(\gamma\) partial agonist.

In contrast to a PPAR\(\gamma\) full agonist with a carboxyl moiety, INT-131 has been shown to weakly interact with the Tyr473 (Helix 12), His323 (Helix 4/5), His449 (Helix 10/11) and Tyr327 (Helix 5) of PPAR\(\gamma\) by a hydrogen bond network and partially stabilizes Helix 12.\(^{13,21}\) On the other hand, KY-903 did not interact with Helix 12, and its tetrazolyl group was suggested to form a salt bridge with Arg288 (Helix 3) and a hydrogen bond with Ser342 (\(\beta\)-strand 3), as shown in its analog.\(^{18}\)

INT-131 exerts insulin-sensitizing effects in obese mice and rats without adverse effects, and reduces HbA1c with minor adverse effects in type 2 diabetic patients.\(^{14,19,20}\) Further studies are needed to establish whether KY-903, a tetrazole-based PPAR\(\gamma\) partial agonist with a different structure and binding mode from INT-131, exerts similar potent and safe anti-diabetic efficacy to INT-131.

The anti-diabetic and adverse effects of KY-903 were compared with pioglitazone, which is clinically and experimentally safer than rosiglitazone for hemodilution and cardiac hypertrophy. In KK-Ay mice, the 4-week administration of KY-903 and pioglitazone similarly reduced plasma glucose and TG levels and increased adiponectin levels. The plasma concentrations of KY-903 administered as a food admixture at 10 and 30 mg/kg reached approximately 1 and 2 \(\mu\)M, respectively, in male KK-Ay mice (unpublished data) and were higher than its EC\(50\) value for PPAR\(\gamma\) agonist activity. KY-903 and pioglitazone are considered to exert similar anti-diabetic effects through the attenuation of insulin resistance via adiponectin enhanced by the activation of PPAR\(\gamma\).

Regarding PPAR\(\gamma\)-related adverse effects, body weight gain was similarly elevated by KY-903 at 10 and 30 mg/kg and pioglitazone at 10 mg/kg, possibly by increases in the weights of organs other than adipose tissue, such as muscle, because they did not affect subcutaneous adipose tissue weight. Pioglitazone at 30 mg/kg markedly increased body weight gain and subcutaneous and visceral adipose tissue weights, suggesting adipogenesis via PPAR\(\gamma\) full activation. A high dose of pioglitazone, but not KY-903, significantly increased heart weight, indicating cardiac hypertrophy by an elevated cardiac preload through fluid retention. PPAR\(\gamma\) agonist-induced fluid retention is attributed to the activation of epithelial Na\(^+\) channels in the collecting ducts and/or to Na\(^+\), K\(^+\) ATPase activation via proximal tubular PPAR\(\gamma\).\(^{22}\) These renal effects may require the full activation of PPAR\(\gamma\). However, pioglitazone did not affect Ht values: hemodilution may be compensatory normalized by increases in red blood cells. In KK-mice, PPAR\(\gamma\) full agonists,
such as pioglitazone, occasionally increased plasma volume assessed by Evans blue methods without reductions in Ht values (unpublished data). Bone loss is a PPARγ-related adverse effect; however, neither KY-903 nor pioglitazone affected BMD in DEXA analyses. Higher doses or longer administration periods of pioglitazone may be needed to induce bone loss by PPARγ full activation in KK-Ay mice.

In KK-Ay mice, KY-903 has been suggested to increase adiponectin production without promoting adipogenesis. This was examined in 3T3-L1 cells, which are rodent pre-adipocytes; KY-903 induced the expression of adiponectin mRNA, but not the adipocyte marker GPDH mRNA, while pioglitazone induced both mRNAs. Furthermore, pioglitazone markedly enhanced GPDH activity; however, KY-903 did not affect GPDH activity, and only inhibited GPDH activity induced by the PPARγ full agonist farglitazar. These results were consistent with those obtained in transactivation assays. Therefore, KY-903 clearly enhanced the induction of adiponectin without causing adipocyte differentiation; the transcription of the adiponectin gene appeared to be more sensitive to PPARγ partial activation than adipocyte-related genes.

