Synthesis and PPAR-γ Ligand-Binding Activity of the New Series of 2′-Hydroxychalcone and Thiazolidinedione Derivatives

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Fifteen chalcones and three thiazolidinedione (TZD) chalcones were prepared to evaluate their peroxisome proliferator-activated receptor-γ (PPAR-γ) ligand-binding activities. Among the three TZDs, one compound possessed PPAR-γ transactivation potential, while the others showed antagonistic activity against PPAR-γ transactivation. Among the chalcones, compound 5 was the most potent, and structure–activity relationship studies indicated that a methoxyl group in position C-4 and hydroxyl group in position C-4′ or 5′ in chalcone plays a key role in determining the potency of PPAR-γ activation.

Key words PPAR-γ; 2′,5′-dihydroxy-4-methoxychalcone; chalconyl-thiazolidinedione

Peroxisome proliferator-activated receptor (PPAR)-γ is a member of the PPAR family and has been the subject of extensive research due to its mechanistic importance in glucose and lipid homeostasis.[1)] It is the predominant molecular target for the insulin-sensitizing thiazolidinedione (TZD) drugs such as troglitazone, pioglitazone, and rosiglitazone.[2,3)] Those TZD derivatives can activate PPAR-γ and improve insulin resistance by increasing the number of small adipocytes resulting in normal function as differentiated from preadipocytes and inducing apoptosis in large adipocytes, which produce and secrete adipocytokines such as leptin, tumor necrosis factor (TNF)-α, and free fatty acid.[4)] Recently, it has been reported that novel sulfonylurea or TZD derivatives containing a flavonoid ring system have antidiabetic activity.[5,6)]

Chalcones, originally isolated from natural plant sources, have been reported to have a variety of biological properties including antioxidant, antiinflammatory, and anticancer activities[7)] and several synthetic chalcone derivatives are known to have inhibitory activity against diabetic complications.[8,9)] It was envisaged that compounds containing these moieties in the same molecule may show enhanced biological activity. It was therefore of interest to synthesize some TZDs with a chalcone moiety (chalconylidene-TZDs) as a substituent at the C-5 position.

In this study, we selected a group of 2′-hydroxychalcones (**3**—**17**) and chalconyl-TZDs (**18**—**20**) and tested them for PPAR-γ ligand-binding activity in the transient reporter assay.

Chemistry

As depicted in Chart 1, the chalcones (**c**) were synthesized by Winget's condensation of an aromatic acetophenone (**a**) with the appropriate aromatic aldehyde (**b**).[10)] The olefin parts of these chalcones were confirmed to have *trans* geometry based on the ^{1}H-NMR spectrum, in which the coupling constant between the two vinylic protons appearing at 6.9—7.2 and 7.8—8.3 ppm is approximately 15 Hz. A comparison of the physical and spectroscopic data of the synthesized compounds with the reported values showed good agreement.

The chalconylidene-TZDs (**18**—**20**) were synthesized as shown in Chart 2. First, dicarboxybenzaldehyde (**d**) was condensed with TZD with anhydrous sodium acetate in acetic acid to form *p*-carboxybenzylidene-TZD (**e**, mp 155—157 °C) (*i.e.*, by Knoevenagel reaction). The Winget condensation gave products that were insoluble yellowish polymers made from **d**. On the other hand, the reaction of appropriate (**a**) compared with aldehyde (**e**) using Winget's condensation gave chalconylidene-TZDs (**f**). In the literature, it was reported that unsubstituted imidazolidinediones and benzaldehydes in acetic medium gave mainly the *Z* isomer.[11)]

In this study, only one isomer was obtained. However, due to the low solubility of the compounds and the difficulties of obtaining suitable crystals for X-ray analysis, the isomer could not be analyzed structurally in detail.

Results and Discussion

The results of the PPAR-γ ligand-binding activity of 2′-hydroxychalcone and chalconylidene-TZD derivatives are presented in Fig. 1, and troglitazone (**2**) and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (PGJ_2) were used as positive controls. We found 2′-hydroxychalcone with 4-methoxyl compounds (**3**—**10**, **12**) to be more active than the other compounds. In particular, compounds **5** (5′-hydroxyl) and **12** (4′-hydroxyl) were the most potent and showed PPAR-γ ligand-binding activity in a concentration-dependent manner. Changing the 4-hydroxyl group (**11**) to the 4-methoxyl group (**5**) improved the activity. These findings support our hypothesis that 2′-hydroxychalcone with a 4-methoxy group has an important functional group for PPAR-γ ligand-binding activity. From the results of PPAR-γ ligand-binding, we demonstrated that a methoxyl group in position C-4 and hydroxyl group in position C-4′ or -5′ were important to PPAR-γ agonistic activity.

