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Design, synthesis, and structure–activity relationship of carbamate-tethered aryl propanoic acids as novel PPARα/γ dual agonists

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Abstract—We have developed a new class of PPARα/γ dual agonists, which show excellent agonistic activity in PPARα/γ transactivation assay. In particular, (*R*)-**9d** was identified as a potent PPARα/γ dual agonist with EC_{50}s of 0.377 μM in PPARα and 0.136 μM in PPARγ, respectively. Interestingly, the structure–activity relationship revealed that the stereochemistry of the identified PPARα/γ dual agonists significantly affects their agonistic activities in PPARα than in PPARγ.

As members of the nuclear hormone receptor super family of ligand-activated transcription factors, the Peroxisome proliferator-activated receptors (PPARs), transduce a wide variety of signals to regulate glucose and lipid homeostasis, sensitivity to insulin, cell proliferation/differentiation, and innate and adaptive immune responses. Up to date, three isotypes, which are designated PPARα, PPARγ, and PPARδ, have been identified and cloned.[1] Recently, the rapid progress in functional analysis of these receptors prompted the scientific and pharmaceutical attention to PPARs due to their biological importance. In particular, the PPAR agonists have intensively been studied as a therapeutic target of type 2 diabetes. As rosiglitazone is a known PPARγ agonist as one of the representative insulin sensitizing thiazolidinediones (Fig. 1),[2] activation of PPARγ induces adipocyte differentiation, lipid uptake, and insulin sensitization, while PPARα regulates lipid homeostasis via control of fatty acid catabolism.[3] The PPARα agonists such as fibrate analogs have been used to treat dyslipidemia by lowering the free triglyceride (TG) plasma concentration.[4,5] Considering the significantly increased risk of complications related to lipid catabolism among the patients with type 2 diabetes, it has been postulated that PPARα/γ dual agonists, such as muraglitazar, might provide outstanding therapeutic effects for the treatment of type 2 diabetes and dyslipidemia (Fig. 1).[6] We herein report identification of the

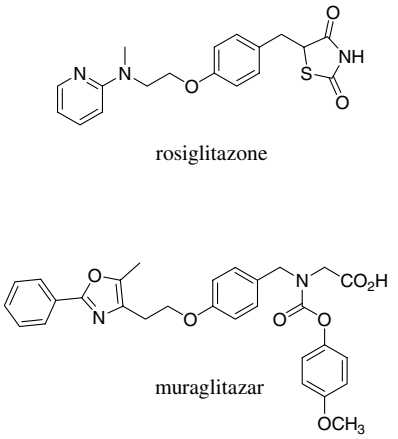

Figure 1. PPARγ agonist (rosiglitazone) and PPARα/γ dual agonist (muraglitazar).

Keywords: Type 2 diabetes; PPARs; PPARα/γ dual agonists.
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novel and potent PPARα/γ dual agonists as well as their structure–activity relationship.

In order to efficiently develop the novel PPARα/γ dual agonists, we initially attempted virtual screening of our chemical library for PPARγ (PDB number: 1K74) and then identified the compound **1** with weak PPARγ agonistic activity through a cell based transactivation assay of the virtual hits. In addition, replacement of benzoic acid moiety of **1** with β-aryl α-(*S*)-ethoxy propanoic acid, a well-known moiety of high binding affinity to PPARs,[7] has led us to discover the lead compound **9a** (Fig. 2) as a potent PPARα/γ dual agonist. Thus, we commenced our studies on development of novel PPARα/γ dual agonists based on the structure of **9a** as well as their structure–activity relationship. The novel series of **9a** consisting of hydrogen bonding donor/acceptor (**A**), linker (**B**), and hydrophobic part (**C**),[8] which are the typical functional regions of the known PPARα/γ dual agonists, were designed and synthesized for the detail investigation of each region.

