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Design and synthesis of benzoxazole containing indole analogs as peroxisome proliferator-activated receptor-γ/δ dual agonists

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ABSTRACT

A series of benzoxazole or benzothiazole containing indole analogs, 6-alkoxyindole-2-carboxylic acids and 5-alkoxy-3-indolylacetic acids, were synthesized as novel candidates of PPARγ/δ dual agonists and their ligand activities for PPAR subtypes (α, γ, and δ) were investigated. In transient transactivation assay, several compounds activated PPARγ and δ with little activity of PPARα. Putative binding mode of the compounds **1a** and **2a** in the active site of PPARγ was similar with that of rosiglitazone and the molecular modeling provides molecular insight to the observed activity.

Peroxisome proliferator-activated receptors (PPARs) belong to a subfamily of ligand-activated nuclear hormone receptors and function as key regulators of glucose, lipid, and cholesterol metabolism.[1] PPARs, upon ligand activation, heterodimerize with retinoid X receptor (RXR) and the PPAR–RXR complex binds to peroxisome proliferator response elements (PPREs) and initiates the transcription process of the target genes. Three PPAR subtypes have been identified to date as PPARα, PPARγ, and PPARδ.[2]

PPARα plays a critical role in the regulation of cellular uptake, activation, and β-oxidation of fatty acid, and its agonists, the fibrates, are used to treat dyslipidemia. PPARγ which is mainly associated with adipose-related functions regulates lipid metabolism, lipid uptake into adipocytes, glucose homeostasis, and insulin sensitivity. PPARγ agonists, specifically the thiazolidinediones, are used as efficient insulin sensitizers in type 2 diabetes. Recently, PPARα/γ dual agonists have been studied intensively to combine the insulin sensitizing potential of PPARγ agonist with the beneficial activity of lipid regulation of PPARα agonist.[3] Most of them, however, have been discontinued in clinical trial due to the adverse toxicity profiles such as kidney and cardiovascular toxicity.[4] Recent research has revealed PPARδ also play a role in the regulation of reverse cholesterol transport and high-density lipoprotein metabolism, and its agonists such as GW501516 may be of use in the treatment of dyslipidemia, obesity, and insulin resistance.

Regarding the beneficial pharmacological effects of each PPAR subtypes, the development of PPARγ/δ dual agonist or PPAR-pan agonist are attractive strategy for the treatment of metabolic syndrome.[5]

We made our endeavor for investigation of structural feature aimed at the discovery of novel PPARγ/δ or PPAR-pan agonists. Despite structural diversity of the PPAR agonists, they share a common structural feature. Most of PPAR agonists compose of five common elements; a polar head group (A) connected to an aromatic ring (C) through a short linker (B), a linker (D), and a hydrophobic tail group (E) (Fig. 1).

Polar head group is a critical binding motif in the active site of PPAR ligand binding domain (LBD). Various groups such as thiazolidinedione ring and α-alkoxy-substituted propionic acids have been investigated as potent polar head groups. However, these polar head groups are prone to racemization under physiological conditions.[6] Therefore, it is important to explore new polar head groups with high affinity and structural stability. Our study was focused on the novel scaffolds of polar head and short linker to aromatic center. To start with, our chemical library was screened to explore new polar head groups for PPAR ligand and molecular docking as a computational tool was also employed for this purpose. In vitro functional assay and docking analysis led us to a number of compounds which showed weak PPAR agonistic activity. One of these was indole based structure, indole-2-carboxylic acid, which was expected to be a good replacement of known acidic head groups. In fact, several indole-based compounds have been reported as PPAR agonists providing novel scaffold of polar head group with planar bicyclic system.[7–9] In the previous indole analog, hydrophobic tail was mostly connected to nitrogen of indole through poly methylene liker.

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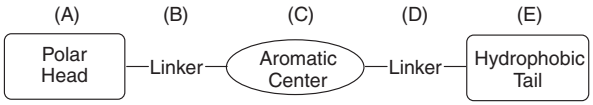
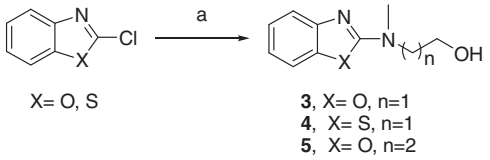


Figure 1. The general topology of synthetic PPAR agonists.



