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Synthesis and Biological Activity of [[(Heterocycloamino)alkoxy] benzyl]-2,4-thiazolidinediones as PPARγ Agonists

Raok Jeon, Yoon-Jung Kim, Yejin Cheon, and Jae-Ha Ryu

College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Korea

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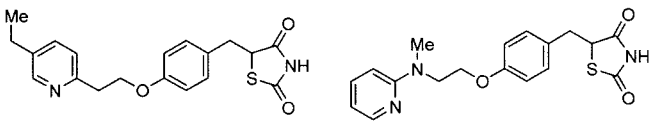
Benzothiazole derivatives of thiazolidinediones (TZD) were synthesized using a modified Mitsunobu reaction of 2-(benzothiazol-2-ylmethylamino)ethanol (**2**) with 5-(4-hydroxybenzyl)-3-triphenylmethylthiazolidine-2,4-dione and assayed for activity on peroxisome proliferator-activated receptor (PPAR) subtypes and inhibitory activity of NO production in lipopolysaccharide-activated macrophages. Most of the tested compounds were identified as potent PPARγ agonists, indicating their potential as drug candidates for diabetes.

Key words: Benzothiazole, Thiazolidinediones, PPARγ, Nitric oxide, Diabetes

INTRODUCTION

More than 90% of diabetic patients suffer from type 2 diabetes, known as noninsulin-dependent diabetes mellitus (NIDDM), which is characterized by insulin resistance and hyperglycemia (Reaven, 1988). Type 2 diabetes gradually develops over the years because of progressive debilitating defects in both the secretion and action of insulin. The discovery of compounds which have insulin-sensitizing effects enables the continued treatment of NIDDM patients without inducing hypoglycemia. A new class of drugs belonging to the thiazolidinedione (TZD) family, whose molecular target was reported to be the peroxisome proliferator-activated receptor-γ (PPARγ) (Lehmann *et al.*, 1995), has been developed recently for the treatment of type 2 diabetes. After the initial launch of troglitazone in the market, two additional TZDs, pioglitazone and rosiglitazone, were introduced as clinically useful agents (Yoshioka *et al.*, 1989; Sohda *et al.*, 1990; Cantello, 1994).



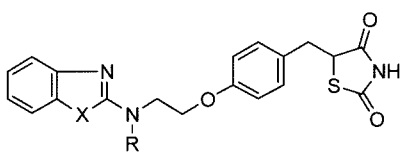
Pioglitazone Rosiglitazone

Fig. 1. Two currently marketed thiazolidinedione antidiabetic drugs

Correspondence to: Raok Jeon, College of Pharmacy, Sookmyung Women's University, Seoul 140-742, Korea
Tel: 82-2-710-9571, Fax: 82-2-715-9571
E-mail: rjeon@sookmyung.ac.kr

Many research groups are still endeavoring to find new antidiabetic drugs (Lohray *et al.*, 1998; Oguchi *et al.*, 2000; Henke *et al.*, 1998). However, despite numerous attempts to replace the thiazolidine-2,4-dione ring with other heterocycles the results have been less than spectacular.

In a previous paper, we reported TZDs featuring benzoxazole, which were modified with benzoxazol-2-ylmethylamino substitution of the BRL 48482 with different *N*-alkyl substituents (Jeon *et al.*, 2004). Continuing the synthesis and evaluation of [[(heterocycloamino)alkoxy]benzyl]-2,4-thiazolidinediones, herein, we report the synthesis of 5-[4-[2-(benzothiazol-2-ylalkylamino)ethoxy]benzyl]thiazolidine-2,4-diones and their activities in a PPAR transactivation assay. In addition, we also investigate PPARγ activator-induced inhibition of NO production since it has been reported that activation of PPARγ induces down regulation of iNOS expression (Heneka *et al.*, 1999).