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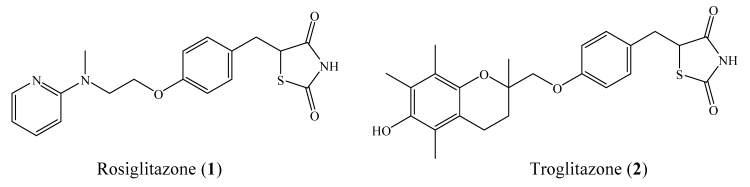
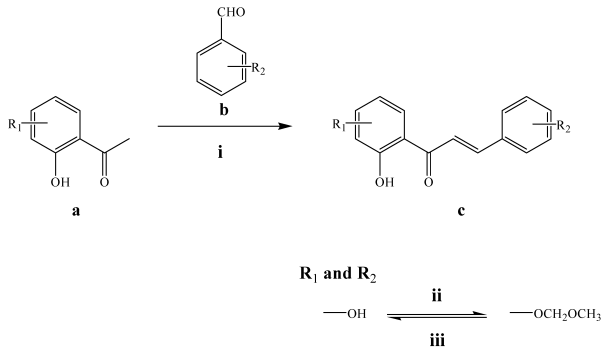


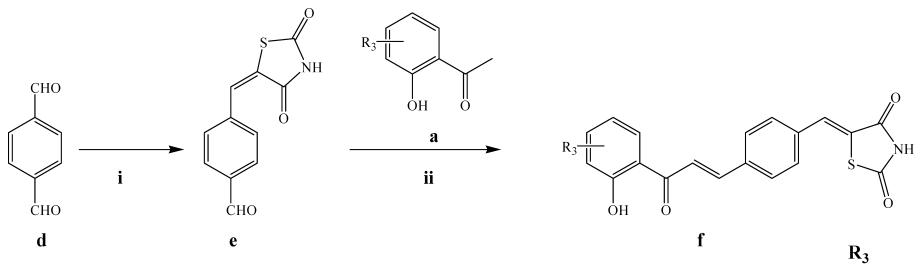
Rosiglitazone (**1**) Troglitazone (**2**)



	R_1	R_2
3	H	4—OCH_3
4	5'—OCH_3	4—OCH_3
5	5'—OH	4—OCH_3
6	5'—CH_3	4—OCH_3
7	5'—Cl	4—OCH_3
8	5'—Br	4—OCH_3
9	6'—OCH_3	4—OCH_3
10	4',5'—diCH_3	4—OCH_3
11	5'—OH	4—OH
12	4'—OH	4—OCH_3
13	4'—OH	3,4—diOH
14	4'—OH	2,4—diOH
15	4'—OH	4—CH_3
16	5'—Cl	3—OH
17	5'—Cl	4—$N(CH_3)_2$

Chart 1. Preparation of 2′-Hydroxychalcone (**3**—**17**)

i) KOH, 95% EtOH. ii) CH_3OCH_2Cl, K_2CO_3, acetone, reflux. iii) 3 N HCl, methaol, reflux.



	R_3
18	H
19	5'—CH_3
20	5'—OCH_3

Chart 2. Preparation of Chalconylidene-TZD (**18**—**20**) Derivatives

i) Thiazolidine-2,4-dione, NaOAc, acetic acid, reflux. ii) KOH, 95% EtDH.

The series of 2′-hydroxychalcone derivatives containing TZDs in the B ring were newly synthesized. Among the three compounds, 2′-hydroxy-5′-methoxychalconylidene-TZD (**20**) was active; on the other hand, the corresponding methylated compound (**19**) or unsubstituted compound (**18**) showed PPAR-γ antagonistic activity.

The cytotoxic effects of the compounds were measured using the MTT assay, and the viability of all treated cells was greater than 95% (data not shown).