Scheme 1. Synthesis of hydrophobic tail, benzoxazolyl- or benzothiazolylmethylaminoalcohol. Reagents and conditions: (a) 2-methylaminoethanol or 3-methylamino-1-propanol, TEA, THF.

In our indole-based analogs, the acidic head group was attached to the C2- or C3-position of indole ring with/without a short linker and hydrophobic tail was connected to the C5- or C6-position of indole through poly methylene liker. It allowed the introduction of extra substituent at the nitrogen of indole leading to the unique feature in comparison with the reported indole-based PPAR agonists. Herein, we report design, synthesis and biological evaluation of the novel indole carboxylic acid and indolylacetic acid analogs with hydrophobic tail of benzoxazole or benzothiazole (Fig. 2). To understand binding mode of the indole analogs into the PPARγ, docking experiments were also performed.

Synthetic routes for the preparation of compounds **1a**–**d** are outlined in Schemes 1 and 2. First, hydrophobic tails, benzoxazolyl- and benzothiazolylmethylaminoalcohol **3**–**5**, were prepared by N-alkylation of 2-methylaminoethanol or 3-methylamino-1-propanol with 2-chlorobenzoxazole or 2-chlorobenzothiazole (Scheme 1).

Preparation of benzoxazole-linked indole-2-carboxylic acids was started from compound **3**. Mesylation of alcohol **3** and O-alkylation of 4-hydroxybenzaldehyde with the mesylate **6** gave aldehyde **7**. Condensation of aldehyde **7** with methyl azidoacetate in the presence of NaOMe and following thermolysis of the resulted 2-azido-phenylacrylate **8** afforded indole **9a**. The N-alkylation of indole **9a** with corresponding alkyl halide in the presence of base gave compounds **9b–d**, which were hydrolyzed to final acids **1b–d** (Scheme 2).

Synthesis of benzothiazole-linked indole-2-carboxylic acids was carried out in the similar way with that of benzoxazole analogs (Scheme 3). 2-Azido-3-phenylacrylate **10** was prepared by condensation of 4-methoxybenzaldehyde with methyl azidoacetate in the presence of NaOMe and converted to 6-methoxy-1*H*-indole-2-carboxylate **11a** via thermolysis. The N-alkylation of indole **11a** and successive demethylation of arylmethyl ether **11a–c** afforded 6-hydroxy-1-alkyl-1*H*-indole-2-carboxylate **12a–c**. Mitsunobu reaction of hydroxyindole **12** with the alcohol **4** in the presence of tributylphosphine and 1,1′-(azodicarbonyl)dipiperidine (ADDP) gave compounds **13a–c** which were hydrolyzed to final acids **1e–f**.

Scheme 4 depicts the synthesis of benzoxazole-linked indolyl-3-acetic acids. Esterification of 4-hydroxyindolyl-3-acetic acid gave methyl ester **14**, which was converted to **15a** and **2d** by Mitsunobu reaction with respective **3** and **5**. Introduction of various substituents to the nitrogen of indoles and the following hydrolysis of the resulted N-alkylated compounds gave the target products **2b**, **2c**, **2g**, and **2i**.

PPARs agonist activities of the synthesized compounds were evaluated by in vitro transient transactivation assays.[11] To compare the efficacy of compounds, GW0746, rosiglitazone and GW0742 were used as reference compounds in the PPARα, γ, and δ transactivation assays, respectively.

We first investigated PPARα, γ, δ transactivation activity of indole carboxylic acid analogs as shown in Table 1. Introduction of the substituents at nitrogen of the indole core led to increased activity for PPAR, especially PPARγ. Replacement of hydrophobic benzoxazole with benzothiazole increased activity as compared **1b** and **1c** with **1f** and **1g**, respectively. Among the indole carboxylic acid analogs, N-ethylated benzothiazole **1g** showed the highest PPARγ/δ transactivation activity (66% efficacy for PPARγ and 69% for PPARδ) with low PPARα activity (17%).