1, X= S
2, X= O

Fig. 2. Structure of thiazolidinedione featuring benzothiazole and benzoxazole

MATERIALS AND METHODS

Materials

Most of the reagents and solvents were purchased from Aldrich chemicals and used without purification, with the following exceptions. Ethyl ether and tetrahydrofuran were distilled from sodium benzophenone ketyl. Acetonitrile, methylene chloride, benzene, toluene, triethylamine, pyridine, dimethyl formamide, and diisopropylamine were distilled from calcium hydride under nitrogen atmosphere. Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco Laboratories (Detroit, MI). Lipopolysaccharide (LPS, *Escherichia coli*, 0127:B8), bovine serum albumin, sodium nitrite, naphthylethylene diamine, sulfanilamide were obtained from Sigma Chemical Co. (St. Louis, MO). Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography (TLC) was performed using Kieselgel 60 F_{254} plates (Merck). Melting points were measured on a Büchi melting point apparatus and were uncorrected. IR spectra were recorded on a JASCO FT/IR 430 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian YH 400 spectrometer as solutions in $CDCl_3$, CH_3OH-d_4 or DMSO-d_6. Chemical shifts are expressed in parts per million (ppm, δ) downfield from an internal standard, tetramethylsilane.

2-(Benzothiazol-2-ylmethylamino)ethanol (3a)

A solution of 2-chlorobenzothiazole (5 g, 29.48 mmol), 2-methylaminoethanol (3.32 g, 44.22 mmol) and triethylamine (4.47 g, 44.22 mmol) in tetrahydrofuran (30 mL) was stirred at 70°C for 18 h. The reaction mixture was diluted with EtOAc and washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (eluent, *n*-hexane/EtOAc = 1:1 v/v) to afford 4.17 g (68%) of the title compound as a yellow oil: R_f= 0.24 (*n*-hexane/EtOAc = 1:1); IR (neat) 3302, 3060, 2930, 1548, 750 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.56 (dd, 1H, *J* = 7.6, 1.2 Hz), 7.52 (d, 1H, *J* = 8.4 Hz), 7.27 (dt, 1H, *J* = 7.6, 1.2 Hz), 7.06 (dt, 1H, *J* = 8.6, 1.2 Hz), 3.92 (t, 2H, *J* = 4.4 Hz), 3.79 (t, 2H, *J* = 4.7 Hz), 3.18 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.6, 151.3, 130.1, 126.2, 121.5, 120.7, 118. 6, 61.5, 56.0, 40.4.

2-(Benzothiazol-2-ylethylamino)ethanol (3b)

This compound was prepared using the same procedure as described for the preparation of **3a**: Yield = 62%; R_f= 0.30 (*n*-hexane/EtOAc = 1:1); IR (neat) 3734, 3326, 3060, 2970, 2931, 2872, 1542, 1053, 751 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.50 (dd, 1H, *J* = 7.6, 1.2 Hz), 7.40 (d, 1H, *J* = 8.4 Hz), 7.22 (dt, 1H, *J* = 8.0, 1.2 Hz), 7.00 (dt, 1H, *J* = 8.0, 1.2 Hz), 3.86 (t, 2H, *J* = 4.4 Hz), 3.72 (t, 2H, *J* = 4.7 Hz), 3.46 (q, 2H, *J* = 7.2 Hz), 1.24 (t, 3H, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.0, 151.7, 130.3, 126.0, 121.3, 120.6, 118.5, 62.4, 53.6, 48.8, 12.7.

