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Design and synthesis of alkoxyindolyl-3-acetic acid analogs as peroxisome proliferator-activated receptor-γ/δ agonists

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This paper is dedicated to Professor Young-Ger Suh on the occasion of his 60th birthday

ABSTRACT

A series of carbazole or phenoxazine containing alkoxyindole-3-acetic acid analogs were prepared as PPARγ/δ agonists and their transactivation activities for PPAR receptor subtypes (α, γ and δ) were investigated. Structure–activity relationship studies disclosed the effect of the lipophilic tail, attaching position of the alkoxy group and *N*-benzyl substitution at indole. Compound **1b** was the most potent for PPARδ and **3b** for PPARγ. Molecular modeling suggested two different binding modes of our alkoxyindole-3-acetic acid analogs providing the insight into their PPAR activity.

Peroxisome proliferators-activated receptors (PPARs) are a group of nuclear receptor proteins that function as a ligand activated transcription factors regulating the expression of gene including cellular differentiation, development and metabolism, therefore they are targets of drugs effective in treatment of metabolic diseases.[1,2] PPARs are a group of three isoforms, PPARα, PPARγ and PPARδ.[3] PPARα which is expressed at high levels in liver, kidney, heart, adipose tissue and others, has been shown to play a critical role in the regulation of cellular uptake, activation and β-oxidation of fatty acid. Increased fatty acid oxidation by activated PPARα lowers circulating triglyceride levels and reduces adiposity, which improves insulin sensitivity.[1] PPARγ which is mainly associated with adipose-related functions, regulates lipid metabolism, lipid uptake into adipocytes, glucose homeostasis and insulin sensitivity, and its agonists, specifically the thiazolidinediones(TZDs) including rosiglitazone and pioglitazone, are used as efficient insulin sensitizers in type 2 diabetes.[4] To combine the beneficial effects of insulin sensitization and lipid regulation,[5] PPARαγ dual agonists including muraglitazar and tesaglitazar have been paid much attention. However, the adverse toxicity profiles PPARαγ dual agonists have been reported and raised critical safety issues, which have led to the discontinuation of clinical development.[6] Recent studies revealed that PPARδ is ubiquitously expressed with highest levels in adipose tissue, skeletal muscle and intestine. PPARδ is also involved in lipid metabolism and glucose homeostasis.[7–9] A number of selective PPARδ agonists have been identified, and GW501516, a potent and selective PPARδ agonist to have entered clinical trials, has shown enhanced fatty acid β-oxidation in skeletal muscle, increased HDL cholesterol level and decreased elevated triglyceride and insulin levels in animal model.[7] Also, PPAR pan agonists which act on all the three subtypes (PPARαγδ) of PPARs, have been reported in an effort to reduce side effects and increase efficacy.[10] Despite the high potential of PPAR agonists, the clinical benefits are nevertheless limited by several adverse effects such as weight gain, renal fluid retention and increased risk of heart failure.[11–13] It is necessary to improve pharmacological profiles of PPAR agonists. PPARαγδ agonists are expected to have superior therapeutic utility for the treatment of altered lipid homeostasis and glucose homeostasis in target organs.

Recently, we reported benzoxazole containing indolylacetic acids and indole carboxylic acid analogs **1** as PPARγ/δ agonists.[14] The representative structures of PPAR agonists and our indole compound depicted in Figure 1. Typically, a number of PPAR agonists have common structural features which include polar head, aromatic ring, proper linker and hydrophobic tail in order (Fig. 2).

In previous study, we have found that indolylacetic acid analogs are more active than the corresponding indole carboxylic acids among our indole compounds. Herein we focused on the influence of alkoxy position at the indolyl-3-acetic acid, the replacement of bicyclic hydrophobic tail with new tricyclic rings and introduction of benzyl substituent at the nitrogen of indole core.

