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RESEARCH ARTICLE

The effect of substituted thiophene and benzothiophene derivates on PPARγ expression and glucose metabolism

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Abstract

Eighteen substituted thiophene and benzothiophene derivatives were studied for their effects on peroxisome proliferator-activated receptor γ (PPARγ) in HepG2 cells. Three derivatives (compounds **5**, 120.97%; **15**, 102.14%; and **17**, 113.82%) were found to transactivate PPARγ *in vitro*. By comparison, the positive control rosiglitazone (**Ros**) transactivated PPARγ by 311.53%. The three compounds were studied for their effects on glucose metabolism *in vivo* in KK/Ay diabetic mice. *In vivo*, the 2-(β-carbonyl/sulfonyl) butyryl-thiophene compounds **5** and **15** significantly decreased blood glucose levels (compounds **5**, to < 15.6 mmol/L; **15**, to < 10 mmol/L), improved glucose tolerance, improved impaired pancreatic islet β-cells, and lowered serum insulin levels.

Keywords: *Peroxisome proliferator-activated receptor γ; antidiabetic drugs; 2-(β-carbonyl/sulfonyl) butyryl-thiophene derivatives; KK/Ay diabetic mice*

Introduction

The global prevalence of type 2 diabetes mellitus is dramatically increasing, and the largest population of newly diagnosed patients is expected to be among adults[1]. It is well recognized that both β-cell dysfunction and insulin resistance are important contributors to the development of type 2 diabetes. One of the functions of β-cells is to produce insulin. The β-cell dysfunction, caused by reductions in β-cell mass, leads to a decline in insulin secretion. One of the actions of insulin is to stimulate cells, particularly muscle and fat cells, to take up and make use of glucose and thus decrease the circulating blood glucose levels (BGL). With insulin resistance, β-cells need to express and secrete more insulin to increase blood insulin levels (BIL)[2]. With progressive β-cell dysfunction and insulin resistance, insufficient insulin is produced by β-cells, and BGL and BIL begin to rise, with abnormal glucose tolerance.

The peroxisome proliferator-activated receptors (PPARs) are members of a nuclear transcription factor superfamily. Three PPAR subtypes including PPARα, δ, and γ have been discovered[3]. PPARγ is predominantly expressed, and plays a fundamental role in adipogenesis and glucose homeostasis[4-6]. Once activated, PPARγ modulates the expression of genes associated with carbohydrate metabolism, which affects glucose uptake and insulin sensitivity.

Thiazolidinediones (TZDs) are high-affinity ligands for PPARγ and are widely used as oral hypoglycemic drugs[7–10]. Rosiglitazone (**Ros**), a TZD in clinical use for the treatment of type 2 diabetes, can protect against declining β-cell function and insulin resistance, decrease BGL and BIL, and increase insulin sensitivity[11]. However, detrimental side effects of TZDs have been frequently reported, including edema and heart failure[12,13]. Therefore, there is a need to generate some novel PPARγ agonists that retain the clinical effects but avoid the side effects associated with the TZDs already in clinical use. Results of our previous study (not provided) indicated that 1-(furan-2-yl) butane-1,3-dione could transactivate PPARγ. To identify the novel agents with antidiabetic effects mediated by transactivating PPARγ, we screened a series of compounds with similar structures to 1-(furan-2-yl)butane-1,3-dione for their effects on transactivating PPARγ in HepG2 cells

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and their effects on improving glucose metabolism in KK/Ay diabetic mice.

Material and methods

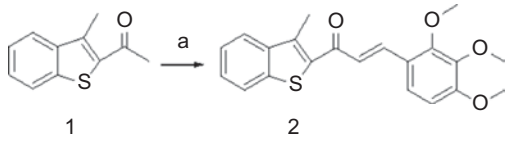
Chemistry

The structures and purities of tested compounds in this article are listed in Table 1. Compounds **2** [(*E*)-1-(3-methylbenzo[b]thiophen-2-yl)-3-(2,3,4-trimethoxyphenyl)prop-2-en-1-one] and **5** [1-(benzo[b]thiophen-2-yl)-2-(methylsulfinyl)ethanone] were synthesized in our laboratory following the routes in Schemes 1 and 2, respectively. To our knowledge, this article is the first report of compound **2**.