2-(Benzothiazol-2-ylpropylamino)ethanol (3c)

This compound was prepared using the same procedure as described for the preparation of **3a**: Yield = 63%; R_f= 0.34 (*n*-hexane/EtOAc = 1:1); IR (neat) 3335, 3060, 2962, 2873, 2360, 1541, 751 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.54 (dd, 1H, *J* = 7.6, 1.2 Hz), 7.50 (d, 1H, *J* = 8.0 Hz), 7.26 (dt, 1H, *J* = 7.6, 1.2 Hz), 7.05 (dt, 1H, *J* = 8.0, 1.2 Hz), 3.90 (t, 2H, *J* = 4.4 Hz), 3.78 (t, 2H, *J* = 4.7 Hz), 3.70 (t, 2H, *J* = 4.7 Hz), 3.39 (t, 2H, *J* = 7.6 Hz), 1.72 (sextet, 2H, *J* = 7.6 Hz), 0.96 (t, 3H, *J* = 7.4 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169. 5, 151.3, 129.9, 126.1, 121.4, 120.6, 118. 5, 62. 6, 56. 2, 54.3, 21.0, 11.2.

2-(Benzothiazol-2-ylbutylamino)ethanol (3d)

This compound was prepared using the same procedure as described for the preparation of **3a**: Yield = 87%; R_f= 0.39 (*n*-hexane/EtOAc = 1:1); IR (neat) 3335, 3060, 2956, 2929, 2870, 2360, 1541, 751 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.53 (dd, 1H, *J* = 7.6, 1.2 Hz), 7.48 (d, 1H, *J* = 8.0 Hz), 7.26 (dd, 1H, *J* = 7.6, 1.2 Hz), 7.04 (dt, 1H, *J* = 8.0, 1.2 Hz), 3.90 (t, 2H, *J* = 4.4 Hz), 3.77 (t, 2H, *J* = 4.7 Hz), 3.41 (t, 2H, *J* = 7.6 Hz), 1.66 (quintet, 2H, *J* = 8.0 Hz), 1.37 (sextet, 2H, *J* = 7.6 Hz), 0.96 (t, 3H, *J* = 7.4 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.4, 151.2, 129.8, 126.3, 121.5, 20.6, 118.5, 62.6, 54.5, 54.3, 29.8, 19.9, 13.8.

5-[4-[2-(Benzothiazol-2-ylmethylamino)ethoxy]benzyl]-3-tritylthiazolidine-2,4-dione (4a)

To a mixture of the alcohol **3a** (62.5 mg, 0.30 mmol), 5-(4-hydroxybenzyl)-3-triphenylmethylthiazolidine-2,4-dione (93.1 mg, 0.20 mmol) and azodicarbonyldipiperidine (151.4 mg, 0.60 mmol) in anhydrous toluene (1 mL 0.60 mmol), was added dropwise tributylphosphine (1.2 mL, 0.60 mmol) in anhydrous toluene, and then the resulting mixture was stirred at room temperature for 6 h. Insoluble materials were filtered and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (eluent, *n*-hexane/EtOAc = 3:1 v/v) to afford 74.7 mg (60%) of the title compound: R_f = 0.51 (*n*-hexane/EtOAc = 3:1); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.59 (d, 1H, *J* = 7.6 Hz), 7.56 (d, 1H, *J* = 8.0 Hz), 7.31-7.03 (m, 19H), 6.82 (d, 2H, *J* = 8.8 Hz), 4.33 (dd, 1H, *J* = 3.8, 9.2 Hz), 4.25 (t, 2H, *J* = 4.8 Hz), 3.88 (t, 2H, *J* = 5.2 Hz), 3.38 (dd, 1H, *J* = 4.0, 14.4 Hz), 3.29 (s, 3H), 3.05 (dd, 1H, *J* = 9.6, 14.4 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.4, 169.8, 168.3, 158.2, 153.3, 141.7, 131.2, 130.8, 128.7, 128.3, 127.8, 126.9, 126.2, 121.3, 120.9, 119.1, 114.9, 66.2, 52.3, 50.8, 40.5, 37.8.