The compounds **9a–h, 10, 11** were conveniently synthesized as outlined in Scheme 1. The key intermediate **6** was synthesized from the commercially available 4-hydroxybenzaldehyde **2**. Sequential *O*-benzylation of phenol and Wittig olefination of aldehyde, followed by hydrogenation of the resulting olefin, provided **3**, which was readily converted to the acid **4** by benzylation and ester hydrolysis. The racemic **4** was transformed into the optically pure ester **5** via a sequence of (*R*)-2-phenylglycinol coupling, separation of the resulting two diastereomers, and amide hydrolysis, followed by ethyl ester formation. The absolute configuration of the ester **5** was confirmed by comparison of its optical rotation with that of the known compound.[9] Triflation of **5**, Sonogashira coupling of the resulting triflate **6** with the racemic or optically pure propargyl alcohol **7**,[10] and then hydrogenation of the triple bond afforded the corresponding optically active or racemic intermediate **8** in optically pure form or as a mixture of two diastereomers, respectively. The analogs **9a–h**, **11** were prepared by coupling of the corresponding intermediate **8** with the appropriate isothiocyanate or isocyanate, followed by ester hydrolysis, while the acid **10** was prepared by direct hydrolysis of the hydroxyl ester **8**. Our design and synthesis of the analogs of **9a** was focused on the thiocarbamate analogs rather than the carbamate because our preliminary studies revealed that the thiocarbamates are much more active than the carbamates for PPARα/γ as shown in the activities of **9a** and **11** of Table 1. The optically active thiocarbamates could also be prepared by employing (*S*)- or (*R*)-**7** by the same procedure. The analog **14** was synthesized from propargyl alcohol by analogy to **9a–h** as depicted in Scheme 2. The thiocarbamate **18** was synthesized from commercially available racemic 1-phenyl-1, 2-ethanediol (**15**). Selective tosylation of the primary alcohol,[11] TBS protection of the remaining secondary alcohol, and then alkylation

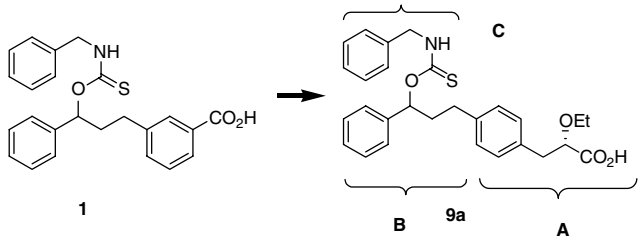
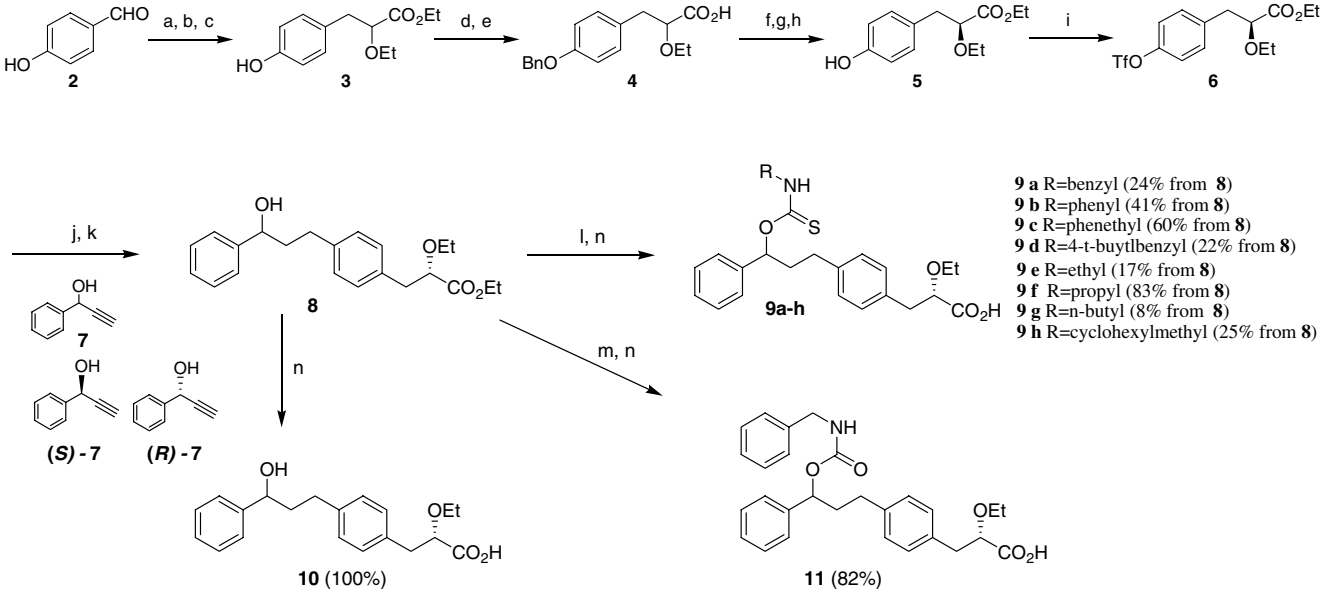


Figure 2. Conversion of hit **1** to lead compound **9a**.