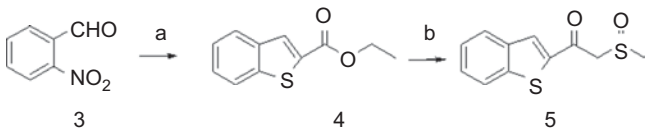
The melting points were determined with an X_6 microscope melting point apparatus and were uncorrected. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury-300 spectrometer. Electron ionization-mass spectrometry (EI-MS) was performed on an AutoSpec Ultima-Tof spectrometer. High resolution (HR)-EI spectra were recorded on an AccuTOF CS mass spectrometer.

[(E)-1-(3-Methylbenzo[b]thiophen-2-yl)-3-(2,3,4-trimethoxyphenyl) prop-2-en-1-one] (compound 2)

A solution of 2-acetyl-3-methyl-benzothiophene (compound **1**, 200 mg, 1.1 mmol) in ethanol was cooled to 0°C, then 0.5 mL of an aqueous solution of sodium hydroxide (53 mg, 1.32 mmol) was added and the mixture was stirred for 5 min, and then 2,3,4-trimethoxyl-benzaldehyde (216 mg, 1.1 mmol) was added. The mixture was stirred for 4 h at 25°C. The reaction mixture was concentrated by evaporation and the residue was dissolved in dichloromethane, and washed with water. The collected organic extract was dried over anhydrous Na_2SO_4 and concentrated. The residue was recrystallized by cyclohexane to obtain a yellow solid (compound **2**, 271 mg, yield 70%). The purity of the solid was 98.5%, as analyzed by the area-normalization high performance liquid chromatography (HPLC) method (column: Dikma ODS 5 μm, 250 × 4. 6 mm; packing material: Platisil ODS 5 μm; mobile phase: methanol:water = 85:15, v/v; flow rate: 1.0 mL/min; ultraviolet (UV) detection wavelength: 260 nm), and the melting point was 104–108°C. ^{1}H NMR ($CDCl_3$) δ: 2.83 (s, 3H), 3.90 (s, 3H), 3.92 (s, 3H), 3.98 (s, 3H), 6.73 (d, *J* = 8.4 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.46–7.5 (m, 3H), 7.88 (t, *J* = 8.4 Hz, 2H), 8.01 (d, *J* = 15.6 Hz, 1H). MS (EI): 368. HR-EI: found 368.1056, $C_{21}H_{20}O_4S$.



Scheme 1. Synthesis of compound **2**. a: 1 equiv. of 2,3,4-trimethoxyl-benzaldehyde and 1.2 equiv. of NaOH.



Scheme 2. Synthesis of compound **5**. a: 1 equiv. of ethyl 2-mercaptoacetate, K_2CO_3, DMF. b: DMSO, NaH.

Ethyl benzo[b]thiophene-2-carboxylate (compound 4)

A mixture of *o*-nitrobenzaldehyde (compound **3**, 4.5 g, 30 mmol) and anhydrous potassium carbonate (5.0 g, 36.2 mmol) in dimethylformamide (60 mL) was cooled to 0°C, and ethyl 2-mercaptoacetate (3.29 mL, 30 mmol) was added. The resulting mixture was stirred for 30 min at 0°C, and then stirred for 12 h at 60°C. The mixture was then poured into ice-water to form a precipitate, which was filtered, and the collected solid was dissolved in chloroform. The resulting solution was dried by anhydrous Na_2SO_4 overnight, filtered, and concentrated to give a crude product which was purified by a silica gel column (petroleum ether:ethyl acetate = 60:7, v:v) to give ethyl benzo[b]thiophene-2-carboxylate (compound **4**, 5.80 g, yield 94%), melting point: 36–38°C (the melting point reported by Kolasa and Brooks[14] was 36–38°C). ^{1}H NMR ($CDCl_3$) δ: 1.42 (t, 3H, *J* = 7 Hz), 4.42 (q, 2H, *J* = 7 Hz), 7.39–7.47(m, 2H), 7.86–7.89 (m, 2H), 8.06 (s, 1H). MS (EI): 206. HR-EI: found 238.0102, $C_{11}H_{10}O_2S_2$. Compound **4** was previously reported by Kolasa and Brooks[14] and is commercially available (CAS 17890-55-0). Our NMR spectral data for compound **4** are consistent with those reported by Kolasa and Brooks.