We then explored indolylacetic acids which have more flexibility at the polar head region by adding a methylene linker between indole core and acidic head, and their PPAR transactivation activities were described in Table 2. In these series, both acidic head group and linker were connected to respective C3- and C5-positions of indole ring instead of C2- and C6-positions in the

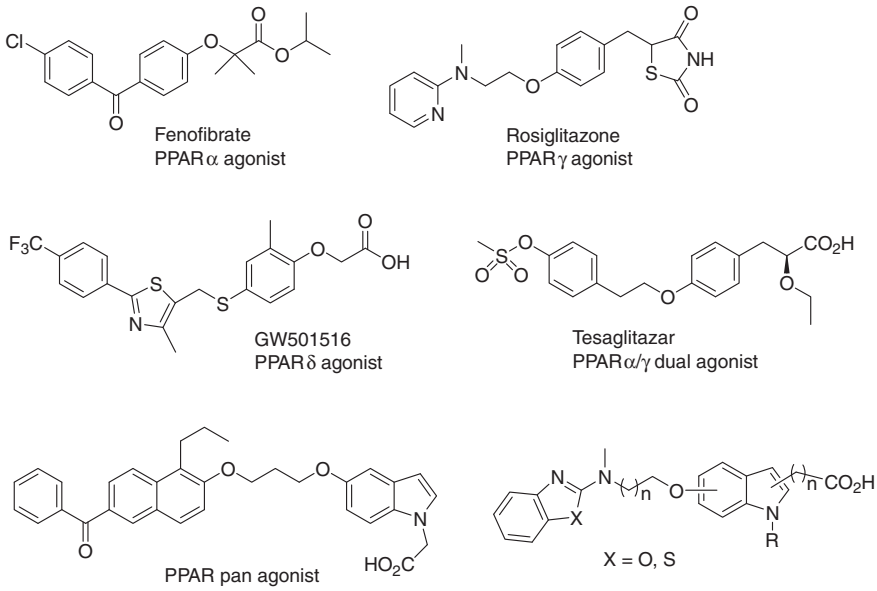
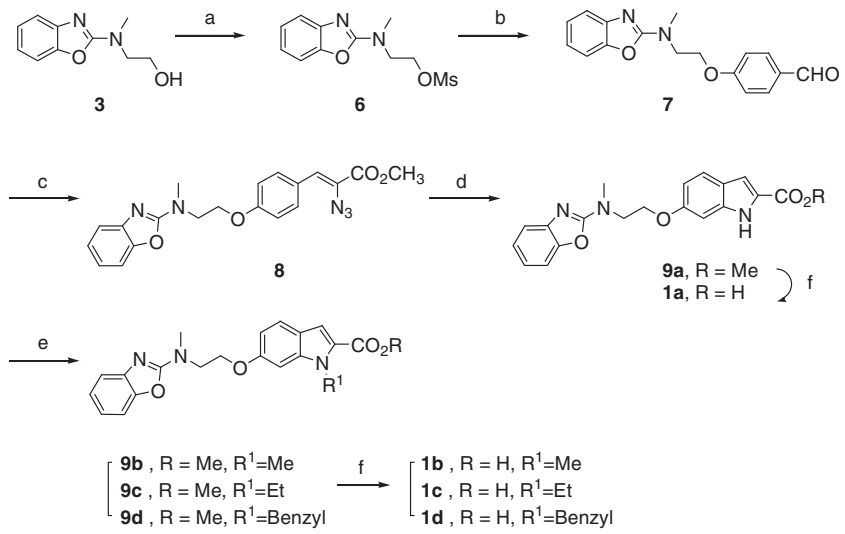
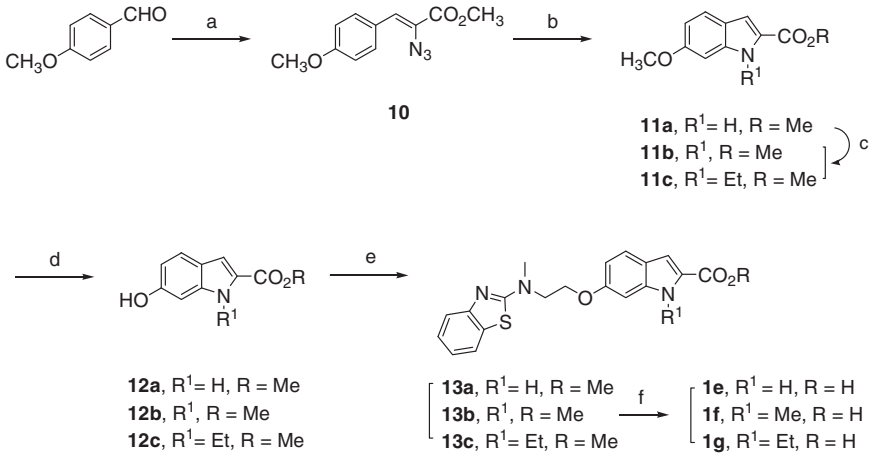