In a recent report,[12] 3-nitro-2′-benzyloxychalcone showed potent insulin-stimulated glucose uptake in a concentration-dependent manner in 3T3-L1 adipocytes in a cell-based glucose uptake screening assay. However, the mechanism of action of this chalcone appears to be different from that of troglitazone. It directly acts on some insulin-signaling pathways. However, in our research, these chalcone derivatives (**3**—**20**) were able to activate or deactivate PPAR-γ ligand-binding activity.

In conclusion, a series of chalcone and chalconylidene-TZD derivatives were synthesized and evaluated for PPAR-γ activity. The potential clinical candidates are **5**, **12,** and **20** that have more potent or similar PPAR-γ agonistic activity compared with troglitazone (**2**) and 15-deoxy-$\Delta^{12,14}$-PGJ_2, which were used as positive controls. The structure–activity relationship study indicated that the introduction of a methoxyl group to the C-4 position, hydroxyl group at the C-4′ or -5′, and 5′-methoxyl group in chalconylidene-TZD plays a key role in determining the potency of PPAR-γ activation.

Further pharmacologic evaluation of these compounds and

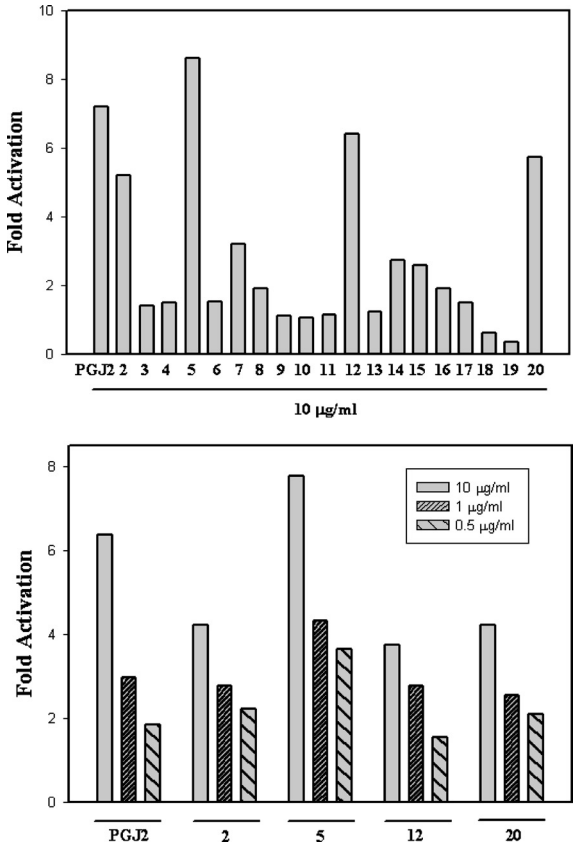


Fig. 1. PPAR-γ Transient Transfection Assay

Activity was determined using transient transfection assays. Luciferase activity was determined and plotted as fold activation relative to untreated cells. PGJ2: 15-deoxy-$\Delta^{12,14}$-PGJ$_2$.

a more detailed structure–activity relationship study of the present series of chalconylidene-TZD derivatives are in progress.

Experimental

Chemistry ^{1}H- (300 MHz) and ^{13}C-NMR (75 MHz) spectra were obtained with a Varian Gemini-2000 spectometer. Chemical shifts (δ) are reported in ppm relative to tetramethylenesilane as internal standard. Melting points were determined on a Mitamura-Riken melting point apparatus (Japan) and are uncorrected. IR spectra were recorded on a JASCO FT/IR-5300 spectrometer, and UV spectra were obtained with a Hitachi U-3210 UV–VIS spectrophotometer. Mass spectra were recorded on a Hewlett Packard model 5989 B GC/MS system and VG70-VSEQ (VG analytical, U.K.).

General Procedure for Synthesis of Chalcone Derivatives To a mixture of 2′-hydroxyacetophenone (3.70 mmol) and potassium hydroxide (18.5 mmol) in 20 ml of 95% ethanol was added 4-methoxybenzaldehyde (3.70 mmol). After stirring for 5 h at room temperature, the reaction was quenched with distilled water. The resulting solution was acidified with 1 N HCl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was purified on column chromatography (silica gel, eluted with 33% ethyl acetate in hexane). In the case of synthesis of compounds with a 2′,4′- or 3,4-dihydroxyl group, the hydroxyl group of the component was protected with methoxymethyl using chloro methylmethyl ether.