Synthetic routes for the preparation of final compounds **1a**–**4c** are outlined in Schemes 1–4. The targeted 5- or 6-alkoxyindolyly-3-acetic acid analogs were prepared by Mitsunobu reaction of the corresponding hydroxyindoles with the various tricyclic-linked alcohols. First, 5-hydroxyindolyl-3-acetic acid methyl ester **5a** was simply prepared by esterification of commercially available 5-hydroxyindole-3-acetic acid (Scheme 1).

For the modification of position of alkoxy-linker at indole ring, 6-hydroxyindolyl-3-acetic acid methyl ester **5b** was prepared by Fukuyama indole synthesis as a key step (Scheme 2).[15] The commercially available 4-amino-3-nitrophenol was treated with sodium nitrite under acidic condition and then potassium iodide to give iodophenol **6,** which was protected with a benzyl group to afford compound **7**. The Heck reaction of compound **7** with

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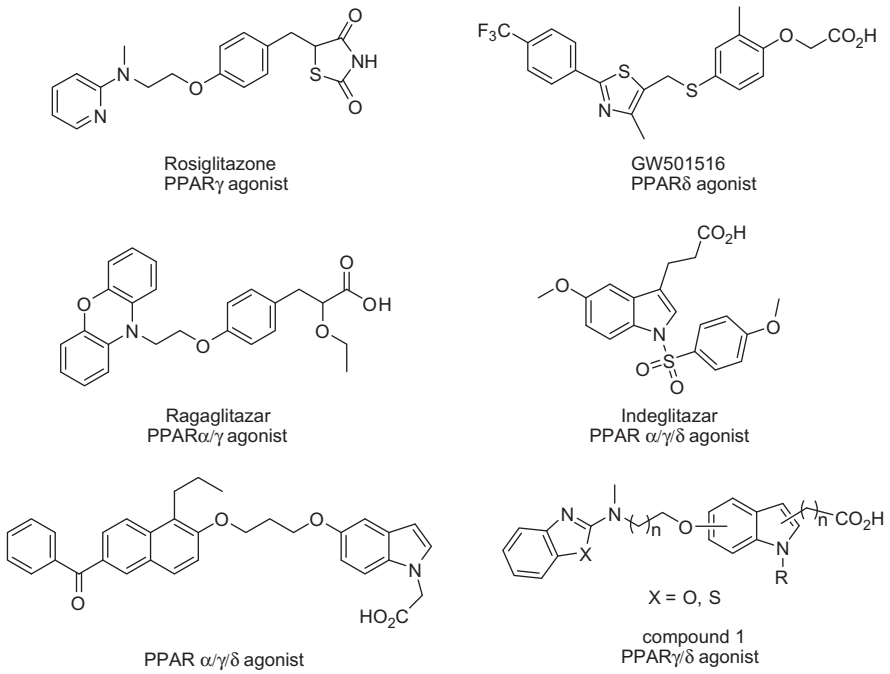


Figure 1. Chemical structures of PPAR agonists.

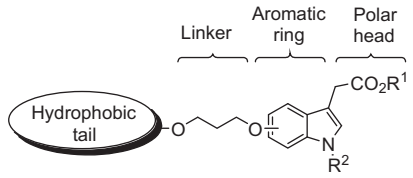
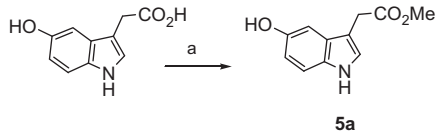


Figure 2. Design of alkoxyindolyl-3-acetic acid based PPARγδ agonist.



Scheme 1. Synthesis of 5-hydroxyindolyl-3-acetic acid methyl ester. Reagent and condition: (a) $SOCl_2$, MeOH, 0 °C–rt.

methyl acrylate gave compound **8** in good yield. Chemoselective reduction of the nitro group of compound **8** afforded amine **9** which was formylated by treating acetoformic anhydride. The compound **10** was treated with triphosgene in the presence of triethylamine to obtain isonitrile **11**. Treatment of the isonitrile **11** with tributyltin hydride in the presence of AIBN gave 3-substituted indole **12**, and followed debenzylation provided the desired 6-hydroxyindole **5b**.