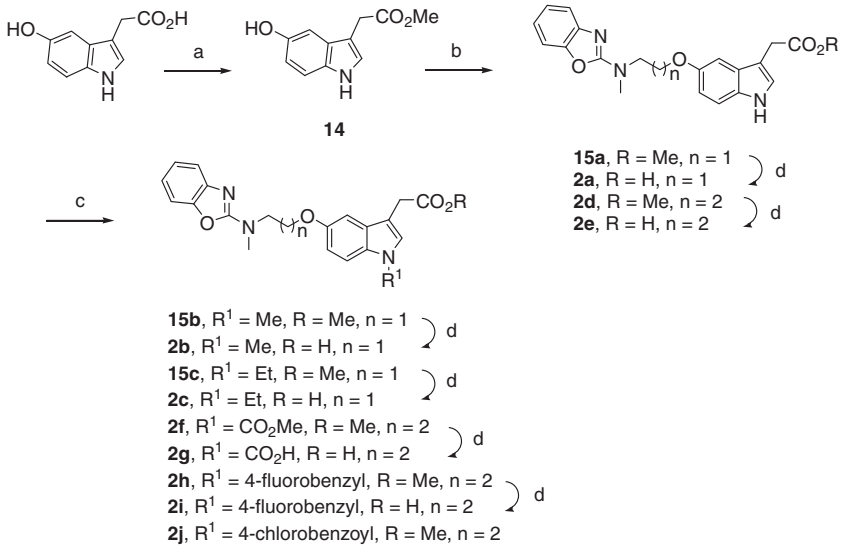
Figure 2. Chemical structures of some known PPAR agonists and the representative structure of target compounds.



Scheme 2. Synthesis of benzoxazole-linked indole-2-carboxylic acids. Reagents and conditions: (a) MsCl, TEA, CH_2Cl_2, rt, 2 h; (b) 4-hydroxybenzaldehyde, NaH, DMF; (c) N_3CH_2COOMe, NaOMe, MeOH; (d) xylene, heat, 6 h; (e) R^1X, NaH (or Cs_2CO_3), DMF; (f) NaOH, THF (or DMF), MeOH, H_2O.

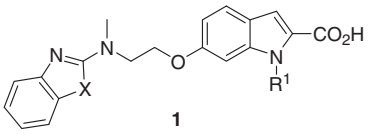


Scheme 3. Synthesis of benzothiazole-linked indole-2-carboxylic acids. Reagents and conditions: (a) N_3CH_2COOMe, NaOMe, MeOH; (b) xylene, heat, 6 h; (c) R^1X, NaH (or Cs_2CO_3), DMF; (d) BBr_3; (e) **4**, ADDP, PBu_3, THF/toluene; (f) NaOH, THF (or DMF), MeOH, H_2O.



Scheme 4. Synthesis of benzoxazole-linked indolyl-3-acetic acids. Reagents and conditions: (a) $SOCl_2$, MeOH; (b) **3** or **5**, ADDP, PBu_3, THF/toluene; (c) R^1X, NaH (or Cs_2CO_3), DMF; (d) NaOH, THF (or DMF), MeOH, H_2O.

