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# Rhodanine derivatives as novel peroxisome proliferator-activated receptor γ agonists[1]

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## Key words

rhodanine derivatives; peroxisome proliferator-activated receptor; structure-activity relationship; adipogenesis

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## Abstract

**Aim:** To characterize the *in vitro* bioactivities of rhodanine derivatives as novel peroxisome proliferator-activated receptor (PPAR) γ modulators, based on a hit (SH00012671) identified during high-throughput screening (HTS) of a diverse synthetic compound library, and to preliminarily elucidate the structure-activity relationship of this class of PPARγ agonists. **Methods:** Full-length PPARγ and retinoid X receptor α (RXRα), biotinylated PPAR response element (PPRE), [$^3$H]BRL49653 (rosiglitazone), and streptavidin-coated FlashPlate or microbeads were used to measure the receptor-binding properties of various compounds based on the scintillation proximity assay (SPA) technology. A recombinant PPRE vector was transiently cotransfected with PPARγ and RXRα plasmids into the African green monkey kidney (CV-1) cells, and the effects of BRL49653 and test compounds on transcription mediated by PPARγ were determined by examining luciferase (reporter) responses. 3T3-L1 cells were employed to determine whether the compounds facilitated adipogenesis upon PPARγ activation. **Results:** Of the 16 000 samples screened with the SPA method, only 1 compound (SH00012671) displayed a similar binding affinity ($K_i$=186.7 nmol/L) to PPARγ as BRL49653, but it was inactive in the cell-based assays. A series of rhodanine derivatives were synthesized based on the core structure of SH00012671 and 8 of them showed agonist activities in both cotransfection and pre-adipocyte differentiation assays. To reduce intrinsic cytotoxicities, the sulphur on the rhodanine was changed to oxygen. This alteration led to a decrease in receptor-binding affinities while modified analogues generally maintained agonist efficacies in the cell-based assays. Of the analogues studied, compound 31 exhibited about 70% the efficacy exerted by BRL49653 in both cotransfection and pre-adipocyte differentiation assays. **Conclusion:** Through minor chemical modifications on the core structure of the initial HTS hit, SH00012671 was transformed to possess both molecular (PPARγ binding) and cellular (adipogenesis) activities. The rhodanine derivatives reported here may represent a new scaffold in further understanding the molecular mechanism of agonism at PPARγ.

## Introduction

Peroxisome proliferator-activated receptor (PPAR) γ, one of the subtypes of PPARs, is a ligand-dependent transcription factor of the nuclear hormone receptor superfamily[1]. PPARγ is mainly present in the adipose tissue, skeletal muscle, and liver[2]. Two other family members include PPARα, which is present in the liver, kidney and heart, and the ubiquitously-expressed PPARδ[3]. Upon ligand binding, PPARs release relevant corepressors and form heterodimers with retinoid X receptors (RXRs)[4]. The heterodimers bind to peroxisome proliferator response elements (PPREs) and recruit co-activators to initiate the transcription of target genes[5,6].

Type 2 diabetes mellitus is a heterogeneous disease resulting from a dynamic interaction between defects in insulin secretion and insulin action[7]. In animal models and

humans affected by this disorder, treatment with thiazolidinediones decreases elevated plasma glucose, triglyceride, and insulin concentrations[8,9]. Their actions also lead to the stimulation of adipogenesis and the recovery of insulin sensitivity in the target tissues[9]. The antidiabetic effects of thiazolidinediones are directly mediated through binding to PPARγ, thereby resulting in an active conformation of the receptor[10].

While the clinical benefits of PPARγ agonists in treating type 2 diabetes have been clearly demonstrated, the current generation of glitazones is associated with undesired side-effects, such as weight gain and edema[11]. Thus, there has been significant interest in the design of novel PPARγ-modulating drugs that retain efficacious insulin sensitizing properties while minimizing potential adverse effects, especially for long-term use.

SH00012671 is a core structure discovered in a high-throughput screening campaign directed towards PPARγ[12]. It displayed a relatively higher binding affinity to the receptor, but was almost inactive in cell-based assays. In this paper, we describe the design, synthesis, and biological evaluation of a series of analogues to SH00012671 with the aim of identifying novel PPARγ agonists.

