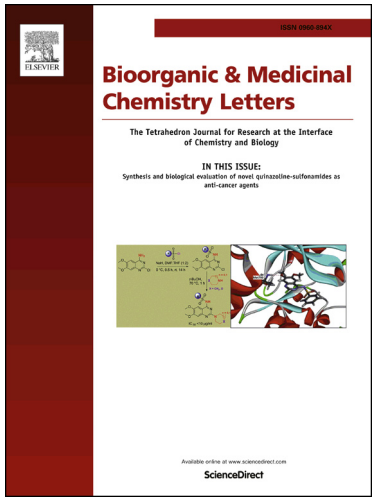

Structure-activity relationships of rosiglitazone for peroxisome proliferator-activated receptor gamma transrepression

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Structure-activity relationships of rosiglitazone for peroxisome proliferator-activated receptor gamma transrepression

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Abstract

Anti-inflammatory effects of peroxisome proliferator-activated receptor gamma (PPRAγ) ligands are thought to be largely due to PPARγ-mediated transrepression. Thus, transrepression-selective PPARγ ligands without agonistic activity or with only partial agonistic activity should exhibit anti-inflammatory properties with reduced side effects. Here, we investigated the structure-activity relationships (SARs) of PPARγ agonist rosiglitazone, focusing on transrepression activity. Alkenic analogs showed slightly more potent transrepression with reduced efficacy of transactivating agonistic activity. Removal of the alkyl group on the nitrogen atom improved selectivity for transrepression over transactivation. Among the synthesized compounds, **3l** exhibited stronger transrepressional activity (IC_{50}: 14 μM) and weaker agonistic efficacy (11%) than rosiglitazone or pioglitazone.

Nuclear receptors (NRs) are ligand-dependent transcription factors that are involved in control of diverse biological functions, including reproduction, differentiation, homeostasis, and immunity. Upon binding of an agonist to the ligand-binding domain (LBD), the NR heterodimer binds to NR response elements (NRE) in promoter regions of specific genes, and helix 12 in the LBD adopts a conformation that closes the ligand binding pocket and forms a groove to which coactivators can bind, allowing gene transcription to occur. This ligand-mediated activation of NRE-dependent gene transcription is called transactivation (Fig. 1a). On the other hand, binding of antagonists results in a conformation that favors binding of corepressors.

Among the NRs, peroxisome proliferator-activated receptors (PPARs) include three subtypes, PPARα, PPARγ and PPARδ. PPARγ plays a critical role in the differentiation of preadipocytes to adipocytes, promotes lipid storage, and enhances glucose disposal to peripheral tissues through binding to PPAR response elements (PPRE) in the promoter region of target genes. Thiazolidinediones (TZDs), such as pioglitazone (**1**) and rosiglitazone (**2**), are PPARγ agonists used clinically to treat type 2 diabetes by enhancing insulin sensitivity in target tissues and lowering glucose and fatty acid levels (Fig. 2). However, despite their proven benefits, clinical application of these drugs has been plagued by adverse effects, such as weight gain, increased rate of bone fractures, fluid accumulation, and pulmonary edema, leading to increased frequency of congestive heart failure.[1,2] These adverse effects are at least partially associated with transactivation of PPARγ.[1,3] Therefore, partial agonists have been developed with the aim of retaining the

beneficial effects while diminishing the adverse effects of full agonists.[4,5] Indeed, phase II clinical trials showed that metaglidasen, a PPARγ partial agonist, significantly improved metabolic parameters without the side effects of fluid retention/edema or weight gain.[2]

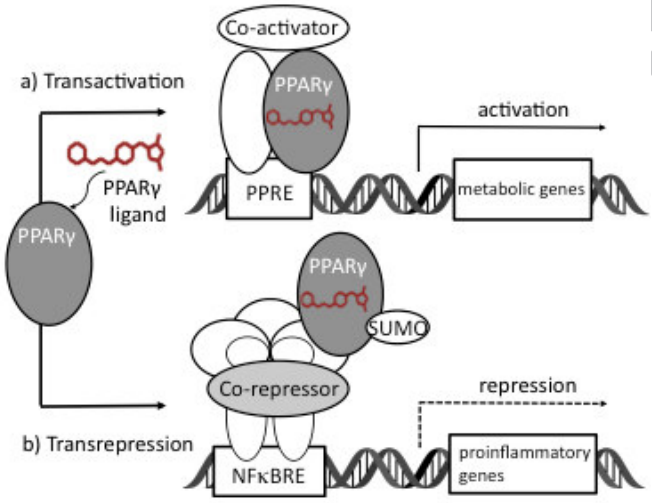


Fig. 1. Proposed mechanisms of a) transactivation and b) transrepression of PPARγ.