5-[4-[2-(Benzothiazol-2-ylethylamino)ethoxy]benzyl]-3-tritylthiazolidine-2,4-dione (4b)

This compound was prepared using the same procedure as described for the preparation of **4a**: Yield = 58%; R_f = 0.52 (*n*-hexane/EtOAc = 3:1); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.47 (d, 1H, *J* = 7.6 Hz), 7.44 (d, 1H, *J* = 8.0 Hz), 7.23-7.69 (m, 19H), 6.73 (d, 2H, *J* = 8.8 Hz), 4.24 (dd, 1H, *J* = 3.8, 9.2 Hz), 4.16 (t, 2H, *J* = 4.8 Hz), 3.86 (t, 2H, *J* = 5.2 Hz), 3.54 (q, 2H, *J* = 7.2 Hz), 3.27 (dd, 1H, *J* = 4.0, 14.4 Hz), 2.95 (dd, 1H, *J* = 9.6, 14.4 Hz), 1.94 (t, 3H, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.1, 168.5, 156.9, 154.3, 142.3, 140.5, 129.5, 127.9, 127.4, 126.5, 126.2, 125.8, 124.9, 120.1, 119.6, 117.6, 113.6, 75.3, 64.8, 49.4, 48.8, 47.2, 36.5, 11.7.

5-[4-[2-(Benzothiazol-2-ylpropylamino)ethoxy]benzyl]-3-tritylthiazolidine-2,4-dione (4c)

This compound was prepared using the same procedure as described for the preparation of **4a**: Yield = 32%; R_f = 0.47 (*n*-hexane/EtOAc = 3:1); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.48 (d, 1H, *J* = 7.6 Hz), 7.45 (d, 1H, *J* = 8.0 Hz), 7.24-6.95 (m, 19H), 6.97 (d, 2H, *J* = 8.8 Hz), 4.26 (dd, 1H, *J* = 3.8, 9.2 Hz), 4.18 (t, 2H, *J* = 4.4 Hz), 3.95 (t, 2H, *J* = 4.7 Hz), 3.47 (t, 2H, *J* = 7.6 Hz), 3.30 (dd, 1H, *J* = 14.4, 4.0 Hz), 2.98 (dd, 1H, *J* = 14.4, 9.6 Hz), 1.70 (sextet, 2H, *J* = 7.6 Hz), 0.89 (t, 3H *J* = 7.4 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.2, 168.6, 156.8, 154.7, 142.9, 140.5, 129.4, 128.3, 127.9, 127.0, 126.2, 124.3, 120.5, 119.7, 117.4, 114.7, 113.6, 75.2, 64.7, 59.4, 49.6, 36.6, 20.0, 18.2, 10.2.

5-[4-[2-(Benzothiazol-2-ylbutylamino)ethoxy]benzyl]-3-tritylthiazolidine-2,4-dione (4d)

This compound was prepared using the same procedure as described for the preparation of **4a**: Yield = 38%; R_f = 0.46 (*n*-hexane/EtOAc = 3:1); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.58 (d, 1H, *J* = 7.6 Hz), 7.55 (d, 1H, *J* = 8.0 Hz), 7.32-7.03 (m, 19H), 6.84 (d, 2H, *J* = 8.8 Hz), 4.35 (dd, 1H, *J* = 9.2, 3.8 Hz), 4.27 (t, 2H, *J* = 4.4 Hz), 3.90 (t, 2H, *J* = 4.7 Hz), 3.56 (t, 2H, *J* = 7.6 Hz), 3.40 (dd, 1H, *J* = 4.0, 14.4 Hz), 3.05 (dd, 1H, *J* = 14.4, 9.6 Hz), 1.74 (quintet, 2H, *J* = 8.0 Hz), 1.41 (sextet, 2H, *J* = 7.6 Hz), 0.97 (t, 3H, *J* = 7.4 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.4, 167.9, 158.2, 153.3, 143.8, 141.7, 131.0 ,130.8, 128.7, 128.2, 127.8, 126.9, 126.0, 121.2, 120.8, 118.9, 114.9, 65.9, 53.7, 50.8, 50.2, 37.9, 31.8, 29.9, 20.3, 14.2.