## Materials and methods

**Reagents** Edetic acid, insulin, and dexamethasone were purchased from Sigma-Aldrich (St Louis, MO, USA). BRL49653 (rosiglitazone) was bought from Cayman Chemical Co (Ann Arbor, MI, USA). [$^3$H]BRL49653 (53 Ci/mmol) was obtained from American Radiolabeled Chemicals (St Louis, MO, USA), FlashPlate and flat-bottom Isoplate were from PerkinElmer (Boston, MA, USA), and the streptavidin-coated microbeads were from Amersham Biosciences UK (Buckinghamshire, England). The plasmids of human nuclear receptors used in this study were from Dr Xu SHEN of Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China) and Dr Sai-juan CHEN of Shanghai Institute of Hematology (Shanghai, China). Full-length PPARγ and RXRα were produced with a baculovirus expression system using fall armyworm (*Spodoptera frugiperda*) immature ovarian (IPLB-SF-21-AE) cells[13]. The PPRE reporter vector was purchased from Panomics (Redwood City, CA, USA), the Steady-Glo luciferase assay system was from Promega (Madison, WI, USA), the FuGene 6 transfection reagent was from Roche Diagnostics (Indianapolis, IN, USA), and the triglyceride (TG) detection kit was from Ningbo Asia-Pacific Biotechnology Co (Zhejiang, China).

**Chemistry** Figure 1 depicts the syntheses of the rhodanine derivatives designed based on SH00012671. Esterification of aromatic acid 1 by methanol and then hydrazinolysis of the resulting methyl ester provided the substituted benzoyl hydrazine 2. The reaction of 2 with carbon disulphide in ethanol in the presence of potassium hydroxide for 2 h resulted in the formation of potassium β-acyldithiocarbazinate 3, which was used without further purification. According to Tadashi and Masaki[14] when 3 was added to a solution of chloroacetic acid and saturated sodium carbonate, stirred at room temperature for 30 min, and acidified by aquous HCl, 3-benzoylamino rhodanine 4 would be separated out and then solidify. However, we found that when acidifying with aquous HCl, the intermediate (2-benzoyl-thiocarbazyl) mercaptoacetic acid, instead of 3-benzoyl-amino rhodanine 4, was separated out as evidenced by $^1$H-NMR (Nuclear magnetic resonance) and ESI (Electrospray ioniza-tion). This intermediate was thus refluxed in dioxane for 1 h to give 3-benzoylaminorhodanine 4, which could also be obtained by dissolving 3-amino rhodanine 5 in pyridine with benzoyl chloride. The final compounds 7–19 were obtained through the condensation of 4 with benzoaldehyde 6 by refluxing in glacial acetic acid in the presence of sodium acetate.

Next, we changed the substitutions on $R_4$ or $R_5$ while $R_1$ or $R_2$ was fixed with chloro (Cl), Br (bromo), or H (hydrogen; Figure 1). We used *p*-hydroxy benzaldehyde or *m*-hydroxy benzaldehyde 43 as the raw material. It was alkylated with different halogenated aralkyl 44 of which the chain length was changed from 1 to 3. Compounds 20–37 were obtained through the condensation of 4 with benzoaldehyde 45 by refluxing in glacial acetic acid in the presence of sodium acetate. Compounds 38–42 were made by substituting thio on the rhodanine with oxygen, in which the former rhodanine was refluxed with excessive chloroacetic acid in ethanol and water (Figure 1).

**Receptor-binding assay** Biotinylated PPRE (2 μL from a stock solution of 10 g/L) was mixed with the assay buffer (10 mL) containing fish sperm DNA (Shanghai Sangon Biotechnology Co, Shanghai, China; 10 μL from a 10 g/L stock solution) and 4 mg streptavidin-coated microbeads in a conical polypropylene centrifuge tube (Corning, Corning, NY, USA) and incubated overnight at 4 °C. The mixture was centrifuged for 10 min at 1 500×*g*. The supernatant was then removed and washed 3 times with 10 mL of the assay buffer. The reaction solution 10 mL containing 700 μg human PPARγ extract protein (70 mg/L), 47 μg human RXRα extract protein (4.7 mg/L), 10 nmol/L [$^3$H]BRL49653, and various concentrations of BRL49653 or test compounds were distributed to each well of the Isoplate (100 μL/well) and incubated at 4°C for 4 h before counting by the MicroBeta counte (PerkinElmer,