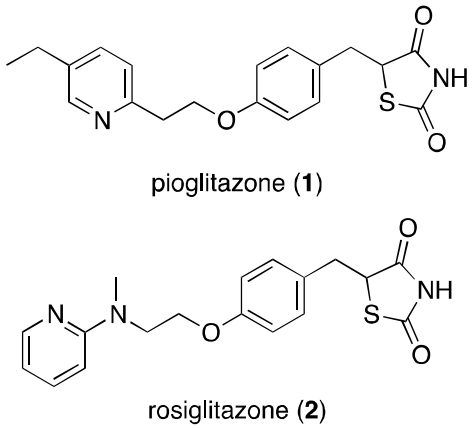


Fig. 2. Chemical structures of PPARγ agonists.

Recent studies have revealed that PPARγ also functions in the regulation of inflammation, by transrepression of proinflammatory genes. For example, clinical efficacy of TZDs on asthma[6] and rheumatoid arthritis[7] has been reported. Several PPARγ agonists inhibit the expression of proinflamatory mediators such as interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS) and tumor necrosis factor α (TNFα) in macrophages stimulated by bacterial infection, TNFα, or lipopolysaccaride (LPS) in vitro.[8,9,10] In the absence of ligand-bound PPARγ, other transcription factors such as NF-κB or AP-1 are able to transactivate target genes through binding to NF-κB response element (NFκBRE) or AP-1 response element in the promoter regions of the genes. In contrast, SUMOylation of PPARγ induced by binding of a PPARγ ligand stabilizes the transrepressional complex, including PPARγ and co-repressor, and prevents NF-κB and AP-1 from binding the target genes, thereby inhibiting expression of these genes[11] (Fig. 1b). This indirect mechanism of action is referred to as transrepression. Because the promoter regions of TNF α, IL-6, iNOS, and other proinflammatory genes do not contain PPRE, the mechanisms of the transactivating and transrepressional actions are clearly different.

Transrepression-selective PPARγ ligands (without agonistic activity or with only partial agonistic activity) should exhibit anti-inflammatory properties with reduced side effects. However, the SARs of rosiglitazone (**2**) focusing on PPARγ transrepression and those of transrepression-selective PPARγ ligands have not yet been fully investigated.[12] Benzothiazolylamino analogs **4f** and **4g** were reported as PPARγ full agonists with

transrepressional activity.[13] On the other hand, transrepressional activities of other NRs, glucocorticoid receptor (GR) and liver X receptor (LXR),[14] have been reported.[15] Separation of transactivating and transrepressional activities was first investigated in the case of GR (Fig. 3).[16,17,18] We have also reported two distinct chemical classes of transrepression-selective LXR ligands.[19,20] We hypothesized that the transactivating and transrepressional activities of PPARγ are also separable. Here, we report SARs of PPARγ agonist rosiglitazone (**2**), focusing on transrepression.

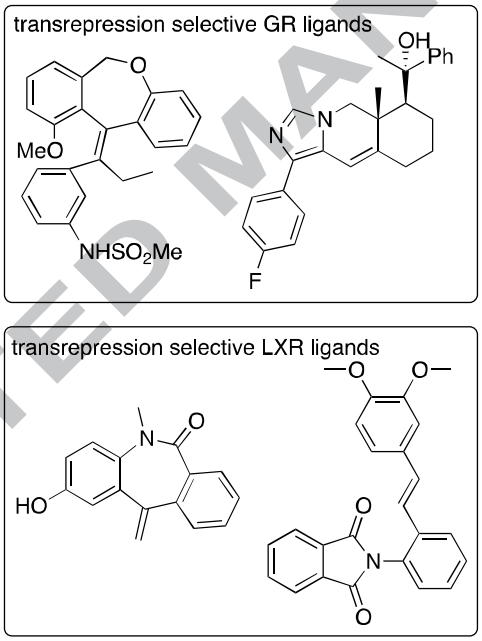


Fig. 3. Transrepression-selective GR and LXR ligands

We selected rosiglitazone (**2**) as a lead compound, because its transrepressional activity has been extensively investigated, including clinical studies. Surprisingly, however, there is relatively little information on SARs for the transactivating activity (PPARγ-mediated