[1-(Benzo[b]thiophen-2-yl)-2-(methylsulfinyl)ethanone] (compound 5)

Compound **4** (200 mg, 0.97 mmol) in tetrahydrofuran (THF, 8 mL) was added into a mixture of 60% sodium hydride (280 mg, 7 mmol) and dimethyl sulfoxide (DMSO; 8 mL), and was stirred at 75°C until no gas was generated. The mixture was then cooled at 0°C, THF was added, and the reaction mixture was stirred at room temperature for 30 min. The mixture was then poured into ice-water, and the pH was adjusted to 3–4 by 2 N hydrochloric acid. Then the mixture was extracted by chloroform; the combined chloroform layers were washed with water, dried over anhydrous Na_2SO_4, and filtered and concentrated to yield a yellow solid (compound **5**, 202 mg, yield 87%) The purity of compound **5** was 98.7%, as analyzed by the area-normalization HPLC method (column: Dikma ODS 5 μm, 250 × 4.6 mm; packing material: Platisil ODS 5 μm; mobile phase: methanol:water = 85:15, v/v; flow rate: 1.0 mL/min; UV detection wavelength: 260 nm), and the melting point was: 120–123°C (the melting point reported by Kolasa and Brooks[14] was 120–123°C). ^{1}H NMR ($CDCl_3$) δ: 2.79 (s, 3H), 4.37 (d, 2H, *J* = 13.2 Hz), 7.43 (t, 1H, *J* = 7 Hz), 7.51 (t, 1H, *J* = 7 Hz), 7.88 (d, 1H, *J* = 7 Hz), 7.94 (d, 1H, *J* = 7 Hz), 8.09 (s, 1H). MS (EI): 238. Compound **5** was previously reported by Kolasa and Brooks[14], and our spectral data for compound **5** are consistent with those reported by Kolasa and Brooks.

Table 1. Transactivation activities of compounds on PPARγ in HepG2 cells.

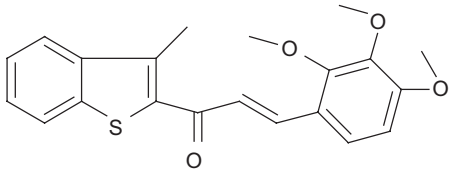
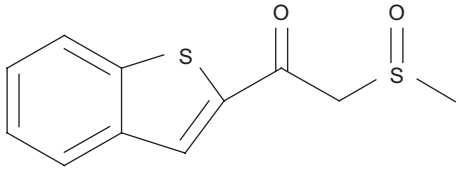
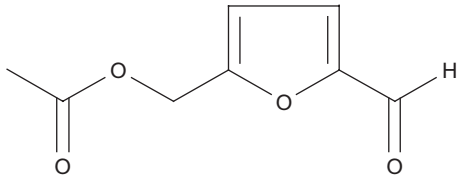
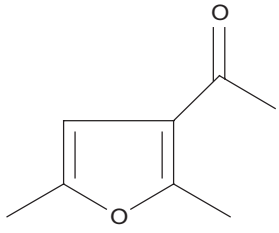
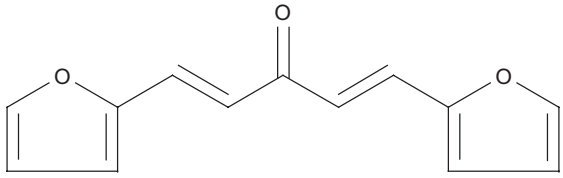
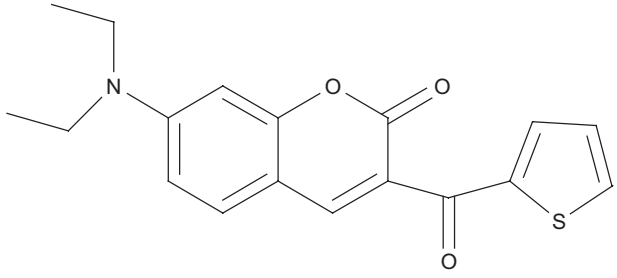
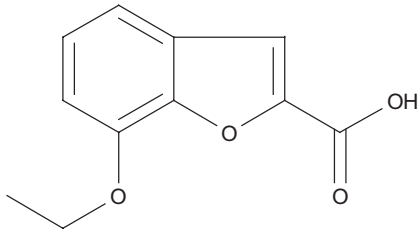
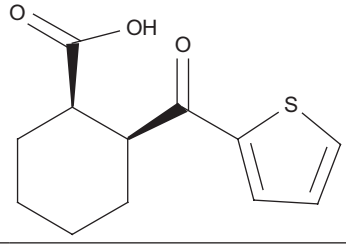
Compound	Structure	Transactivation activity (%)	Purity (%)
2		70.08	98.5
5		120.97	98.7
6		70.75	97
7		59.74	98
8		79.44	98
9		40.59	99
10		80.55	98
11		70.12	99

Table 1. Continued on next page.