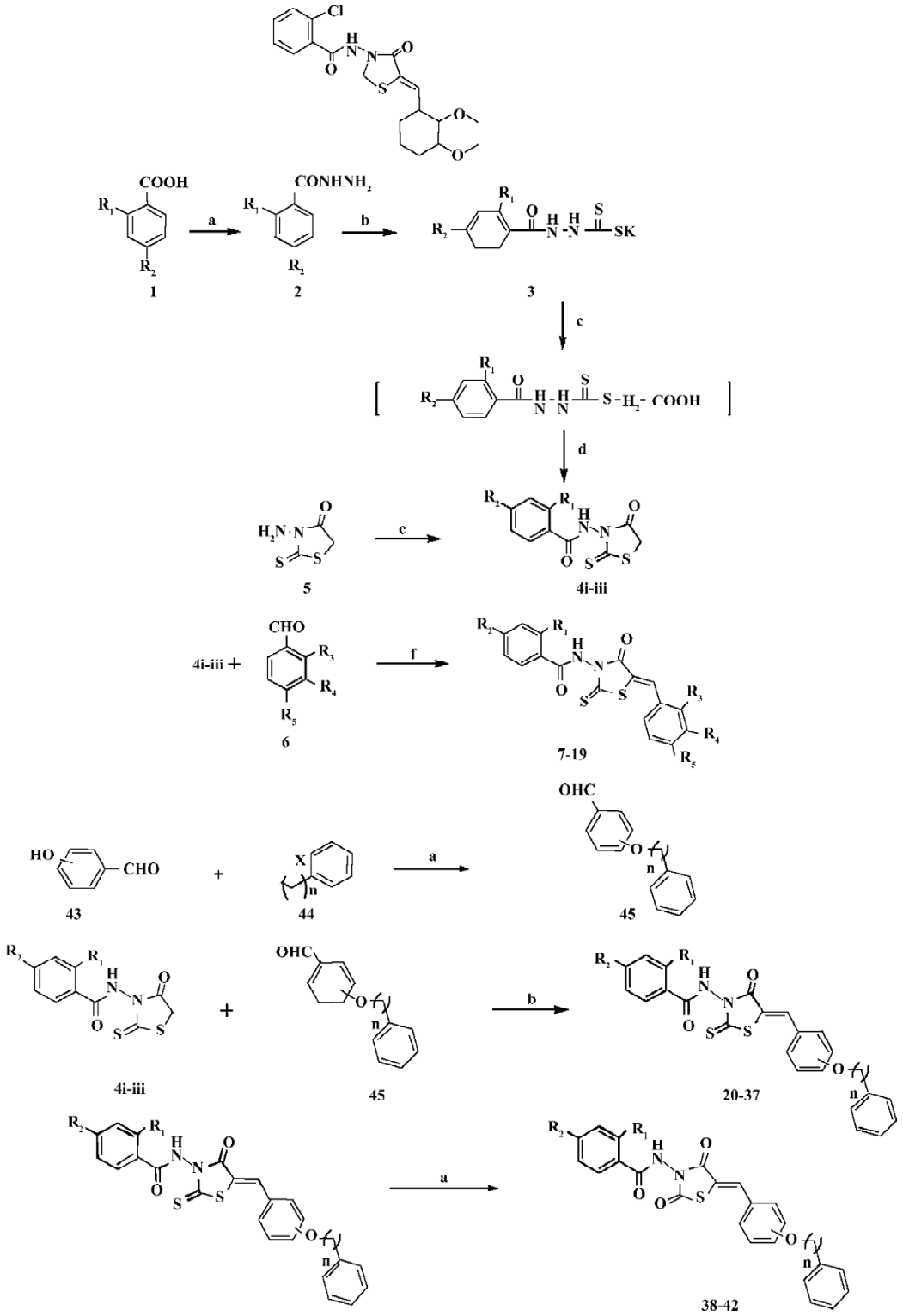

**Figure 1.** Top**,** structure of the initial hit SH00012671. Reagents and conditions. a, methanol containing a few drops of concentrated sulfuric acid, reflux for 18 h, and then the addition of 99% hydrazine hydrate followed by further reflux for 10 h; b, potassium hydroxide in ethanol before the addition of carbon disulphide; c, chloroacetic acid/saturated sodium carbonate; d, 1,4-dioxane reflux for 1 h; e, benzoyl chloride in pyridine; f, sodium acetate/acetic acid (HAc) reflux for 1 d; g, potassium carbonate and benzyl triethyl ammonium chloride (TEBA) in ethyl acetate or alcohol, reflux for 1 d; h, sodium acetate/HAc, reflux for 1 d; i, chloroacetic acid/ethanol/water.