transcription) of rosiglitazone,[21,22,13,23] probably because the molecular target of TZDs was unknown when these compounds were discovered. Acidic hydrogen on the thiazolidinedione ring of **2** forms a hydrogen bond with the phenol group of Tyr473 on helix 12 (Fig. 4a), and this interaction is important for PPARγ agonistic activity and receptor binding.[23] Thus, this acidic hydrogen on the thiazolidinedione ring was fixed with the aim of maintaining PPARγ binding affinity. On the other hand, the pocket to which the pyridyl group binds is relatively wide and includes unoccupied space (Fig. 4b). Therefore, we focused on the methylpyridylamino moiety, and designed several heterocyclic analogs including a benzothiazole analog.[13] In addition, introduction of a double bond at the 5-position of the thiazolidinedione was planned to remove the asymmetric carbon, which is prone to isomerization.[24]

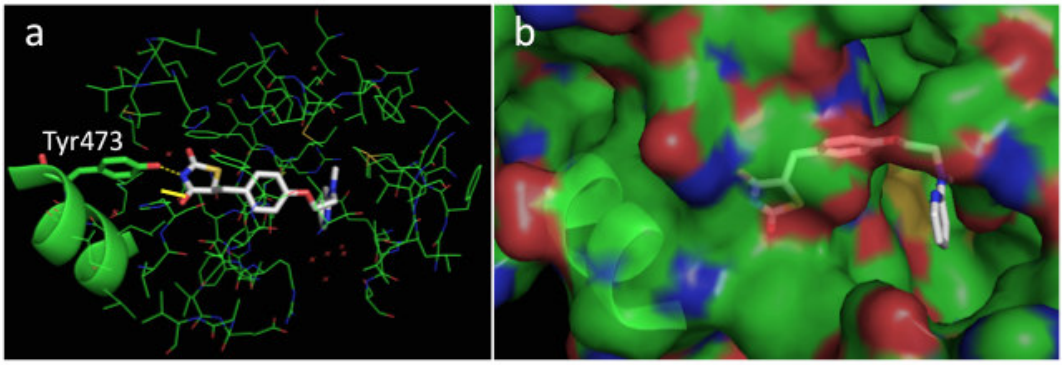
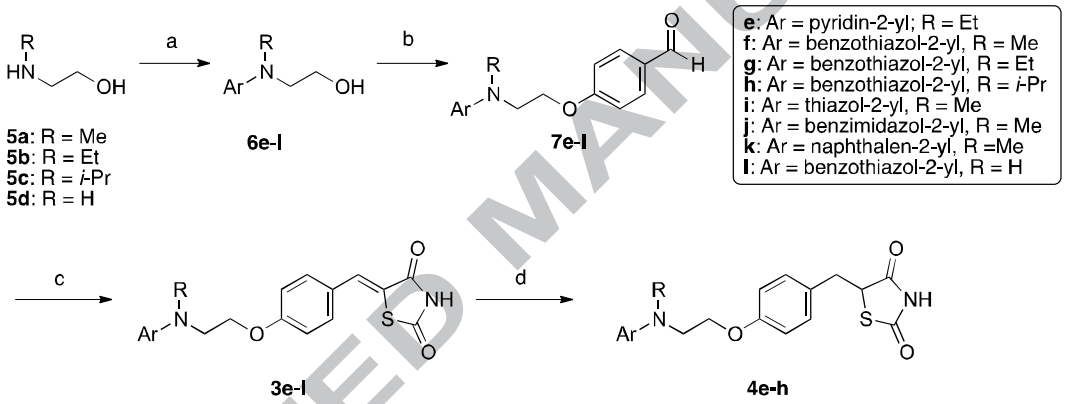


Figure 4. X-Ray crystal structure of PPARγ (green) and rosiglitazone (white stick) complex (PDB ID: 1FM6). Helix 12 is shown in cartoon form. a) Only amino acids within 8 angstroms of the compound are shown. The important hydrogen bond is shown as a yellow line. Tyr473 is shown in stick form. b) The binding pocket of the pyridyl group of rosiglitazone.