Table 1. Continued.

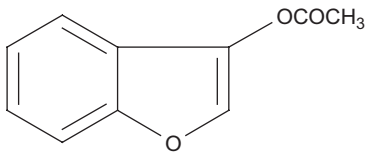
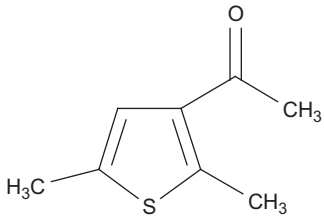
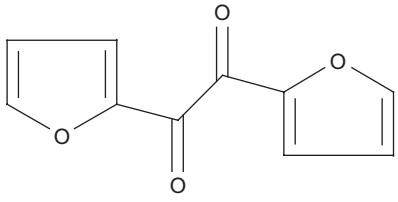
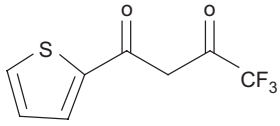
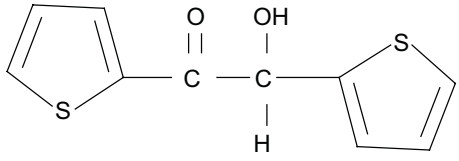
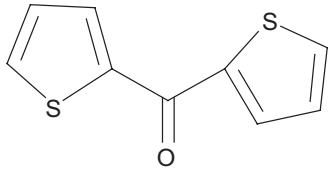
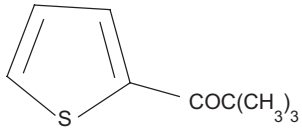
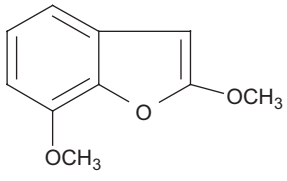
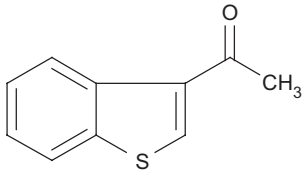
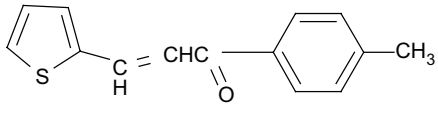
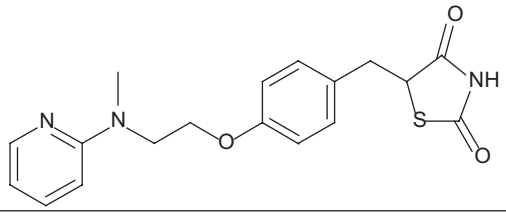
Compound	Structure	Transactivation activity (%)	Purity (%)
12		73.81	98
13		87.20	99
14		76.97	98
15		102.14	99
16		86.93	97
17		113.82	98
18		80.98	98
19		75.43	97
20		52.50	98
21		18.23	98

Table 1. Continued on next page.

Table 1. Continued.

Compound	Structure	Transactivation activity (%)	Purity (%)
Ros		311.53	99.22

Compounds **1, 6, 7, 8, 9, 10**, and **11** were purchased from J&K Chemica Ltd.; compounds **12, 13, 14, 15, 16, 17, 18, 19, 20**, and **21** were purchased from Alfa Aesar China (Tianjin); compound **3** and **Ros** were purchased from Nanjin Tianzun Chemical Co. Ltd.

Pharmacological screening

Plasmids

The cDNA encoding the ligand binding domain of human PPARγ (amino acids 174–475) was amplified from total mRNA of human fat cells (Clontech, #7128-1) by reverse transcriptase-polymerase chain reaction (RT-PCR) and cloned into the 3' end of the GAL4 DNA binding domain in pBIND (Promega, Madison, WI, USA) at an open reading frame to generate the GAL4-hPPARγ (LBD) chimeric receptor expression vector. The reporter vector pGL3-promoter-GAL4 (firefly luciferase) was constructed by inserting five copies of the GAL4 response element into the upstream of the SV40 promoter and the luciferase reporter gene of the pGL3-promoter vector (Promega).