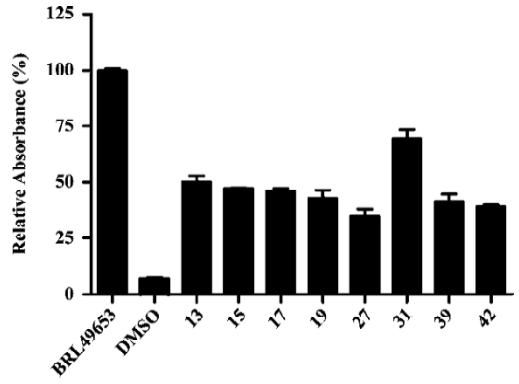


**Figure 2.** Effects of the rhodanine analogues on pre-adipocyte 3T3-L1 differentiation. The cells were induced to differentiate following stimulation with BRL49653 (0.5 μmol/L; control) or the rhodanine analogues (5 μmol/L). Triglyceride quantification was performed 7 days afterwards. Data presented are the means±SEM.

USA).

**Cotransfection assay** The CV-1 cells ($1\times10^6$ per well) were inoculated into 6 cm culture plates and incubated in 5% $CO_2$/air at 37 °C for 6 h. Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) and 10 mL/L penicillin-streptomycin (5 000 IU/mL and 5 000 mg/mL, respectively; Gibco, USA) was used as the culture medium. The cells were transiently transfected with PPRE, PPARγ, and RXRα expression vectors using FuGene 6. Sixteen hours after transfection, the cells were inoculated into 96-well plates, and following 2 h incubation in 5% $CO_2$/air at 37°C, the test compounds were added and the cells were further incubated for 48 h. Luciferase assay substrate (Promega, USA) was introduced to each well and the intensity of emitted luminescence was determined using EnVision (PerkinElmer, USA). PPARγ transcriptional activity was expressed as the relative luminescence intensity to that of the control. For antagonist effects, the test compounds were added to the cells 30 min before the addition of BRL49653 (1 μmol/L) and incubated for 24 h. Luciferase activity was determined as above.

**Adipogenesis assay** 3T3-L1 cells (20 000 cells/well) were planted onto 24-well culture plates and maintained for 2 d after reaching confluence. The culture medium (DMEM, Gibco, USA) was then changed with the differentiation medium (DMEM containing 10% FBS, 1 μmol/L dexamethasone, and 1 μg/mL insulin). The cells were treated with BRL49653 (0.5 μmol/L) or the test compounds (5 μmol/L) for 2 d. Following the change of the medium containing the compounds, the cells were cultured for an additional 3 d. The differentiation medium was replaced by the adipocyte growth medium (DMEM supplemented with 10% FBS and 1 μg/mL insulin) and incubated for a further 2 d. The cells were washed with phosphate-buffered saline and lysed by 0.1% Nonidet P-40 (Sigma-Aldrich, USA). The supernatant of the preparation was examined by the TG detection kit at a wavelength of 500 nm on VERSA$^{max}$ (Molecular Devices, Sunnyvale, CA, USA).

**Data analysis** Data were analyzed using GraphPad Prism software (GraphPad, San Diego, CA, USA). Non-linear regression analyses were performed to generate dose–response curves. $K_i$ values were calculated from the half maximal inhibitory concentration ($IC_{50}$) using the equation of Cheng and Prusoff[15]:

$$K_i = \frac{IC_{50}}{1+\frac{[\text{radioligand}]}{K_d}}$$

## Results

A group of 16 analogues to SH00012671 were synthesized initially (Figure 1) with various substitutions at $R_1$–$R_5$, of which only compounds 13, 15, and 17 showed submicromolar binding affinities to PPARγ, but not as potent as the initial hit (Table 1). However, they displayed some agonist activities with less than 50% efficacies when compared to rosiglitazone in the cotransfection assay (see Table 4 below). We next synthesized another group of 18 rhodanine derivatives by making small modifications on the $R_1$ or $R_2$ (substitution of H with Cl or Br in *meta*- or *para*-site) and with different chain lengths. In this case (Table 2), significant improvement in PPARγ binding was observed among one-half of these compounds (21, 23, 25, 27, 29, 31, 33, 35, and 37), to the extent that some of them displayed $K_i$ values similar to that of rosiglitazone (21, 27, 31, and 37). In the cotransfection assay, compounds 21, 25, 31, and 37 exhibited similar half maximal effective concentration ($EC_{50}$) values compared to rosiglitazone with lower efficacies (23%–69%; see Table 4 below). When tested in the antagonist mode, compounds 27, 31, 29, and 37**,** with good binding affinities and modest transactivation efficacies, were unable to suppress luciferase activity induced by rosiglitazone (1 μmol/L).