Rosiglitazone analogs **3e**-**l** and **4e**-**h** were synthesized as outlined in Scheme 1. Nucleophilic substitution of aryl chlorides with aminoethanols **5a**-**d** afforded **6e**-**l**. Subsequent nucleophilic substitution of 4-fluorobenzaldehyde with alcohols **6e**-**l** yielded **7e**-**l**. Aldol reaction of aldehydes **7e**-**l** and 2,4-thiazolidinedione gave **3e**-**l**. Reduction of **3e**-**l** with $CoCl_2$·DMG (dimethylglyoxime) and $NaBH_4$[24] gave **4e**-**h**.

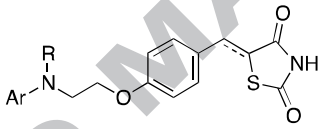


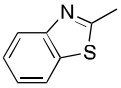
Scheme 1. Reagents and conditions: a) ArCl, 120 ºC, 6-99%, b) 4-fluorobenzaldehyde, KO*t*-Bu, DMF, r.t., 9-78%, c) 2,4-thiazolidinedione, pyrrolidine, MeOH, 45 ºC, 38-74%, d) $CoCl_2$·DMG, $NaBH_4$, MeOH, 35 ºC, 20-33%.

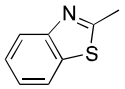
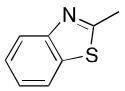
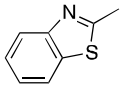
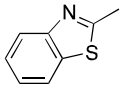
Cell-based transrepressional activity was evaluated with an NF-κB reporter system in HEK293 (human embryonic kidney) cells transiently expressing human PPARγ under stimulation with TNFα.[25] The positive controls pioglitazone (**1**) and rosiglitazone (**2**) showed transrepressional activity with IC_{50} values of 22 μM (Table 1). PPARγ-transactivating agonistic activity was measured with the Gal4N-human PPARγ

LBD reporter system in HEK293 cells, as previously reported.[26] Percent efficacy is estimated as the maximal stimulatory response in relation to the maximal activity of **1**. The positive controls **1** and **2** showed transactivating agonistic activity with EC_{50} values of 1.1 μM (Table 1). In both these assays, cells were assayed for luciferase activities and β-galactosidase activities after 7 h (for transrepression) and 24 h (for transactivation) treatment with test compounds. None of our compounds reduced β-galactosidase activity under these conditions, suggesting that none of them is toxic at the concentrations tested.

Table 1. SARs of rosiglitazone



Compound	R	Ar	Bond[a]	Transactivating agonistic activity		Transrepression
				EC_{50} (μM)	% efficacy[b]	IC_{50} (μM)
1	-	-	-	1.1	100	22
2	Me		s	1.1	94	22
4e	Et		s	0.54	110	21
3e	Et		d	0.36	38	15
4f	Me		s	0.057	120	20

3f	Me		d	0.31	40	13
4g	Et		s	0.052	130	25
3g	Et		d	0.82	44	17
4h	*i*-Pr		s	0.53	92	20

[a] s, single bond; d, double bond. [b] Emax values are given relative to positive control **1**.

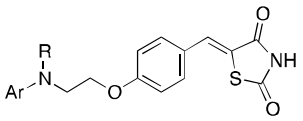
As regards transactivating agonistic activity, we found that benzothiazolyl analogs **4f** and **4g** showed 19- and 10-fold increased EC_{50} values compared with pyridyl analogs **2** and **4e**, respectively, whereas maximum efficacy was almost the same, in accordance with the previous report.[13] Next, we examined the effect of the alkyl group on transactivation. Ethyl analogs **4e** and **4g** showed more potent transactivating agonistic activity (EC_{50}) than the methyl analogs **2** and **4f**, and *i*-Pr analog **4h**. On the other hand, transrepressional activity was roughly equal among these analogs **2** and **4e**-**g** (IC_{50} in the range of 20-25 μM). These results indicate that substituents on the amino group influence transactivating activity more sensitively than transrepressional activity.