Cell-based reporter assays

The HepG2 cell line (China Center for Type Culture Collection) was cultured in RPMI-1640 medium (from Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (from Hyclone) at 37°C in a humidified 5% CO_2 incubator. Cells were seeded into 96-well plates at a concentration of 3×10^4 cells/well. After 24 h, cells at 90% confluence were cotransfected with the expression vector pBIND-PPARγ-LBD and the reporter vector pGL3-promoter-GAL4 using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's instructions. Negative control (0.1% DMSO), positive control (**Ros**, 28 μM), or the screening compound (28 μM) was added 6 h after transfection. After incubation for 24 h, the cells were lysed, and luciferase activity was detected as relative luminescence units (RLUs) using the Luciferase Assay System (Promega). The transactivation activities of the substituted thiophene and benzothiophene derivatives (STBD) on PPARγ were calculated using the following formula:

$$\text{Transactivation activity} = [A/B] \times 100\%$$

where A = RLUs for the STBD of interest and B = RLUs for DMSO.

Animals and treatment

Female KK/Ay mice (9–10 weeks old) were obtained from the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC). All mice were housed individually in plastic cages at 25°C with illumination for 12 h. The mice were given a high-fat diet with water freely available. Animals were treated with the compounds by gavage once daily for 23 days (from day 0 to day 22) at a dose of 75 mg/kg body weight in a vehicle of 0.5% methylcellulose (MC), similar to the method described elsewhere[15]. **Ros** (4 mg/kg body weight/day) was administered as a positive control. The control group received the vehicle alone by gavage. Each group consisted of five animals. All experimental procedures were approved and carried out in compliance with the guidelines of the Institute of Laboratory Animal Sciences, CAMS and PUMC.

Metabolic measurements

Fasting blood glucose levels were measured on days–2 (2 days before treatment), 0, 3, 10, 14, and 22. Blood samples were collected from the tail vein 6 h after treatment. Blood glucose levels were measured by an Accu-Chek® Active blood glucose meter (Roche Diagnostics Corp.). Fasting serum insulin levels were measured on day 22 after treatment. Orbital vein blood samples were collected by removal of the eyes of the mice. The serum insulin levels were measured by radioimmunoassay.

Glucose tolerance test

On day 18, after fasting for 16 h, the glucose tolerance test was executed[16]. Glucose (2 g/kg) was administered orally, and blood was collected from the tail vein at 0, 0.5, 1, and 2 h to determine blood glucose levels using the Accu-Chek® Active glucose meter.

Histological analysis

Samples of pancreatic islet were fixed in Bouin's fixative solution (75 mL of saturated trinitrophenol solution, 25 mL of 10% neutral-buffered formalin, and 5 mL of glacial acetic acid), embedded in paraffin, cut and stained by Gomori's aldehyde-fuchsin method, and then analyzed microscopically and morphologically.

Statistical analysis

Quantitative data are expressed as mean ± standard deviation. Statistical significance of the data was evaluated by SPSS. Values $p < 0.05$ were considered significant.

Results and discussion

In vitro results

Transactivation activities of STBD on PPARγ in vitro

Eighteen compounds were evaluated *in vitro* (Table 1); 15 (including compounds **7, 8, 20, 19**, etc.) did not transactivate PPARγ, while three (compounds **5, 15**, and **17**) transactivated PPARγ with transactivation activities > 100%. Of note, compound **5** transactivated PPARγ by 120.97%. The transactivation activity of the positive control, **Ros**, was 311.53%.