To reduce intrinsic cytotoxicities associated with this class of molecules, the atom thio on the rhodanine was changed to oxygen (Figure 1). This alteration led to a decrease in receptor-binding affinities (Table 3) while the modified analogues maintained efficacies in the cotransfection assay, with the exception of compound 42 (42 *vs* 37; Table 4).

Representative compounds from each of the above 3 groups were evaluated in the pre-adipocyte differentiation assay. All of the 8 compounds tested were able to induce adipogenesis in 3T3-L1 cells with compound 31 being the

**Table 1.** PPAR γ binding affinities of the rhodanine derivatives.

| Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | hPPARγ binding $K_i$ (nmol/L) |
|---|---|---|---|---|---|---|
| 4i | Cl | H | | | | NA |
| 4ii | H | OH | | | | NA |
| 4iii | H | H | | | | NA |
| 7 | Cl | H | $OCH_3$ | H | $OCH_3$ | NA |
| 8 | H | OH | $OCH_3$ | H | $OCH_3$ | NA |
| 9 | Cl | H | H | $OCH_3$ | 2-furancarboxy | NA |
| 10 | H | OH | H | $OCH_3$ | 2-furancarboxy | NA |
| 11 | Cl | H | H | $OCH_2CH_3$ | $OCH_2CONH_2$ | NA |
| 12 | H | H | H | $OCH_2CH_3$ | $OCH_2CONH_2$ | NA |
| 13 | H | H | H | benzyloxy | $OCH_3$ | 767.2 |
| 14 | H | OH | H | benzyloxy | $OCH_3$ | NA |
| 15 | Cl | H | H | benzeneethoxy | $OCH_3$ | 321.2 |
| 16 | H | H | H | benzeneethoxy | $OCH_3$ | NA |
| 17 | Cl | H | H | $OCH_3$ | benzeneethoxy | 689.3 |
| 18 | H | H | H | $OCH_3$ | benzeneethoxy | NA |
| 19 | H | OH | H | $OCH_3$ | benzeneethoxy | 3789.5 |
| SH00012671 | | | | | | 186.7 |
| Rosiglitazone | | | | | | 47.4 |

The compounds were evaluated for their binding abilities to PPARγ in competition with [$^3$H]BRL49653. Each value represents the mean of the triplicate determinations obtained in the $K_i$ studies. NA, not active.

**Table 2.** PPARγ binding affinities of the rhodanine derivatives.

| Compound | $R_1$ | $R_2$ | *m* or *p* | *n* | hPPARγ binding $K_i$ (nmol/L) |
|---|---|---|---|---|---|
| 20 | H | H | *p* | 1 | 572.1 |
| 21 | H | H | *m* | 1 | 58.4 |
| 22 | H | H | *p* | 2 | 28800 |
| 23 | H | H | *m* | 2 | 118.5 |
| 24 | H | H | *p* | 3 | 9071 |
| 25 | H | H | *m* | 3 | 85.3 |
| 26 | Cl | H | *p* | 1 | 826.2 |
| 27 | Cl | H | *m* | 1 | 30 |
| 28 | Cl | H | *p* | 2 | 598.7 |
| 29 | Cl | H | *m* | 2 | 76 |
| 30 | Cl | H | *p* | 3 | 421.7 |
| 31 | Cl | H | *m* | 3 | 39 |
| 32 | H | Br | *p* | 1 | NA |
| 33 | H | Br | *m* | 1 | 78.3 |
| 34 | H | Br | *p* | 2 | 1432 |
| 35 | H | Br | *m* | 2 | 76 |
| 36 | H | Br | *p* | 3 | 3261 |
| 37 | H | Br | *m* | 3 | 34 |
| Rosiglitazone | | | | | 47.4 |

The compounds were evaluated for their binding abilities to PPARγ in competition with [$^3$H]BRL49653. Each value represents the mean of the triplicate determinations obtained in the $K_i$ studies. NA, not active.