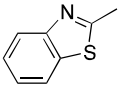
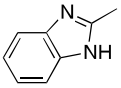
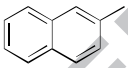
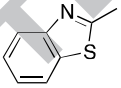
Alkenic analogs **3e**-**g** showed slightly increased transrepressional activity compared with the saturated analogs **4e**-**g**, and **3f** showed an IC_{50} value of 13 μM. On the other hand, EC_{50} values of **3e**-**g** were decreased compared with the saturated analogs **4e**-**g**. In addition,

3e-**g** exhibited weak efficacy of around 40% compared to the full agonist pioglitazone. These results indicate that introduction of the double bond into the 5-position of thiazolidindione increases the selectivity for transrepression over transactivation.

These results prompted us to synthesize alkenic analogs bearing other heterocycles. Thiazol-2-yl **3i**, benzoimidazol-2-yl **3j**, and 2-naphthyl **3k** analogs showed decreased EC_{50} values and efficacy compared with **3f** (Table 2). But these analogs **3i**-**k** also showed diminished transrepressional activity (IC_{50} in the range of 38-42 μM). On the other hand, a smaller alkyl substituent (**3f**) tends to show slightly increased transrepressional activity with lower efficacy compared to **3g** (Table 1). Therefore, we synthesized non-substituted analog **3l** in an attempt to improve the selectivity. In the range of 0.03-30 μM, compound **3l** exhibited a more potent EC_{50} value (0.053 μM) but lower efficacy of 11% than methyl analog **3f**. On the other hand, the transrepressional activity of **3l** (14 μM) was comparable to that of **3f**. It has been proposed that the molecular mechanisms of PPAR**γ** partial agonism are stabilization of helix 3 and β sheet in the PPARγ LBD, based on X-ray crystallographic studies, docking studies, and amide H/D exchange kinetics (Fig. 5).[27,28] The molecular mechanism of PPARγ partial agonism of **3l** is unclear, but the SAR described here indicates that the substituent on the amino group and the location of the aromatic heterocycle near helix 3 and the β sheet might be important for partial agonism.

Table 2. SARs of benzylidene-thiazolidine-2 4-diones



Compound	R	Ar	Transactivating agonistic activity		Transrepression
			EC_{50} (μM)	% efficacy[a]	IC_{50} (μM)
3f	Me		0.31	40	13
3i	Me		0.70	31	42
3j	Me		3.4	12	38
3k	Me		1.6	8.7	40
3l	H		0.053	11	14

[a] Emax values are given relative to the positive control **1**.

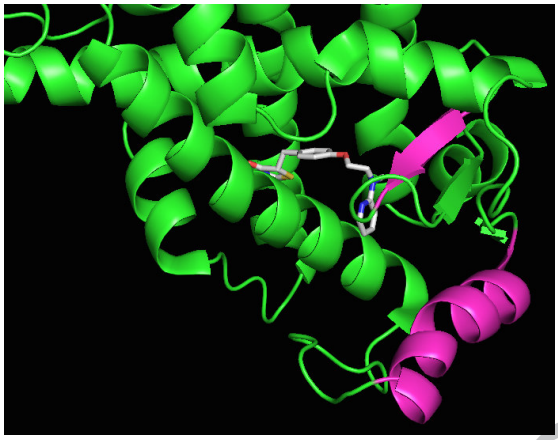


Fig. 5. Location of helix 3 (magenta) and the β sheet (magenta) proposed to be important for partial agonism. PPARγ: green, rosiglitazone: white stick (PDB ID: 1FM6).

TNFα transmits inflammatory signaling intracellularly through the TNFα receptor in the plasma membrane, and this induces degradation of the transrepressional complex on the promoter region of proinflammatory genes (e.g., IL-6 and iNOS), thereby activating gene transcription. So, inhibitory activity toward TNFα-induced NF-κB inhibition is also exhibited by TNF receptor antagonists and TNFα-signaling inhibitors. Therefore, we examined whether the NF-κB luciferase inhibitory activity of our compounds was PPARγ-dependent. As shown in Figure 6, inhibition of TNFα-induced NF-κB luciferase by **3l** (IC_{50}: 29 μM) was partially abolished in the absence of transfection of PPARγ. This partial abolition of NF-κB inhibitory activity would be due to endogenous expression of PPARγ in HEK293 cell lines. This result indicates that PPARγ contributes to the inhibition of NF-κB luciferase by **3l** at least in part, although we cannot exclude the

possibility that our compounds also inhibit TNFα-induced NF-κB transcription through other mechanisms.