Table 1
In vitro functional transactivation activity of 6-alkoxyindole-2-carboxylic acids on murine PPAR[a]

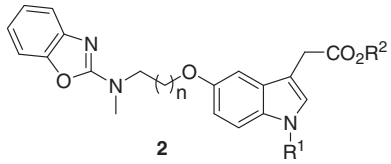


1

Compd	X	R^1	% Max[b] (20 μM)		
			mPPARα	mPPARγ	mPPARδ
1a	O	H	NA	NA	NA
1b	O	Me	NA	NA	NA
1c	O	Et	NA	23.3	NA
1d	O	Bn	10.0	43.9	33.4
1e	S	H	NA	NA	14.6
1f	S	Me	13.6	29.6	27.9
1g	S	Et	16.7	66.4	68.8

NA means not active, which is for compounds producing transactivation activity lower than 10% at 20 μM.
[a] Compounds were tested in at least three separate experiments.
[b] Fold activation relative to maximum activation obtained with GW0746 (1 μM) for PPARα, with rosiglitazone (1 μM) for PPARγ, and with GW0742 (0.1 μM) for PPARδ.

Table 2
In vitro functional transactivation activity of 5-alkoxy-3-indolylacetic acids on murine PPAR [a]



2

Compd	*n*	R^1	R^2	% Max[b] (20 μM)		
				mPPARα	mPPARγ	mPPARδ
2a	1	H	H	NA	28.1	NA
2b	1	Me	H	28.0	58.6	NA
2c	1	Et	H	NA	66.7	32.9
2d	2	H	Me	NA	42.1	NA
2e	2	H	H	NA	18.3	NA
2f	2	CH_2CO_2Me	Me	NA	37.1	NA
2g	2	CH_2CO_2H	H	NA	NA	NA
2h	2	$CH_2(4\text{-}F\text{-}C_6H_4)$	Me	NA	NA	NA
2i	2	$CH_2(4\text{-}F\text{-}C_6H_4)$	H	13.3	15.8	NA
2j	2	$CO(4\text{-}Cl\text{-}C_6H_4)$	Me	NA	28.9	18.3

NA means not active, which is for compounds producing transactivation activity lower than 10% at 20 μM.
[a] Compounds were tested in at least three separate experiments.
[b] Fold activation relative to maximum activation obtained with GW0746 (1 μM) for PPARα, with rosiglitazone (1 μM) for PPARγ, and with GW0742 (0.1 μM) for PPARδ.

indole carboxylic acids. Subsequently, the distance between carboxylic acid and oxygen of the linker was kept in both seriese of indole carboxylic acids and indolylacetic acids. As expected, indolylacetic acids **2a–c** revealed higher activity than corresponding indole carboxylic acids **1a–c**. Similarly with indole carboxylic acids (**1a–c**), *N*-methyl (**2b**) or ethyl (**2c**) substituted analogs of indole ring revealed higher PPARγ transactivation activity than un-substituted indole (**2a**) did. However, introduction of *para*-substituted benzyl (**2h–j**), carboxy ester (**2f**), or carboxyl group (**2g**) was not favorable for the activity. Effect of lengthening (**2e**) of the linker between indole core and benzoxazole on the activity was not clear. Most of the active compounds showed higher activity for PPARγ over PPARδ. PPARα transactivation activity was ignorable.

To help interpretation of SAR data and to rationalize the different activities between indole carboxylic acids and indolylacetic

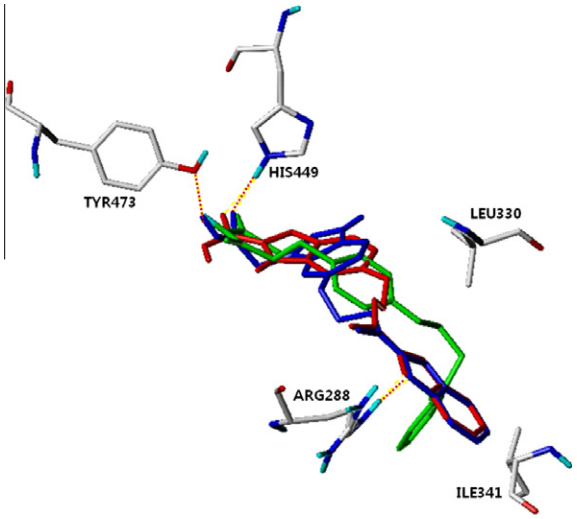


Figure 3. Superposition of the structure of **1a** (red), **2a** (blue) and crystallized rosiglitazone (green) in the binding pocket of PPARγ.