Scheme 1. Reagents and conditions: (a) benzylchloride, K_2CO_3, DMF, 80 °C, 93%; (b) (1,2-diethoxy-2-oxyethyl)triphenylphosphoniumchloride, tetramethylguanidine, CH_2Cl_2, 0 °C to rt, 93%; (c) H_2, Pd/C, MeOH, 95%; (d) benzylchloride, K_2CO_3, DMF, 120 °C, 75%; (e) LiOH·H_2O, MeOH/THF/H_2O, 94%; (f) EDCI, HOBT, DIEA, CH_2Cl_2, then (*R*)-phenylglycinol, 0 °C to rt, 30% (desired diastereomer); (g) concd-H_2SO_4, H_2O/1,4-dioxane, reflux; (h) TMSCl, EtOH, 45% for two steps; (i) Tf_2O, Et_3N, CH_2Cl_2, 0 °C to rt, 92%; (j) (*S*)- or (*R*)-**7** for **9a, 9c**, and **9d**, and **7** for **9b, 9e–h, 10, 11**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, TBAI, DMF, 50 °C, 83%; (k) H_2, Pd/C, MeOH, 100%; (l) NaH, alkyl or arylisothiocyanate, THF; (m) BF_3·OEt_2, benzylisocyanate, toluene; (n) LiOH·H_2O, MeOH/THF/H_2O.

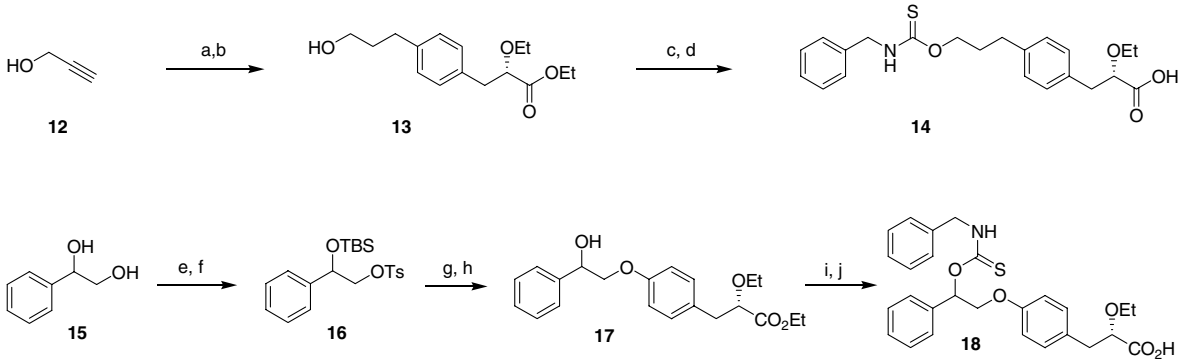
Table 1. In vitro activities of the synthesized compounds in cell-based GAL4-PPARα/γ transactivation assay

Compound[a]	Human PPARα		Human PPARγ	
	EC_{50} (μM)	Efficacy[b] (%)	EC_{50} (μM)	Efficacy[b] (%)
Rosiglitazone	3.46	56	0.03	100
Gemfibrozil	193.30	100	147.80	79
(***R***)-**9a**	0.26	101	0.35	113
(***S***)-**9a**	0.07	234	0.58	159
9b	5.25	154	4.58	204
(***R***)-**9c**	2.77	112	1.05	190
(***S***)-**9c**	0.88	120	0.49	177
(***R***)-**9d**	0.38	71	0.14	197
(***S***)-**9d**	3.27	66	0.16	187
9e	20.40	130	5.39	182
9f	3.94	128	3.48	152
9g	7.26	104	3.60	118
9h	10.70	117	1.06	145
10	1.51	108	0.30	119
11	6.05	164	1.42	160
14	1.47	118	1.40	117
18	6.21	136	7.17	138