Alcohols **13** and **14** were obtained from commercially available carbazole and phenoxazine by alkylation with 3-iodopropanol in the presence sodium hydride. Mitsunobu reaction of hydroxyindoles **5** with alcohols **13** and **14** in the presence of tributylphosphine and 1,1′-(azodicarbonyl)dipiperidine (ADDP) gave compounds **15–18** which were hydrolyzed to final acids **1a–4a** (Scheme 3). *N*-benzylation of compounds **15–18** and the following hydrolysis gave the final acids **1b–4c** as shown in Scheme 4.

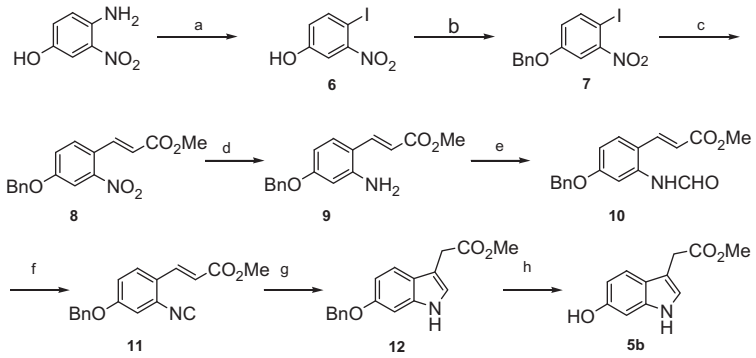
PPAR agonist activities of the synthesized compounds were evaluated by in vitro transient transactivation assays. The efficacy of the tested compounds was compared to the reference compounds GW0746, rosiglitazone and GW0742 in the PPARα, γ and δ transactivation assays, respectively. PPARα, γ and δ transactivation activity of alkoxyindolyl-3-acetic acid analogs were described in Table 1.

To compare the effect of hydrophobic ring system, tricyclic carbazole or phenoxazine was conjugated into 5- or 6-position of indole scaffold through C3-methylene linker. Phenoxazine-linked indole analogs **3a** and **3b** showed slightly increased activity for PPARγ or δ as compare with carbazole-linked analogs **1a** and **1b**, respectively.

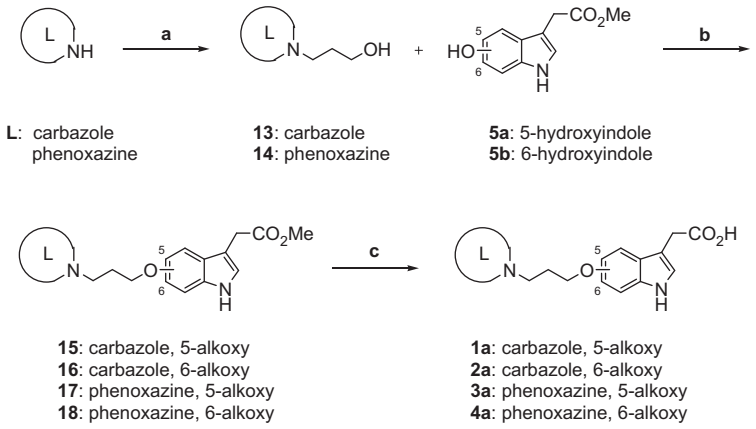
The activity was also influenced by attaching position of the alkoxy-linker at indole ring. In general, 5-alkoxyindole analogs showed higher PPARγ or δ activity than those of 6-alkoxyindole analogs. 5-Alkyoxy analogs **1a**, **1b** and **3a** were more active for both PPARγ and δ transactivation than those of corresponding 6-alkoxy analogs **2a**, **2b** and **4a** with exception of phenoxazine containing N-benzylated 5-alkoxyindole **3b**.

As expected from the previous study, introduction of the benzyl group at nitrogen of indole core led to improved activity for PPARγ and δ as compared **1a**, **3a** and **4a** with **1b**, **3b** and **4b**, respectively. However, carbazole-linked 6-alkoxy analogs were not active even after introduction of *N*-benzyl group while phenoxazine linked 6-alkoxy analogs brought up the higher activity for PPARγ and/or δ by N-benzylation. Among the tested compounds, the most active compounds were carbazole containing N-benzylated analog **1b** for PPARδ and phenoxazine containing N-benzylated analog **3b** for PPARγ.