In subsequent experiments, the hypolipidemic and adverse effects of KY-903 were compared with those of pioglitazone in OVX/HFD rats. OVX rats show BMD reductions, weight gain, and hyperlipidemia, but not hyperglycemia,23–25 and are used as an experimental model of postmenopausal syndrome, such as obesity and osteoporosis. However, OVX rats show mild obesity with increases in adipose tissue weight and, thus, they were fed HFD. OVX/HFD rats showed slightly higher plasma TG and adiponectin levels, significantly higher body weight gain, plasma leptin levels, and adipose tissue weight, and markedly lower BMD than Sham/HFD rats. In OVX/HFD rats, KY-903 and pioglitazone similarly reduced plasma TG, NEFA, and leptin levels and increased plasma adiponectin levels, suggesting insulin sensitization via adiponectin and improvements in leptin resistance. Insulin reduces plasma TG and NEFA levels by inhibiting very-low-density lipoprotein (VLDL) secretion from hepatocytes and uptake into adipocytes via lipoprotein lipase activation.26 PPARγ agonists have been shown to reduce TG and NEFA levels by insulin sensitization in diabetic rats.27

Regarding PPARγ-related adverse effects, pioglitazone did not signiﬁcantly increase body weight gain or white adipose tissue weight, while it markedly increased brown adipose tissue and heart weight without affecting Ht values. Pioglitazone may not have further increased adipose tissue weight under obese conditions because adipose tissue weight was already elevated. As described for KK-Ay mice, volume expansion by pioglitazone may have led to cardiac hypertrophy, but hemodilution may not have occurred as an adaptive response.

In OVX/HFD rats, KY-903 reduced body weight gain and adipose tissue weight, between which a correlation was observed. KY-903 may attenuate adipogenesis by antagonizing activity against endogenous or diet-derived PPARγ ligands, resulting in decreases in body weight. The PPARγ antagonist GW9662 has been reported to exert anti-obesity effects in mice fed HFD.28

In OVX/HFD rats, KY-903 did not affect BMD in the femur cortical bone and slightly reduced that in the trabecular bone, while pioglitazone markedly reduced BMD in both bones. Insulin promotes osteoblastogenesis and type 2 diabetic patients are at a high risk of bone fractures.29 PPARγ agonists increase the risk of bone fractures in diabetic patients: rosiglitazone and pioglitazone were previously reported to increase the risk of bone fractures by decreasing BMD in aged women,30 which may be due to a shift from osteoblast differentiation to adipocyte differentiation in bone marrow stem cells.31 Rosiglitazone was shown to increase fat volume in the bone marrow cavity and decrease BMD.32 Pioglitazone significantly increased fat volume in the bone marrow cavity, which may be, at least in part, related to reductions in BMD.

In mesenchymal stem cell-like ST2 cells, KY-903 partially and pioglitazone almost completely inhibited osteoblast differentiation. Pioglitazone, but not KY-903, markedly increased differentiation to adipocytes, as observed by increases in GPDH and PPARγ2 mRNA levels. These results suggest that pioglitazone reduced BMD by increasing adipocyte differentiation and decreasing osteoblast differentiation. However, KY-903 partially inhibited osteoblast differentiation without affecting adipocyte differentiation. PPARγ-related reductions in osteoblast differentiation may not be solely explained by a shift to adipocyte differentiation.

In conclusion, KY-903, a newly synthesized tetrazole derivative, is a PPARγ partial agonist and has similar efficacy to, but weaker adverse effects than pioglitazone in KK-Ay mice and OVX/HFD rats. Among the adverse effects exerted by pioglitazone, increases in body and adipose tissue weights and bone loss are attributed to adipogenesis induced by PPARγ activation. In contrast to PPARγ full agonists, KY-903 increases adiponectin levels without adipocyte differentiation, leading to potent efficacy without adverse effects. Furthermore, KY-903 exerts anti-obesity effects against OVX/HFD-induced obesity, possibly by PPARγ antagonism against endogenous or diet-derived PPARγ ligands. More detailed investigations on the effects and mechanisms of action of KY-903 are needed.

Conflict of Interest The authors are all employees of Kyoto Pharmaceutical Industries, Ltd.

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