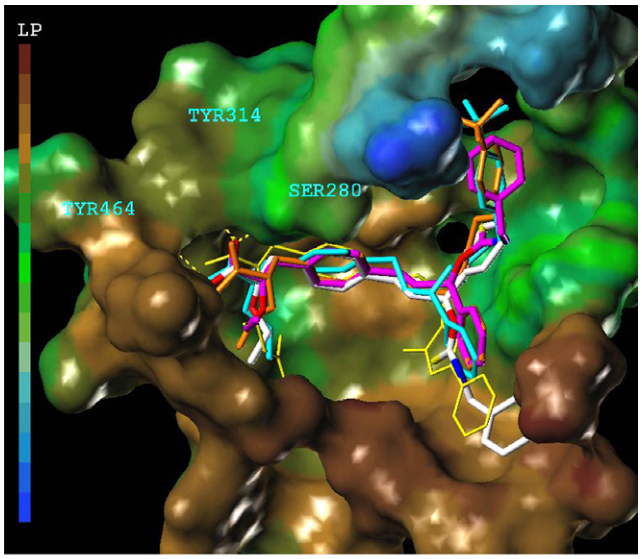
[a] (*R*) or (*S*)-**9a**, **9c** and **9d** as well as **14** are optically active, while **9b**, **9e**–**h**, **10**, **11**, and **18** are 1:1 diastereomeric mixtures.
[b] The relative maximum efficacy to the percentage of the standards.

of the intermediate **5** with the resulting tosylate **16** gave the ether **17** after desilylation. Reaction of **17** with benzylisothiocyanate and ester hydrolysis provided **18**.

The in vitro activities of the synthesized analogs are summarized in Table 1.[12] Generally, the aryl thiocarbamates (**9a, 9c**, and **9d**) exhibited higher agonistic activity than the alkyl thiocarbamates (**9e**–**h**) in both PPARα and PPARγ transactivation assays. In particular, the analog **9a** possessing benzyl thiocarbamate moiety displayed potent activities for PPARα/γ regardless of the stereochemistry. However, it decreased as the chain length of **C**-region decreased (**9b**) or increased (**9c**). Interestingly, the similar trend was observed for the alkyl thiocarbamates and the propyl turned out to be a linker of optimal length. It is noteworthy that (*R*)-**9d** possessing the bulkiest substituent in the lipophilic moiety (**C**-region) showed the most potent activity for PPARγ with EC_{50} of 0.14 μM. These results might imply that the bulky substituents in the lipophilic region are beneficial for the high PPARγ activation and the similar results were observed for the alkyl thiocarbamates as the analog **9h** with cyclohexylmethyl substituent exhibited the most potent activity for PPARγ. Our preliminary work revealed that the thiocarbamates are much more active than the carbamates for PPARα/γ, as it was confirmed by the activities of **9a** (EC_{50}: 0.26–0.58 μM) and **11** (EC_{50}: 1.42–6.50 μM). Replacement of the carbon linker with an ether linkage decreased the activity as shown in the activities of **9a** and **18**. Importantly, the agonistic activity of the thiocarbamate analogs sensitively changes according to the stereochemistry at the benzylic carbon, particularly for PPARα. The (*S*)-isomer of **9a** was more potent than the corresponding (*R*)-isomer for PPARα, while both exhibited the similar activity for PPARγ. The (*S*)-isomer of **9c** is slightly more potent than the (*R*)-isomer of **9c** in both PPARα and γ. In case of the compound **9d** showing the most potent activity for PPARγ, the (*R*)-isomer of **9d** was 10-fold more potent than the (*S*)-isomer for PPARα. The docking studies with Surflex–Dock™,[13] shown in Fig. 3, provided a rationale in part for the stereochemistry/substituent-dependent selectivity for the PPAR isotypes. The benzylthiocarbamoyl moiety of (*S*)-**9a** seems to fit well to the lipophilic region of the active site of PPARα, while the corresponding moiety of (*R*)-**9a** orients toward the hydrophilic region instead of the lipophilic region, which is enforced by the stereochemistry. In addition, the calculated binding energies revealed the lower binding energy for (*S*)-**9a** compared to that for (*R*)-**9a**. However, *p*-*tert*-butyl benzyl group of **9d** appears to be too bulky to fit to the lipophilic pocket of PPARα in both (*R*)- and (*S*)-isomers and subsequently the bulky lipophilic moiety to orient toward the spacious hydrophilic pocket. As the results, the activities of (*R*)-**9a**, (*S*)-**9d**, and (*R*)-**9d** seem to be reduced by the inadequate orientations of their lipophilic moiety. Removal of the phenyl moiety of the **B**-region of **9a** significantly drops the activity for both PPARα and PPARγ as shown in the activities of **14**. This would be understood in terms of formation of the favorable conformation for the high activity, which is induced