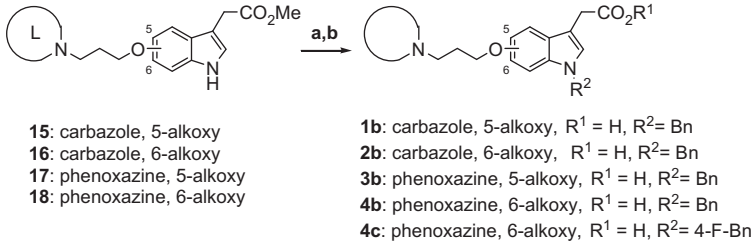
To rationalize the different activities between 5-alkoxy and 6-alkoxyindolyl-3-acetic acids and effect of N-benzylation, docking experiments into the hPPARγ receptor binding domain were carried out using Surflex Dock interfaced with SYBYL-X version 1.3. The crystal structure of the human PPARγ in complex with rosiglitazone (PDB code: 2PRG)[16] was employed for the docking study. In this automated docking program, the flexibility of the ligand 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



Scheme 2. Synthesis of 6-hydroxyindolyl-3-acetic acid methyl ester. Reagents and conditions: (a) $NaNO_2$, HCl, then KI, rt, 89%; (b) BnBr, K_2CO_3, acetone, rt, 95%; (c) methyl acrylate, $Pd(OAc)_2$, $(Tol)_3P$, TEA, MeCN, reflux, 84%; (d) Zn, AcOH, rt, 72%; (e) AcOCHO, Py, CH_2Cl_2, rt, 65%; (f) triphosgene, TEA, CH_2Cl_2, rt, 73%; (g) Bu_3SnH, AIBN, Toluene, 80 °C, then HCl, 84%; (h) H_2, $Pd(OH)_2$/C, AcOH, rt, 87%.



Scheme 3. Synthesis of carbazole or phenoxazine linked indole analogs **1a**–**4a**. Reagents and conditions: (a) 3-iodopropanol, NaH, DMF, rt; (b) ADDP, $P(Bu)_3$, THF/toluene, rt; (c) NaOH, THF/MeOH/H_2O, rt.



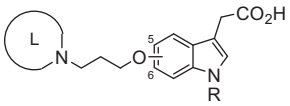
Scheme 4. Synthesis of carbazole or phenoxazine linked N-benzylated indole analogs **1b**–**4c**. Reagents and conditions: (a) benzyl or *p*-fluorobenzylbromide, NaH, THF, rt; (b) NaOH, THF/MeOH/H_2O, rt.

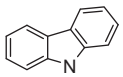
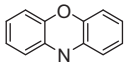
Gasteiger–Huckel charge with 20,000 iterations of conjugate gradient method in convergence criterion of 0.05 kcal/mol. The docking analyses of compound **1a**, **3a** and **3b** were performed using the internal default parameters for all the variables.

Most of PPARγ agonists including rosiglitazone interact with critical residues in helix H12 of the LBD. Thiazolidinedione ring of rosiglitazone forms H-bonding with Try473, His449, His323 and Ser289, where Tyr473 was located in the AF-2 helix as a key residue for the stabilization of conformation favoring the binding of co-activators.[16] As expected, **1a** and **3a** were bound to the active site of PPARγ co-crystallized with rosiglitazone through the known key interaction between specific residues of the protein and the ligands (Fig. 3). The carboxyl group of both compounds **1a** (red) and **3a** (magenta) interacted with His323(1a) or 449(3a), Tyr473 and Gln286 and NH of indole ring interacted with Ser289 residue. The lipophilic tails were inserted into the hydrophobic pocket of the binding site. In there, phenoxazine analog **3a** showed additional H-bonding interaction between the oxygen of phenoxazine and Ser342, whereas the carbazole analog **1a** did not.