5-[4-[2-(Benzothiazol-2-ylmethylamino)ethoxy]benzyl] thiazolidine-2,4-dione (1a)

A mixture of **3a** (74.7 mg, 0.12 mmol) and trifluoroacetic acid (0.8 mL) was stirred at room temperature for 3 h. The reaction mixture was diluted with EtOAc, neutralized by the addition of saturated potassium carbonate solution, and extracted with EtOAc. The combined organic extracts were washed with water, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (eluent, *n*-hexane/EtOAc = 3:1 v/v) to afford 27.6 mg (56 %) of the title compound as a yellow oil: IR (neat) 3204, 3063, 3030, 2930, 2877, 2752, 1697 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.98 (br s, 1H), 7.57 (d, 1H, *J* = 7.6 Hz), 7.51 (d, 1H, *J* = 8.0 Hz), 7.27 (dt, 1H, *J* = 8.0, 1.2 Hz), 7.10 (d, 2H, *J* = 8.6 Hz), 7.04 (dt, 1H, *J* = 8.0, 1.2 Hz), 6.80 (d, 2H, *J* = 8.8 Hz), 4.45 (dd, 1H, *J* = 9.2, 3.8 Hz), 4.14 (t, 2H, *J* = 4.4 Hz), 3.95 (t, 2H, *J* = 5.2 Hz), 3.38 (dd, 1H, *J* = 14.4, 4.0 Hz), 3.27 (s, 3H), 3.09 (dd, 1H, *J* = 14.4, 9.6 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.4, 170.6, 168.4, 158.2, 153.1, 131.0, 130.7, 128.2, 126.2, 121.3, 120.9, 118.9, 114.9, 66.1, 53.8, 52.4, 40.5, 37.8; HRMS-FAB$^+$ (*m/z*) M$^+$ calculated for $C_{20}H_{20}N_3O_3S_2$ 414.0946, was found to be 414.0944.

5-[4-[2-(Benzothiazol-2-ylethylamino)ethoxy]benzyl] thiazolidine-2,4-dione (1b)

This compound was prepared using the same procedure as described for the preparation of **1a**: Yield = 44%; R_f = 0.43 (*n*-hexane/EtOAc = 3:1); IR (neat) 3198, 3063, 2930, 2877, 2752, 1697, 1541, 752 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.59 (br s, 1H), 7.51 (dd, 1H, *J* = 7.6, 1.2 Hz), 7.45 (d, 1H, *J* = 8.0 Hz), 7.21 (dt, 1H, *J* = 8.0, 1.2 Hz), 7.04 (d, 2H, *J* = 8.6 Hz), 6.97 (dt, 1H, *J* = 8.0, 1.2 Hz), 6.74 (d, 2H, *J* = 8.8 Hz), 4.38 (dd, 1H, *J* = 9.2, 3.8 Hz), 4.10 (t, 2H, *J* = 4.4 Hz), 3.86 (t, 2H, *J* = 4.7 Hz), 3.55 (q, 2H, *J* = 7.2 Hz), 3.30 (dd, 1H, *J* = 14.4, 4.0 Hz), 3.01 (dd, 1H, *J* = 14.4, 9.6 Hz), 1.24 (t, 3H, *J* = 7.1 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 175.1, 171.3, 167.9, 158.2, 152.7, 130.6, 128.1, 121.4, 120.9, 118.7, 116.1, 114.8, 66.0, 54.0, 50.1, 48.6, 37.8, 12.9; HRMS-FAB$^+$ (*m/z*) M$^+$ calculated for $C_{21}H_{22}N_3O_3S_2$ 428.1103, was found to be 428.1098.