2′-Hydroxy-4-methoxychalcone (**3**): Yield, 33.0%. mp 87—90 °C (in the literature,[13] 95 °C).

2′-Hydroxy-4,5′-dimethoxychalcone (**4**): Yield, 43.0%. mp 85—86 °C (in the literature,[14] 87—89 °C).

2′,5′-Dihydroxy-4-methoxychalcone (**5**): Yield, 33.0%. mp 176—178 °C (in the literature,[15] 178—80 °C).

2′-Hydroxy-4-methoxy-5′-methylchalcone (**6**): Yield, 62.1%. mp 94—96 °C (in the literature,[16] 92—94 °C).

5′-Chloro-2′-hydroxy-4-methoxychalcone (**7**): Yield, 38.0%. mp 188—190 °C (in the literature,[13] 191—192 °C).

5′-Bromo-2′-hydroxy-4-methoxychalcone (**8**): Yield, 81.0%. mp 102—104 °C (in the literature,[16] 104 °C).

2′-Hydroxy-4,6′-dimethoxychalcone (**9**): Yield, 48.5%. mp 96—98 °C (in the literature,[13] 98—100 °C).

2′-Hydroxy-4-methoxy-4′,5′-dimethylchalcone (**10**): ^{1}H-NMR (DMSO-d_6) δ: 12.63 (1H, s), 7.88 (1H, d, J=15.3 Hz), 7.51 (1H, d, J=15.3 Hz), 7.66 (1H, s), 6.95 (1H, s), 7.62 (2H, d, J=8.7 Hz), 6.93 (2H, d, J=8.7 Hz), 3.86 (3H, s), 2.27 (3H, s), 2.25 (3H, s). Tlc, *Rf* 0.57 (5 : 3 hexane/EtOAc). mp 142—144 °C. MS *m*/*z*: 282 (M^+). Yield: 67.6%. Brown needles. IR (KBr) ν_{max} cm^{-1}: 3403, 1623.

4,2′,5′-Trihydroxychalcone (**11**): Yield, 21.1%. mp 191—192 °C (in the literature,[17] 191—192 °C).

2′,4′-Dihydroxy-4-methoxychalcone (**12**): Yield, 24.1%. mp 188—190 °C (in the literature,[18] 186 °C).

2′,3,4,4′-Tetrahydroxychalcone (**13**): Yield, 21.5%. mp 250—252 °C (in the literature,[19] 251—252 °C).

2,2′,4,4′-Tetrahydroxychalcone (**14**): Yield, 28.6%. mp 138—140 °C (in the literature,[20] 140 °C).

2′,4′-Dihydroxy-4-methylchalcone (**15**): Yield, 16.0%. mp 102—104 °C (in the literature,[21] 104—106 °C).

5′-Chloro-2′,3-dihydroxychalcone (**16**): ^{1}H-NMR (DMSO-d_6) δ: 12.86 (1H, s), 8.28 (1H, d, J=2.7 Hz), 7.99 (1H, d, J=15.3 Hz), 7.86 (1H, d, J=15.3 Hz), 8.63 (1H, s) 7.01 (1H, dd, J=2.1, 8.7 Hz), 7.2—7.4 (2H, m), 7.53 (1H, dd, J=2.4, 9.0 Hz), 6.96 (1H, m). Tlc, *Rf* 0.41 (5 : 3 hexane/EtOAc). mp 149—150 °C. MS *m*/*z*: 274 (M^+). Yield: 21.8%. Yellow needles. IR (KBr) ν_{max} cm^{-1}: 3344, 1641.

5′-Chloro-2′-hydroxy-4-dimethylaminochalcone (**17**): Yield, 52.0%. mp 132—134 °C (in the literature,[22] 134—135 °C).

General Procedure for Synthesis of TZD Chalcone Derivatives A mixture of 2′-hydroxyacetophenone and 50% aqueous potassium hydroxide solution in 95% ethanol was added to 5-(4-formylphenylmethylene)-2,4-thiazolidinedione. After stirring for 5 h at room temperature, the reaction was quenched with distilled water. The solution was acidified with 1 N HCl and diluted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified on column chromatography (silica gel with 20% ethyl acetate in hexane) to give a yellow powder.