However, *N*-benzylated indole analogs did not fit into the same binding site of PPARγ which was occupied by rosiglitazone and compounds **1a** and **3a** (Fig. 4). Hydrophilic head of compound **3b** occupied other binding pocket which was away from the helix H12 and thereby louse the critical H-bonding interaction. Docking

Table 1
In vitro functional transactivation activity of 5- or 6-alkoxyindolylacetic acids on murine PPAR[a]



Compd no.	L	Alkoxy position	R	%Max[b] (10 μM)		
				*m*PPARα	*m*PPARγ	*m*PPARδ
1a		5	H	10.1	NA	10.5
1b		5	Bn	NA	28.7	41.3
2a		6	H	NA	NA	NA
2b		6	Bn	NA	NA	NA
3a		5	H	NA	29.4	16.0
3b		5	Bn	12.8	43.1	21.7
4a		6	H	NA	NA	NA
4b		6	Bn	NA	NA	27.3
4c		6	*p*-F-Bn	NA	23.8	36.6

NA means not active, which is for compounds producing transactivation activity lower than 10% at 10 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.

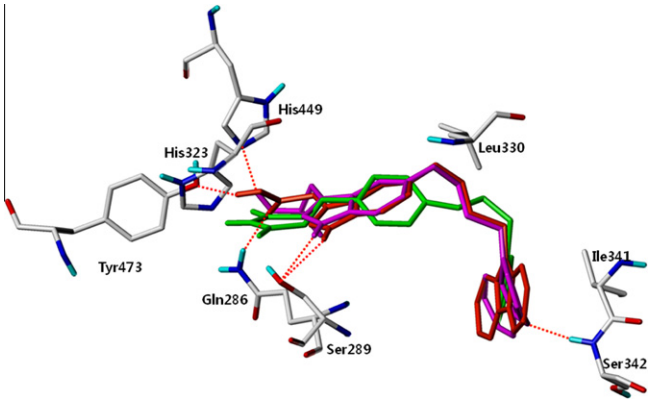


Figure 3. Putative binding modes of **1a** (red), **3a** (magenta) in the binding pocket of PPARγ co-crystallized with rosiglitazone (green) (PDB code: 2PRG). Only critical amino acids interacted with the docked ligand are displayed and labeled. Several hydrogen bonds discussed in the text are depicted as dashed red lines.

studies suggested that there might be different modes for the binding of our indole analogs, especially *N*-benzyl substituted analogs, in the active pocket of PPARγ.

Recently, crystallographic analyses of PPARγ co-crystallized with serotonin-metabolites and indomethacin, which contain indole acetate group as a common moiety, and fatty acid was reported (PDB code: 3ADX).[17] They provided two distinct subpockets; one is namely AF-2 pocket adjacent to helix H12 for binding of indole acetate-containing ligands and the other is Ω pocket for the binding of fatty acid metabolites. We employed this crystal structure of LBD complex with indole acetate-containing ligand and fatty acid ligand for the docking analysis of our indole compounds. Docking result indicated that indolylacetic acid moiety occupied AF-2 pocket with the polar interaction and the lipophilic tail occupied Ω pocket (Fig 5).

The carboxyl groups of both compounds **3a** and **3b** interacted with Gln286 and compound **3b** additionally with His449 in AF-2 pocket and the hydrophobic tail phenoxazine occupied similarly Ω pocket where fatty acid bound. In comparison of non-benzylated indole analog **3a** to benzylated analog **3b**, the NH of indole ring of