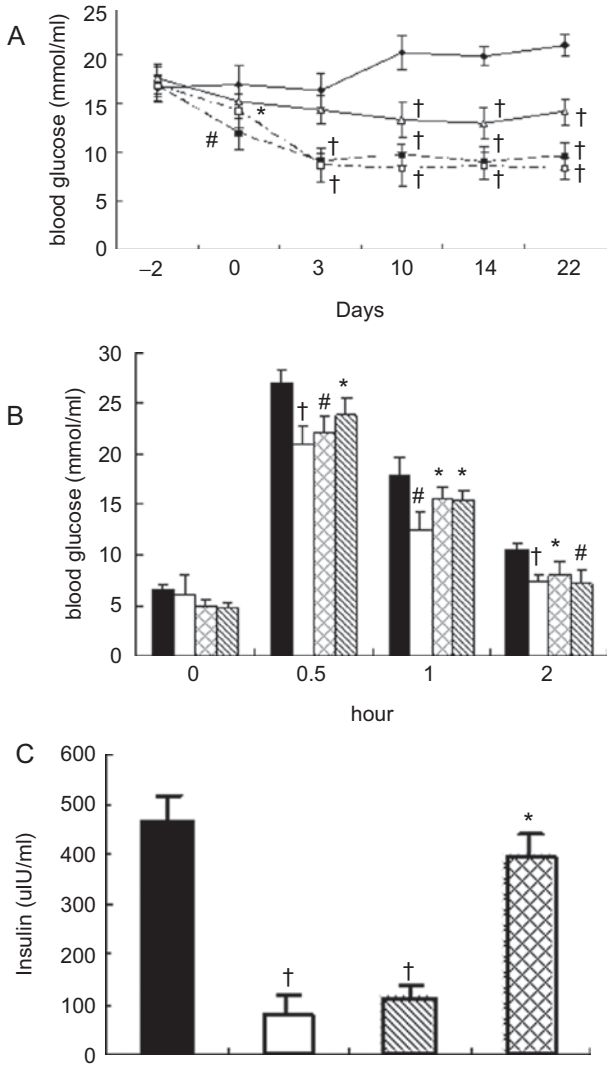


Figure 1. Effects of compounds (methylcellulose (MC), **Ros, 15, 5**) on fasting glucose profile (A), glucose tolerance (B), and insulin profile (C) of KK/Ay diabetic mice. Parameters for (A) were measured 2 days prior to and for 22 days during treatment with MC (0.5%, 20 mL/kg body weight/day; black diamonds), **Ros** (4 mg/kg body weight/day; black squares), **15** (75 mg/kg body weight/day; white squares), **5** (75 mg/kg body weight/day; white triangles). (B) Response to a glucose load in KK/Ay mice treated with MC (black bar), **Ros** (white bar), compound **5** (double hatched bar), or compound **15** (hatched bar). (C) Insulin level on day 22 (last day of treatment) in KK/Ay mice treated with MC (black bar), **Ros** (white bar), compound **15** (hatched bar), or compound **5** (double hatched bar) Values are means ± SD for groups of five mice. *$p < 0.05$, #$p < 0.01$, †$p < 0.001$ vs. MC-treated KK/Ay mice.

In vivo results

Effects of compounds 5 and 15 on BGL and serum insulin levels

There were no differences in BGL in the mice before treatment. After treatment with **Ros**, compound **5**, or compound **15**, the BGL of the diabetic mice decreased markedly (Figure 1A), whereas the groups treated with the other compounds showed no significant change (data not shown). On day 0, after dosing, compared with MC-treated mice (17.02 ± 1.85 mmol/L), the mice treated with **Ros** (11.98 ± 1.55 mmol/L, $p < 0.01$) and compound **15** (14.26 ± 1.76 mmol/L, $p < 0.05$) showed significantly reduced BGL. On day 3, compound **15** had decreased the BGL to less than 10.6 mmol/L, and maintained this level until the end of the study. Compound **15** had glucose-lowering effects that were comparable with those for **Ros** (with no statistical difference). After 3 days of treatment, the BGL was lower in **Ros**-treated mice (9.14 ± 0.76 mmol/L, $p < 0.001$) compared with MC-treated mice (16.38 ± 1.79 mmol/L), and the BGL was maintained until the end of the study. After 10 days of treatment, compound **5** had markedly reduced BGL (13.36 ± 1.75 mmol/L, $p < 0.001$) compared with MC-treated mice (20.24 ± 1.74 mmol/L), and maintained comparable BGL until the end of the study.

Oral glucose tolerance tests showed that compounds **5** and **15** improved glucose tolerance, with lower glucose levels at all time-points after glucose loading compared with MC-treated mice (Figure 1B). **Ros** (79.276 ± 35.812 μIU, $p < 0.001$) and compounds **15** (111.99 ± 24.56 μIU, $p < 0.001$) and **5** (395.32 ± 41.36 μIU, $p < 0.05$) decreased the serum insulin levels (SIL) compared with MC-treated mice (465.55 ± 49.72 μIU) (Figure 1C).