5-{4-[3-(2′-Hydroxyphenyl)-3-oxo-propenyl]-benzylidene}-thiazolidine-2,4-dione (**18**): ^{1}H-NMR (DMSO-d_6) δ: 12.8 (1H, s), 8.18 (1H, d, J=15.6 Hz), 7.67 (1H, d, J=15.6 Hz), 8.01 (1H, d, J=8.1 Hz), 7.02 (1H, d, J=8.1 Hz), 7.6—7.7 (1H, m), 6.99 (1H, d, J=8.1 Hz), 7.71 (2H, d, J=8.4 Hz), 7.36 (2H, d, J=8.4 Hz), 6.98 (1H, s). ^{13}C-NMR (DMSO-d_6) δ: 127.0, 162.0, 117.9, 136.6, 121.1, 130.0, 193.6, 123.5, 147.8, 135.8, 131.1, 130.5, 135.9, 143.8, 119.4, 168.8, 169.5. Tlc, *Rf* 0.36 (5 : 3 MC/MeOH). mp 186—189 °C. MS *m*/*z*: 351 (M^+). Yield: 36.0%. Yellow powder. IR (KBr) ν_{max} cm^{-1}: 3287, 1651.

5-{4-[3-(2′-Hydroxy-5′-methylphenyl)-3-oxo-propenyl]-benzylidene}-thiazolidine-2,4-dione (**19**): ^{1}H-NMR (DMSO-d_6) δ: 13.1 (1H, s), 8.08 (1H, d, J=15.3 Hz), 7.81 (1H, d, J=15.3 Hz), 7.67 (1H, d, J=9.0 Hz), 7.54 (1H, d, J=9.0 Hz), 7.46 (1H, d, J=9.0 Hz), 7.62 (2H, d, J=9.0 Hz), 6.94 (2H, d, J=9.0 Hz), 6.88 (1H, s), 3.81 (3H s). ^{13}C-NMR (DMSO-d_6) δ: 124.2, 152.0, 118.9, 121.0, 156.1, 113.5, 193.1, 123.7, 143.5, 131.5, 130.5, 130.0, 134.8, 137.8, 168.8, 169.5, 55.9. Tlc, *Rf* 0.46 (5 : 3 MC/MeOH). mp 123—126 °C; MS *m*/*z*: 381 (M^+). Yield: 35.0%. Yellow powder. IR (KBr) ν_{max} cm^{-1}: 3269, 1641.

5-{4-[3-(2′-Hydroxy-5′-methoxyphenyl)-3-oxo-propenyl]-benzylidene}-thiazolidine-2,4-dione (**20**): ^{1}H-NMR (DMSO-d_6) δ: 12.3 (1H, s), 8.08 (1H, d, J=15.3 Hz), 7.83 (1H, d, J=15.3 Hz), 7.76 (1H, d, J=9.0 Hz), 7.47 (1H, d, J=8.7 Hz), 7.33 (1H, d, J=8.7 Hz), 7.62 (2H, d, J=8.7 Hz), 7.01 (2H, d, J=8.7 Hz), 6.90 (1H, s), 2.49 (3H, s). ^{13}C-NMR (DMSO-d_6) δ: 123.2, 160.1, 117.6, 137.3, 130.3, 131.2, 193.5, 126.9, 143.5, 135.8, 128.2, 127.9, 136.0, 143.5, 120.5, 168.8, 169.4, 20.9. Tlc, *Rf* 0.56 (5 : 3 MC/MeOH). mp 154—157 °C; MS *m*/*z*: 365 (M^+). Yield: 25.0%. Yellow powder. IR (KBr) ν_{max} cm^{-1}: 3273, 1651.

***In Vitro* Biological Activity** The PPAR-γ ligand-binding activity of the chalcones (**3**—**17**) and chalconylidene-TZD (**18**—**20**) was determined using transient transfection assays.[23] The CV-1 cells were plated in 96-well plates at 1.2×10^4 cells/well and cultured until 70—80% confluency for 16 h. The cells were transiently transfected with pCDM8-mPPARγ, pcDNA3.1-mRXRa, pPPRE (×3) tk-luc, and pcDNA3.1-lacZ by liposomal delivery using a GenePORTER2 transfection kit (GTS). After 16-h incubation, the cells were washed and changed to fresh complete medium with the indicated

compounds. After 24-h treatment, the cells were harvested in luciferase lysate buffer and cellular luciferase activity was determined using a luciferase assay kit (Promega). The results were normalized to the β-galactosidase activity to correct the transfection efficiencies.

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