5-[4-[2-(Benzothiazol-2-ylpropylamino)ethoxy]benzyl] thiazolidine-2,4-dione (1c)

This compound was prepared using the same procedure as described for the preparation of **1a**: Yield = 52%; R_f = 0.46 (*n*-hexane/EtOAc = 3:1); IR (neat) 3183, 3058, 2926, 2875, 2760, 1698, 1541, 753 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.79 (br s, 1H), 7.51 (dd, 1H, *J* = 7.6, 1.2 Hz), 7.46 (d, 1H, *J* = 8.0 Hz), 7.22 (dt, 1H, *J* = 8.0, 1.2 Hz), 7.06 (d, 2H, *J* = 8.6 Hz,), 7.00 (dt, 1H, *J* = 8.0, 1.2 Hz), 6.77 (d, 2H, *J* = 8.8 Hz), 4.41 (dd, 1H, *J* = 9.2, 3.8 Hz), 4.18 (t, 2H, *J* = 4.4 Hz), 3.91 (t, 2H, *J* = 4.7 Hz), 3.46 (t, 2H, *J* = 7.6 Hz), 3.33 (dd, 1H, *J* = 9.2, 3.8 Hz), 3.03 (dd, 1H, *J* = 14.4, 9.6 Hz), 1.73 (sextet, 2H, *J* = 7.6 Hz), 0.92 (t, 3H, *J* = 7.4 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.3, 170.5, 168.0, 158.3, 153.1, 131.0, 130.6, 128.1, 126.9, 121.3, 120.8, 118.8, 115.8, 65.9, 60.7, 53.8, 50.2, 37.8, 29.9, 21.8, 13.4; HRMS-FAB$^+$ (*m/z*) M$^+$ calculated for $C_{22}H_{24}N_3O_3S_2$

442.1259, was found to be 442.1263.

5-[4-[2-(Benzothiazol-2-ylbutylamino)ethoxy]benzyl] thiazolidine-2,4-dione (1d)

This compound was prepared using the same procedure as described for the preparation of **1a**: Yield = 58%; R_f = 0.50 (*n*-hexane/EtOAc = 3:1); IR (neat) 3200, 3063, 2955, 2928, 2870, 2750, 1698, 1541, 752 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.19 (s, 1H), 7.54 (d, 1H, J = 7.6 Hz), 7.49 (d, 1H, J = 8.0 Hz), 7.26 (dt, 1H, J = 8.0, 1.2 Hz), 7.10 (d, 2H, J = 8.6 Hz,), 7.02 (dt, 1H, J = 8.0, 1.2 Hz), 6.81 (d, 2H, J = 8.8 Hz), 4.45 (dd, 1H, J = 9.2, 3.8 Hz), 4.20 (t, 2H, J = 4.4 Hz), 3.93 (t, 2H, J = 4.7 Hz), 3.52 (t, 2H, J = 7.6 Hz), 3.37 (dd, 1H, J = 14.4, 4.0 Hz), 3.07 (dd, 1H, J = 14.4, 9.6 Hz), 1.71 (quintet, 2H, J = 8.0 Hz), 1.39 (sextet, 2H, J = 7.6 Hz), 0.95 (t, 3H, J = 7.4 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.1, 170.4, 167.9, 158.3, 153.1, 130.9, 130.6, 128.1, 126.9, 121.3, 120.8, 118.8, 114.9, 65.9, 60.7, 53.8, 50.2, 37.8, 29.9, 21.3, 20.3, 14.4; HRMS-FAB$^+$ (*m/z*) M^+ calculated for $C_{23}H_{26}N_3O_3S_2$ 456.1416, was found to be 456.1414.

PPAR Transactivation assay

GAL4 fusions were made by fusing murine PPAR ligand binding domain to the C-terminal end of yeast GAL4 DNA binding domain. CV-1 cells were seeded at 2×10^4 cells/well and cultured for 24 h at 37°C. Cells were cotransfected for 3 h at 37°C with pUAS, pRL-TK, and pCMX-GalPx. Transfected cells were treated with 2 μM of the test compounds for 24 h. DMSO (0.1%) was used as a blank. GW409544, which is a potent full agonist on both PPARα and PPARγ was used as positive control. Luciferase activity was determined as 'fold activation' relative to positive control.