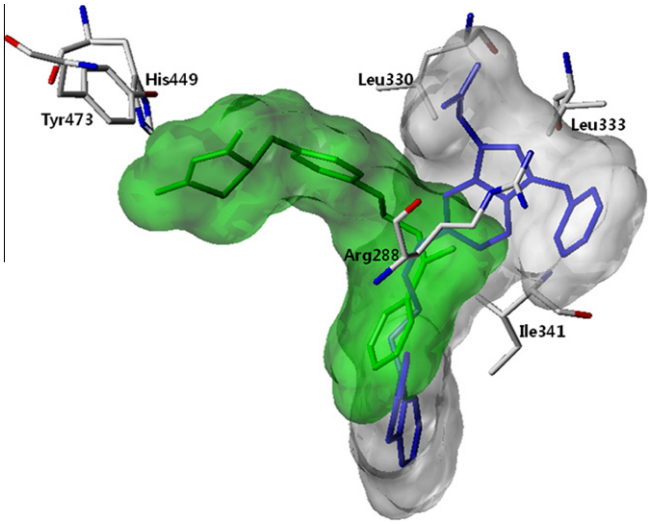


Figure 4. Comparison of the docked conformation of compound **3b** (blue) on the PPARγ-bound conformation of rosiglitazone (green) (PDB code: 2PRG). The connolly surface was generated around the ligand.

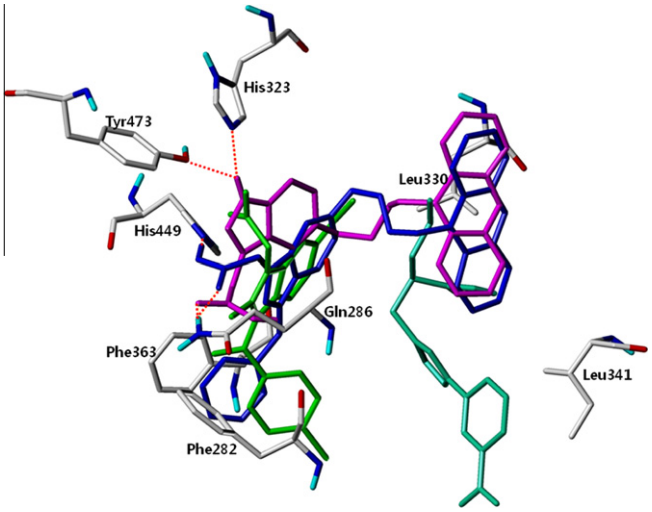


Figure 5. Putative binding modes of **3a** (magenta) and **3b** (blue) in the binding pocket of PPARγ co-crystallized with indomethacin (green) and fatty acid ligand (cyan) (PDB code: 3ADX). Only critical amino acids interacted with the docked ligand are displayed and labeled. Several hydrogen bonds discussed in the text are depicted as dashed red lines.

compound **3a** interacted with His323 and Tyr473. In contrast, introduction of *N*-benzyl substituent turned the indole ring over and revealed similar binding mode with indomethacin. Benzyl group of compound **3b** occupied hydrophobic cavity covered with Phe282, Phe360 and Phe363 in a similar way to *p*-Cl-benzoyl group of indomethacin. This result suggested that the additional hydrophobic interaction of benzyl group in the hydrophobic pocket of the binding site might be responsible to higher activity of *N*-benzylated indole analogs than those of non-benzylated analogs. These docking results guide further rational drug design of *N*-substituted indolylacetic acid analogs as a novel PPAR agonist.

In summary, we designed and synthesized carbazole or phenoxazine containing 5- or 6-alkoxyindolyl acetic acid analogs as PPARγδ dual agonist. In general, 5-alkoxyindole analogs were more active than the corresponding 6-alkoxyindole and phenoxazine was more favorable for hydrophobic moiety than carbazole. We propose two putative binding modes of our indole analogs by

docking analysis employing two crystal structures of PPARγ co-crystallized with rosiglitazone or indole acetate-containing ligands. *N*-benzyl substitution at indole increased PPARγδ activity and docking analysis indicated that our indole analog might have a different binding mode in LBD of PPARγ as compared to the well known PPARγ lignad such as rosiglitazone. We are exploring the further SAR studies and development of PPARγδ agonists by utilizing alkoxyindolylacetic acid template. We suggest that alkoxyindolylacetic acid might be useful not only as chemical tools to study PPAR function as well as development of a drug candidate for the treatment of metabolic disease.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.033.

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