**Table 3.** PPARγ binding affinities of the thiazodinediones.

| Compound | $R_1$ | $R_2$ | *m* or *p* | *n* | hPPARγ binding $K_i$ (nmol/L) |
|---|---|---|---|---|---|
| 38 | Cl | H | *p* | 3 | 885.1 |
| 39 | Cl | H | *m* | 3 | 123.6 |
| 40 | Cl | H | *m* | 2 | 106.7 |
| 41 | Cl | H | *m* | 1 | 137.5 |
| 42 | H | Br | *m* | 3 | 155.2 |
| Rosiglitazone | | | | | 47.4 |

The compounds were evaluated for their binding abilities to PPARγ in competition with [$^3$H]BRL49653. Each value represents the mean of the triplicate determinations obtained in the $K_i$ studies.

best inducer relative to BRL49653, consistent with its performance in both the PPARγ-binding and cotransfection assays (Figure 2; Tables 2, 4).

## Discussion

In a previous study[12], we reported the discovery of 12 hit compounds with selective PPARγ binding properties, of which 2 belong to the thiazodinedione class, including SH00012671 with a relatively lower $IC_{50}$ value (0.2 μmol/L).

**Table 4.** Activity of compounds in cell-based co-transfection assay.

| Compound | hPPARγ agonist | | Compound | hPPARγ agonist | |
|---|---|---|---|---|---|
| | $EC_{50}$ (μmol/L) | Eff. (%) | | $EC_{50}$ (μmol/L) | Eff. (%) |
| 13 | 27.0±1.5 | 36.5±3.1 | 33 | >100 | 25.3±1.5 |
| 15 | 24.5±4.5 | 53.4±10 | 37 | 0.9±0.1 | 22.9±7.2 |
| 17 | 11.7±3.8 | 43.9±4.5 | 38 | 26.5±7.3 | 14.4±2.8 |
| 19 | 1.7±0.1 | 17.3±3.1 | 39 | 0.8±0.1 | 49.0±4.8 |
| 21 | 1.1±0.1 | 23.0±2.5 | 40 | 2.9±1.7 | 36.1±0.7 |
| 23 | >100 | 15.8±1.1 | 41 | 2.4±1.4 | 18.1±6.4 |
| 25 | 0.8±0.5 | 30.3±1.1 | 42 | 4.2±1.1 | 49.0±1.5 |
| 27 | 2.4±0.4 | 28.5±6.8 | SH00012671 | 2.1±0.8 | 11.0±4.0 |
| 29 | 2.2±0.4 | 38.6±6.6 | Rosiglitazone | 0.7±0.1 | 100 |
| 31 | 1.1±0.6 | 68.8±2.3 | | | |

The agonist activities of the compounds were assessed in the CV-1 cells co-transfected with PPARγ, RXRα and PPRE-driven luciferase genes. Values shown represent means±SEM obtained from at least two independent experiments. Eff., efficacy using rosiglitazone as control (100%).

When tested for its effects on cells, it acted either as a poor activator in the cotransfection assay (Table 4) or was inactive in stimulating pre-adipocyte (3T3-L1) differentiation. We thus started some limited chemistry efforts by modifying the rhodanine core structure to better understand the molecular basis of this discrepancy. Three compounds synthesized initially (4i, 4ii, and 4iii) did not bind to PPARγ at all (Table 1), suggesting that benzylidene is required for receptor-binding properties. Therefore, the 5-substituted benzylidene group was introduced, and simple substitutions at $R_3$, $R_4$ and $R_5$ with methoxy, ethoxy, 2-furancarboxy, or aminocarbonylmethoxy did not change the binding situation (Table 1). However, when $R_4$ or $R_5$ was substituted with benzyloxy or benzeneethoxy, 4 derivatives (13, 15, 17, and 19) possessed both receptor-binding and PPARγ agonist properties (Tables 1, 4). Hydroxy in $R_2$ (14) abolished the receptor-binding ability of 13, while chloride in $R_1$ may be responsible for PPARγ agonist activity (15 *vs* 16 and 17 *vs* 18; Tables 1, 4). Cytotoxicity was noted in the cells treated with 19 (data not shown) and this might have led to the lower agonist efficacy observed (Table 4).