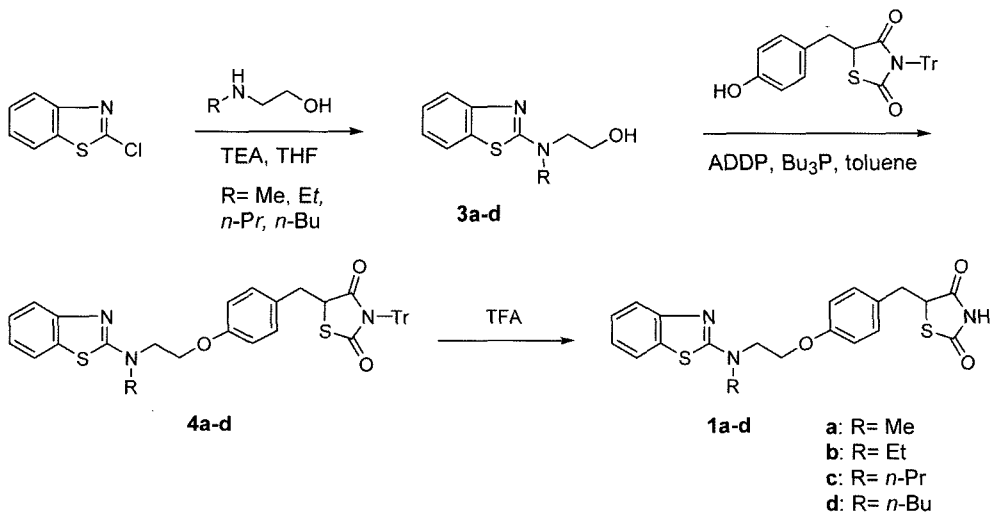
Nitrite assay

Murine macrophage cell line (RAW 264.7) was obtained from American Type Culture Collection (Rockville, MD). Cells were cultured in DMEM containing 10% fetal bovine serum (FBS), 2 mM glutamine, 1 mM sodium pyruvate, penicillin (100 U/mL) and streptomycin (10 μg/mL). Cells were grown at 37°C, in an atmosphere of 5% CO_2 in fully humidified air, and were split twice a week. RAW 264.7 cells were seeded at 5×10^5 cells/mL in 24-well plates and activated for 20 h by the incubation in 1% FBS medium containing lipopolysaccharide (LPS, 1 μg/mL) and various concentrations of test compounds dissolved in DMSO (final 0.1% in media). The supernatant was collected as a source of secreted NO.

NO released from macrophages was assessed by the determination of NO_2^- concentration in culture supernatant. Samples (100 μL) of culture media were incubated with 150 μL of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine in 2.5% phosphoric acid solution) at room temperature for 10 min in 96-well microplates (Green *et al.*, 1982). Absorbance at 540 nm was read using an ELISA plate reader. Standard calibration curves were prepared using sodium nitrite as standard.

RESULTS AND DISCUSSION

The methods used to prepare the TZDs (**1**) are outlined in Scheme 1. 2-Chlorobenzothiazole was treated with the appropriately substituted alkylamino ethanols to give the amino alcohols (**3**). Mitsunobu reaction of **3** with 5-(4-hydroxybenzyl)thiazolidine-2,4-dione, which was prepared from 4-hydroxybenzaldehyde by modification of the reported method (Morita *et al.*, 1998), in the presence of azodicarbonyldipiperidine and tributylphosphine, gave compound **4**. Removal of the trityl protecting group by



Scheme 1. Synthesis of 5-[4-[2-(benzothiazol-2-ylalkylamino)ethoxy]benzyl]thiazolidine-2,4-diones

treating with trifluoroacetic acid, gave the desired products (**1**).