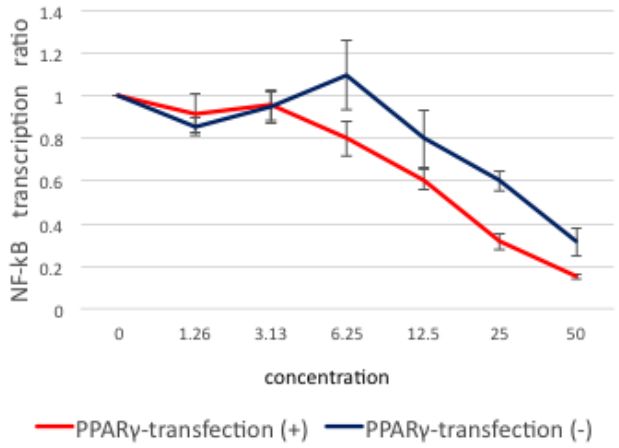


Fig. 6. PPARγ dependency of NF-kB-inhibitory activity of compound **3l**. Red: PPARγ transfection (+); blue: PPARγ transfection (-).

To examine the separability of transactivation and transrepression, we plotted –Log (IC_{50}) against –Log (EC_{50}) (Fig. 7). Neither –Log (EC_{50}) nor Emax was correlated to –Log (IC_{50}), (R^2 = 0.2 and 0.07), despite the similarity of chemical structures. This result indicates that strong transactivation is not necessary for strong transrepression. Thus, it can be anticipated that transrepression and transactivation should be separable by chemical modification of PPARγ ligands.

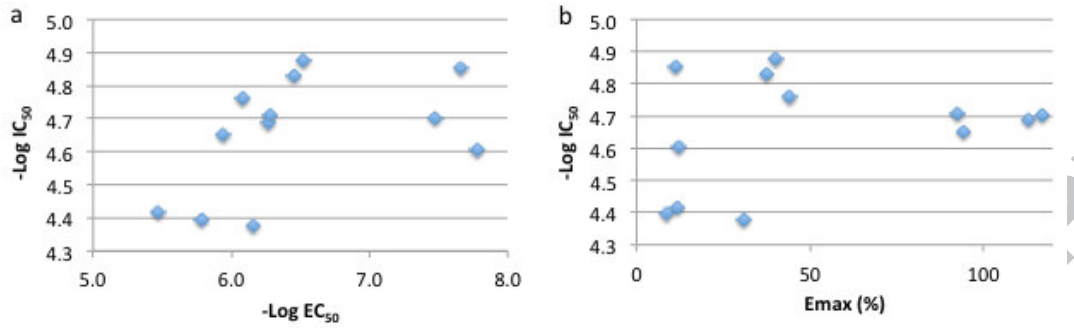


Fig. 7. Correlation between transactivation and transrepression among risoglitazone analogs. (a) –Log (IC_{50}) and –Log (EC_{50}). (b) –Log (IC_{50}) and Emax.

In summary, we investigated SARs of the PPARγ agonist rosiglitazone, focusing on transrepressional activity. Among the synthesized compounds, **3l** showed PPARγ transrepressional activity with low transactivating efficacy (11%). Therefore, this compound might be a promising candidate for exhibiting anti-inflammatory properties with reduced side effects. Biological evaluations of **3l** and further chemical modifications to improve selectivity are in progress.

ACKNOWLEDGMENT

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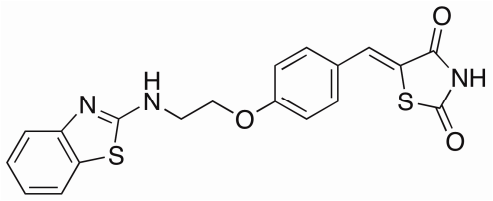

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