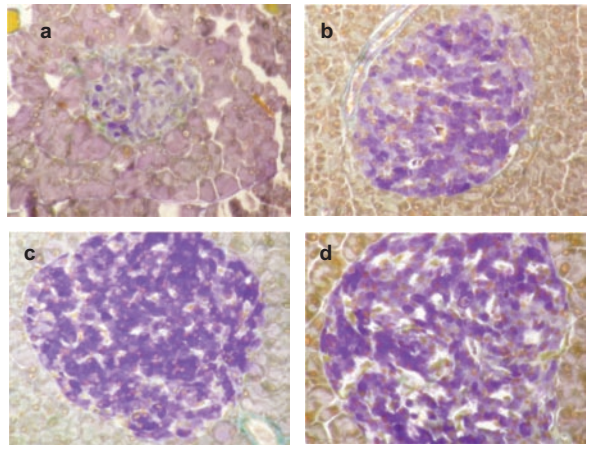


Figure 2. Effects of MC (a), compound **15** (b), compound **5** (c), or **Ros** (d) on pancreatic islet β-cells. Gomori's aldehyde-fuchsin stain. (See colour version of this figure online at www.informahealthcare.com/enz).

Effects of compounds 5 and 15 on pancreatic β-cells

Impairment of pancreatic islet β-cells, as a result of diabetes, was markedly improved by the administration of **Ros**, and compounds **5** and **15** (Figure 2).

In vitro, of the 18 STBD tested in this study, three (compounds **5, 15**, and **17**) compounds showed transactivating effects on PPARγ, whereas the other 15 compounds did not transactivate PPARγ. A comparison of the structures and transactivation activities indicated that the 2-carbonyl group of the STBD and the polarity and bulk of substituents near the carbonyl moiety might play important roles in transactivating PPARγ. The compounds that could transactivate PPARγ were all substituted with carbonyl at the C_2 position, whereas compounds without the 2-carbonyl group (as seen in compounds **7, 8, 12, 13, 19, 20**, and **21**) did not transactivate PPARγ. When substituents with greater polarity (as seen in compounds **10** and **16**), or bulky substituents (as seen with compound **18**), were in close proximity to the carbonyl, the compounds were unable to transactivate PPARγ. Similarly, when substituents with greater polarity and bulky substituents were located near the 2-carbonyl group, the compound was unable to transactivate PPARγ. For example, the transactivation activity of compound **11** was 70.12% in the *in vitro* assay. This compound has cyclohexyl, a bulky group, and a carboxylic group, a highly polarized moiety, near the 2-carboxyl. Furthermore, if a double-bond appears near the 2-carbonyl moiety in a compound, the number of carbon atoms between the double-bond and 2-carbonyl moiety, i.e. the distance between the double-bond and the 2-carbonyl moiety, seems to affect the transactivation activity *in vitro*. When the number is 0, i.e. the double-bond is directly connected to the 2-carbonyl (i.e. compounds **2, 9**, and **14**), the compound is unable to transactivate PPARγ *in vitro*. In contrast, if the number is 1, i.e. there is one carbon atom between the double-bond and the 2-carbonyl moiety (i.e. compounds **5** and **15**), the compound transactivates PPARγ *in vitro*.

Accordingly, the *in vitro* data indicated that the 2-carbonyl heterocycle or benzo-heterocycle are essential frameworks for the transactivation of PPARγ, and a substituent near the 2-carbonyl with appropriate polarity and bulk may help to activate PPARγ, and if there is a double-bond near the 2-carbonyl, the number of carbon atoms between the double-bond and 2-carbonyl seems to play some role in transactivating PPARγ.

HepG2 is a perpetual cell line that was derived from the liver tissue of a 15-year-old Caucasian male with well-differentiated hepatocellular carcinoma. The screening assay at cellular level involved a *trans*-reporter assay based on luciferase, reflecting the effect of protein–protein interaction. However, in nature, it is just an *in vitro* simulation, and not an endogenous procedure in the cell. In other words, it is a useful method to explore the preliminary biological phenomenon, but further validation is necessary to determine the real interactions and mechanisms involved. Therefore, we used a diabetic mouse model to detect the effects of the STBD on improving the glucose metabolism *in vivo*.