All synthesized compounds were evaluated for their ability to activate murine PPARα and PPARγ in a trans-activation assay in CV-1 cells. The results are shown in Table I compared with those of the previously reported benzoxazole analogs. All compounds tested showed significant PPARγ activities without PPARα activities. Although it would be too early to differentiate the benzothiazoles from the benzoxazoles in a meaningful way, with the exception of **1c**, the benzothiazole analogs showed higher activities than benzoxazole did.

Compound **1a**, which was substituted with a methyl on the exocyclic nitrogen, was the most potent PPARγ agonist as was the case of the benzoxazole analog. As *N*-substituents were lengthened, transactivation of PPARγ decreased proportionally.

In addition, the amount of NO_2^- accumulation in LPS-activated RAW 264.7 cells was measured. As expected, all of the compounds tested showed an inhibitory activity against NO production in a dose dependant manner, although the tendency was not consistent with that of PPARγ activation. Benzothiazole analogs such as **1b-d** were shown to be more effective than benzoxazole analogs. Methyl substituted analogs in both benzoxazole and benzothiazole series revealed the lowest inhibitory activity.

Cells were incubated with LPS (1 μg/mL) in the presence or absence of each of the compounds (5, 10, and 20 μM). After treatment, NO_2^- accumulation was measured. Unstimulated cells served as controls. Each value is the mean ± S.D. of three determinations.

Table I. *In vitro* PPAR activities of the compounds[a]

Compound	X	R	Transactivation RR %[b]	
			PPARα	PPARγ
1a	S	Me	NA[c]	120
1b	S	Et	NA	107
1c	S	*n*-Pr	NA	77
1d	S	*n*-Bu	NA	54
2a	O	Me	NA	113
2b	O	Et	NA	78
2c	O	*n*-Pr	NA	86
2d	O	*n*-Bu	NA	51
GW409544			100	100
KRP-297			NA	63

[a]Compounds were assayed for agonist activity on PPAR-GAL4 chimeric receptors in transiently transfected CV-1 cells as described in the Materials and Methods section. [b]Data are represented as relative response (%), which is [(test drug-negative control)/(positive control-negative control)]x100. GW409544 and DMSO were used as positive and negative controls, respectively. [c]NA: no activity.

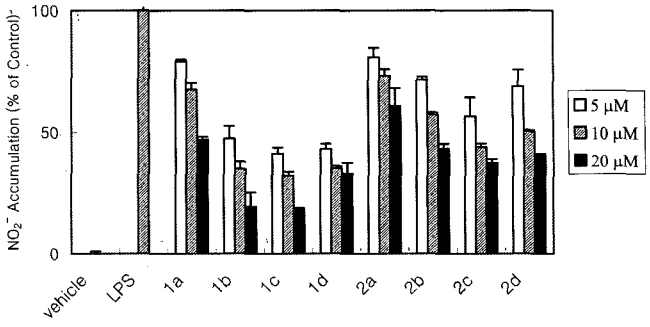


Fig. 3. Inhibitory activities of the compounds on NO_2^- accumulation in LPS-activated macrophages. Cells were incubated with LPS (1 μg/mL) in the presence or absence of the compounds (5, 10, and 20 μM). After treatment, NO_2^- accumulation was measured. Unstimulated cells served as controls. Each value is the mean ± S.D. of three determinations.

In summary, we synthesized 5-[4-[2-(benzothiazol-2-yl-alkylamino)ethoxy]benzyl]thiazolidine-2,4-diones with various lengths of alkyl chains on the exocyclic nitrogen in order to determine the steric influence at the active site of PPARγ. The lengthening of the *N*-alkyl substituent of [[(benzothiazolamino)alkoxy]benzyl]thiazolidinedions lowered the activation of PPARγ but increased the inhibitory action of NO production. Replacement of oxygen in benzoxazole by sulfur merely affected *in vitro* PPARγ activation but it did increase the inhibition of NO production. Even though these two biological results were not correlated proportionally, the results strongly suggest that a PPARγ activator might be a useful therapeutic candidate for the treatment of inflammatory diseases through the inhibition of overproduction of NO.

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