acids, docking experiments into the hPPARγ receptor binding domain were carried out using Surflex Dock interfaced with SYBYL-X version 1.2 on Linux. The crystal structure of the human PPARγ in complex with rosiglitazone (PDB code: 2PRG)[10] was employed for the docking study. In this automated docking program, the flexibility of the ligands is considered while the protein is considered as a rigid structure. The molecules for docking were sketched in the SYBYL and energy minimizations were performed using Tripos Force Field and Gasteiger–Huckel charge with 20,000 iterations of conjugate gradient method with convergence criterion of 0.05 kcal/mol. The docking analyses of compounds **1a** and **2a** were performed using the internal default parameters for all the variables.

According to the crystal structure of rosiglitazone in the LBD of PPARγ, thiazolidinedione ring of rosiglitazone interacts with residues His323, His449 and Tyr473. Many reports have suggested that the specific polar interactions are important for the activities of PPARγ agonists, as this H bonding network could stabilize the AF-2 helix in a conformation favoring the binding of co-activators to PPARγ.[10] In the predicted binding orientation, both **1a** and **2a** were occupied the same spatial position and had specific interactions with the critical amino acids in the binding pocket as the

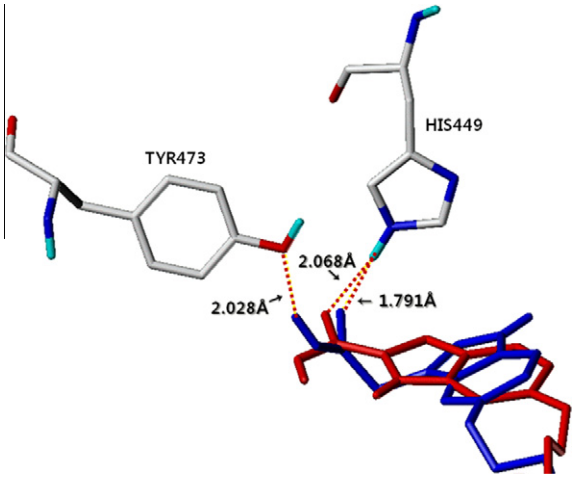


Figure 4. View of important polar interactions between **1a** (red) and **2a** (blue) and the critical residues of the PPARγ.

crystallized rosiglitazone did (Fig. 3). Compared the predicted binding mode of **1a** with **2a**, the carboxy group of **1a** made H-bond only with Tyr473, whereas the carboxy group of **2a** made two H-bonds with Tyr473 and His449 (Fig. 4). This docking result might provide the structural insight for the higher PPARγ transactivation activity of indolylacetic acid analogs **2a–c** than those of indole carboxylic acid analogs **1a–c**. Interestingly, an additional polar interaction was observed between the oxygen of benzoxazole ring and Arg288 in the region positioned by hydrophobic tail.

In summary, series of novel indole carboxylic acids and indolylacetic acids were prepared as PPARγ/δ agonists and carefully investigated their structural feature. In general, indolylacetic acids were more active than the corresponding indole carboxylic acids. Docking analysis imply that indolylacetic acids likely form more favorable H bond network in the LBD of PPARγ. We suggest that indolylacetic acid structure might act as a versatile template for the creation of novel PPAR agonists and further SAR exploration is underway.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.027.

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11. *Transient transfection and luciferase assay:* CV-1 cells were seeded in 48-well plate at a density of 1.5×10^5 cells/well in DMEM with 10% FBS. The cells were transiently transfected with plasmid mixtures containing PPARs expression vector and tk-PPRE-luciferase (Luc) vector for 6 h, and then treated with samples for 24 h. To normalize transfection efficiency, β-galactosidase plasmid was cotransfected. The luciferase activities in cell lysates were measured using luciferase assay system (Promega Corp., Madison, WI) and the β-gal activities were measured as the absorbance at 410 nm by using an ELISA plate reader. Data are reported as relative luciferase activity divided by the β-galactosidase activity. All the constructs were kindly gifted by Dr. Ronald M. Evans at The Salk Institute (La Jolla, CA).