In vivo, of the three tested compounds (**5, 15**, and **17**), compounds **5** and **15** significantly improved the diabetic syndrome in tested animals. On the first day of treatment, compound **15** and **Ros** dramatically decreased glucose levels, and from day 3 to day 22, both lowered the glucose level to below 11 mmol/L. On day 10, compound **5** reduced the BGL markedly, and maintained the BGL below 16 mmol/L to the end of the study.

β-Cells synthesize and release insulin, a hormone that controls the BGL. When diabetes occurs, β-cell mass[17,18] and function are not sufficient to cope with insulin resistance[19,20]. The most striking functional defect is a loss of acute glucose-induced insulin secretion, which leads to abnormality in glucose tolerance tests. In the oral glucose tolerance test, compounds **5**, **15**, and **Ros** dramatically improved glucose tolerance compared with that in the MC-treated mice. As sections of pancreatic islet showed, **Ros**, and compounds **5** and **15**, improved the mass of β-cells, indicating that compounds **5** and **15** can improve impaired β-cell mass and function. According to the pancreatic islet sections, the effect of **Ros** is more pronounced than that of compounds **5** and **15**; the effects of compounds **5** and **15** were comparable. **Ros** may improve the β-cell mass and function by reducing the toxicity of blood glucose and blood fat[21], and by direct action on β-cells[22]. Based on the fact that compounds **5** and **15**, as well as **Ros**, transactivated PPARγ, compounds **5** and **15** may improve the β-cell mass and function in a manner similar to that of **Ros**.

Insulin is a hormone that stimulates most of the body cells (including liver, muscle, and fat tissue cells) to take up glucose from the blood, and store it as glycogen in the liver and muscle. When control of the circulating insulin level fails, diabetes mellitus arises[23,24]. Type 2 diabetes is often associated with hyperinsulinemia. After 23 days of treatment, compounds **5** and **15** notably decreased the serum insulin levels in mice compared with the MC-treated mice. There are some differences between the structrues of compounds **5** and **15**, and compound **15** was more efficient than compound **5** in decreasing the serum insulin levels. The structure of compound **5** includes a benzothiophene ring, and is more rigid than compound **15**, which only has a thiophene ring. Furthermore, the side chain of compound **5** is less polar than that of compound **15**. Because of the difference in structure, compound **15** may be metabolized *in vivo* in a different way compared with compound **5**, which may be responsible for the greater improvements in serum levels with compound **15** compared with compound **5**. Accordingly, compound **15** may affect the serum insulin levels in a more advantageous manner, which may lead to better affinity to the PPARγ receptor or a greater quantity to bind the PPARγ receptor compared with compound **5**.

A comparison of the *in vitro* and *in vivo* results showed that compound **15** with transactivation percentage

102.14% had higher activity *in vivo* than compound **5** with transactivation percentage 120.97%, which indicates that high *in vitro* activity does not necessarily predict high *in vivo* activity. Indeed, compound **17**, for which the *in vitro* transactivation activity was 113.82%, showed no antidiabetic effects *in vivo*, which indicates that active compounds with *in vitro* activity are not necessarily active *in vivo*. When compared with compounds **5** and **15**, the structure of compound **17** differs in that there is a thiophenic ring connected to the 2-carbonyl moiety. Accordingly, it is possible that the thiopenic ring causes compound **17** to be metabolized in a different way, and the compound cannot be transformed to an active form to be effective *in vivo*. Therefore, it can be concluded that the lack of *in vivo* activity observed for compound **17** may be attributed to potential metabolic instability, poor oral bioavailability, or other drug metabolism pharmacokinetic characteristics of the compound. Further studies of compounds **5** and **15** are warranted to examine the effect of other doses of these compounds on glucose lowering.

Conclusion

Compounds **5**, **15**, and **17** were able to transactivate PPARγ *in vitro* and compounds **5** and **15** showed potent antidiabetic effects *in vivo*. With their simple structure and small molecular weight, compounds **5** and **15** could be modified in many ways. Therefore, further studies are of interest to improve the antidiabetic activities of compounds **5** and **15** by structural modification and to fully investigate the mechanism by which these compounds affect glucose metabolism *in vivo*. Compounds **5** and **15** contain either 2-(β-carbonyl) butyryl-thiopene or 2-(β-sulfonyl) butyryl-thiopene; therefore, these structures are of particular interest in future studies of novel antidiabetic agents which transactivate PPARγ.

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