In the receptor-binding assay, we found that the removal of methoxy in 5-substituted benzylidene would markedly improve binding activity (Table 2). Among the 18 compounds in this group, *meta*-substitution behaved better than *para*-substitution (21 *vs* 20, 23 *vs* 22, 25 *vs* 24, 27 *vs* 26, 29 *vs* 28, 31 *vs* 30, 33 *vs* 32, 35 *vs* 34, and 37 *vs* 36; Table 2). The chain length also affected the receptor-binding activity. Compounds with $n$=1 or 3 in the chain length bound to PPARγ more potently than compounds with $n$=2 in the chain length (21, 25 *vs* 23 and 27, and 31 *vs* 29; Table 2). When $R_1$ or $R_2$ was substituted with halogen, the receptor-binding activity was also improved (27 *vs* 21; 29, 35 *vs* 23; 31, and 37 *vs* 25; Table 2). In the PPARγ cotransfection assay, both the chain length and halogen substitution affected the agonist properties. The activities of compounds with $n$=3 in the chain length were better than compounds with $n$=1 or 2 in the chain length (25, 31, 37, and 39; Table 4). The halogen (eg Cl) group also increased the cotransfection activity (27, 29, and 31; Table 4).

To reduce intrinsic cytotoxicities, the atom thio on the rhodanine was changed to oxygen. This alteration led to a decrease in receptor-binding affinities while modified analogues generally maintained efficacies in cell-based assays, except compound 42 (42 *vs* 37; Table 4). The underlining mechanism of this phenomenon deserves further investigation.

According to Amit and colleagues[16], a 3-D quantitative structure–activity relationship model of PPARγ agonist thiazolidine-2,4-dione has 3 biophoric centers, A, B, and C, corresponding to carbonyl oxygen of thiazolidine-2,4-dione, sulphur atom of thiazolidine-2,4-dione, and the oxygen atom attached to the phenyl ring, respectively. These are electron-rich sites capable of donating electrons and may be involved in electrostatic, ionic, and π-π interactions. Site C is essential to the agonistic action of PPARγ, whereas the carbonyl group of thiazolidinediones (site A) may be responsible for the formation of hydrogen bonds with 2 histidine residues, His 323 and 449, of PPARγ. This information could certainly help us understand why the benzyloxy, phenylethoxy, and phenylpropoxy groups are important to the PPARγ agonists described in the present study.

It is conceivable that the modest transactivation efficacies observed with these compounds (68.8% maximum compared to rosiglitazone) were due to partial agonist effects.

The fact that they did not behave as antagonists in the same assay system suggests that their potencies at PPARγ should be further improved. Likewise, we noted some inconsistencies relative to transactivation versus adipogenesis assays in terms of agonist effects. Although some agonists have relatively potent agonistic activities in the cotransfection assay (eg 39), they acted poorly on pre-adipocyte differentiation. This is in agreement with previous studies on a group of PPARγ agonists, including KR-62980, GW0072, FMOC-L-leucine, PAT5A and nTZDpa[17]. Biochemical and structural studies with PPARγ revealed that different ligands may occupy the PPARγ ligand-binding pocket in different manners, thereby inducing different allosteric changes in its conforma-tion. Such alterations would result in different kinds of interactions between receptor and cofactor proteins, such as steroid receptor co-activator 1 (SRC1), nuclear receptor co-activator TIF2, cAMP response element (CRE) binding protein (CBP), TRAP220/DRIP205, nuclear receptor co-repressor (NcoR), and the silencing mediator of the retinoid and thyroid hormone receptors (SMRT)[18]. Therefore, the effect of PPARγ modulators on the downstream adipogenesis and glucose uptake will depend on the context of interaction between the ligand and the receptor.

In summary, 5-benzylidene was required for the receptor-binding properties. Simple substitutions on benzylidene with methoxy, ethoxy, 2-furancarboxy, or amino-carbonylmethoxy did not change the binding affinity. However, when benzylidene was substituted with aralkyl, both receptor-binding and PPARγ agonist activities were demonstrable. *Meta*-substitution was better than that of *para*. The chain length also affected the PPARγ-binding and agonist activities. The best chain length studied was 3. Finally, the substitution of halogen in $R_1$ or $R_2$ improved binding activity. Of the analogues studied, compound 31 exhibited about 70% the efficacy exerted by BRL49653 in both the cotransfection and pre-adipocyte differentiation assays, in addition to its weak agonist action on hPPARα (data not shown). High receptor binding potency ($K_i$=47.4 nmol/L) and less than optimal agonist efficacy ($EC_{50}$=700 nmol/L) relative to rosiglitazone create an opportunity to use rhodanine derivatives as a new scaffold in further understanding the molecular mechanism of agonism at PPARs.

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