Scheme 2. Reagents and conditions: (a) **6**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, TBAI, DMF, 60 °C, 45%; (b) H_2, Pd/C, MeOH, 76 %; (c) NaH, benzylisothiocyanate, THF, 0 °C to rt; (d) LiOH·H_2O, MeOH/THF/H_2O, 65% for two steps; (e) Bu_2SnO, *p*-toluenesulfonylchloride, Et_3N, CH_2Cl_2, 0 °C to rt, 94%; (f) TBSCl, imidazole, DMAP, DMF, 65 °C, 85%; (g) **5**, K_2CO_3, DMF, 130 °C, (h) 2 N-HCl, THF, 50 °C, 40% for two steps; (i) NaH, benzylisothiocyanate, THF; (j) LiOH·H_2O, MeOH/THF/ H_2O, 60% for two steps.



Compound	*(S)*-**9a**	*(R)*-**9a**	*(S)*-**9d**	*(R)*-**9d**
Energy*	-8.86	-8.69	-7.94	-8.66

Figure 3. Docking model of (*S*)-**9a** (white), (*R*)-**9a** (magenta), (*S*)-**9d** (cyan), (*R*)-**9d** (orange), and the co-crystallized ligand as a reference molecule (yellow) with PPARα (PDB No. 1K7L). LP, lipophilicity; dotted line, hydrogen bonding. *Note.* *Log value (kcal/mol).

by the phenyl substituent. On the other hand, elimination of the thiocarbamate moiety of the thiocarbamate **9a** significantly reduced the activity for PPARα as shown in the activity of the alcohol **10** while the activity for PPARγ remained. This result obviously supports that the thiocarbamate moiety is essential for the high activity of our series for PPARα.

In summary, we have identified the novel and potent PPARα/γ dual agonists and established SAR of the carbamate-tethered propanoic acids through the computational and synthetic chemistry. The stereochemistry/substituent-dependent activation of the particular isotype of PPARs by our carbamate series was elucidated on the basis of the binding mode of the ligands in the active site. Further work for the development of the therapeutically useful PPARα/γ dual agonists based on our current results is in good progress.

Acknowledgments

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12. In vitro transactivation assays: the ligand binding domains (LBD) of the human PPARα/γ receptors were fused to the DNA binding domain (DBD) of the yeast transcription factor GAL4. CV-1 cells were transiently transfected with an expression vector for the respective PPAR chimera along with a reporter construct containing five copies of the GAL4DNA binding site and pRL-TK as a control vector (Promega). The test compounds were dissolved in DMSO and diluted 1:1000 in media. Cells were treated with the compounds at seven concentrations ranging from 0.03 to 100 μM for 24 h followed by dual luciferase assay using Dual-Glo luciferase reagent (promega). EC_{50} values were calculated by nonlinear regression using SigmaPlot 4.0 (SPSS).
13. The X-ray structures of PPARα and PPARγ complexed with the ligand were taken from the RCSB Protein Data Bank (PDB code: 1K7L for PPARα/1K74 for PPARγ). The protein was prepared for docking, using the protein preparation and refinement utility provided by Surflex–Dock™ suite in SYBYL 7.0. Water molecules of the crystal structure were removed from the complexes, and the hydrogen atoms were added computationally at appropriate positions. Calculations for the docking studies between the prepared PPARα protein and the selected compounds were performed using SYBYL 7.0 software.