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Design and synthesis of novel *N*-sulfonyl-2-indole carboxamides as potent PPAR-γ binding agents with potential application to the treatment of osteoporosis

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Abstract—The synthesis and structure–activity relationships of a novel series of *N*-sulfonyl-2-indole carboxamides that bind to peroxisome proliferator-activated receptor gamma (PPAR-γ) are reported. Chemical optimization of the series led to the identification of **4q** (IC_{50} = 50 nM) as a potent binding agent of PPAR-γ. Also reported is preliminary cell based data suggesting the use of these compounds in the treatment of osteoporosis.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily.[1] The PPARs have generated much interest in the pharmaceutical industry due to their effects in a widerange of therapeutic areas, and a number of agonists of this class have progressed through the clinic and to marketed anti-diabetic drugs.[2] Although the initial effort in this area was to find agonists, recent data have suggested that partial agonists and antagonists of PPARs (namely PPAR-γ) could be potentially beneficial in a number of areas. Over the past several years, PPAR-γ modulators have attracted increasing attention as potential treatments for osteoporosis. Recent evidence in both genetic and pharmacological studies suggests that attenuation of PPAR function can alter the balance between adipogenesis and osteogenesis, such that bone formation may potentially be enhanced.[3] In fact, using various animal models, the thiazolidinedione, rosiglitazone (a full PPAR-γ agonist), was shown to reduce bone mineral density and increase bone marrow adipocytes.[4] Thus, the regulation of PPAR-γ activation may control differentiation of mesenchymal stem cells allowing for osteoblast formation.[5] Due to this, there have been a number of reports in the literature regarding synthetic PPAR-γ modulators that affect adipocyte differentiation.[3,6]

There have been a number of reports in the literature about indole-derived PPARγ binding agents, and it was with these reports that our scaffolds were derived.[7] The synthesis of the *N*-sulfonyl-2-indole carboxamide compounds is outlined in Scheme 1.[8] Starting from the appropriately substituted ethyl indole-2-carboxylate, the 3-Ar-substituted compounds (**2a–f**) were synthesized by bromination (NBS, THF) followed by N-alkylation (K_2CO_3, DMF, R^3-Br) to yield **1**. Next, palladium-catalyzed Suzuki–Miyaura cross-coupling[9] ($Pd(PPh_3)_4$, 2 M Na_2CO_3, EtOH, toluene, $ArB(OH)_2$) gave the 3-Ar-substitution which was then carried on to the acid via saponification of the ethyl ester (3 M KOH, THF/EtOH, reflux). Finally, the target compounds, **2a–f**, were realized by coupling with a variety of aryl- or alkylsulfonamides (EDCI, DMAP, Et_3N, CH_2Cl_2) in good overall yield (58–93%). The indoles without substitution at the 3-position (3-H) were synthesized in an analogous fashion. Thus, the ethyl indole-2-carboxylate was N-alkylated (K_2CO_3, DMF, R^3-Br), the ester saponified (3 M KOH, THF/EtOH, reflux) followed by coupling

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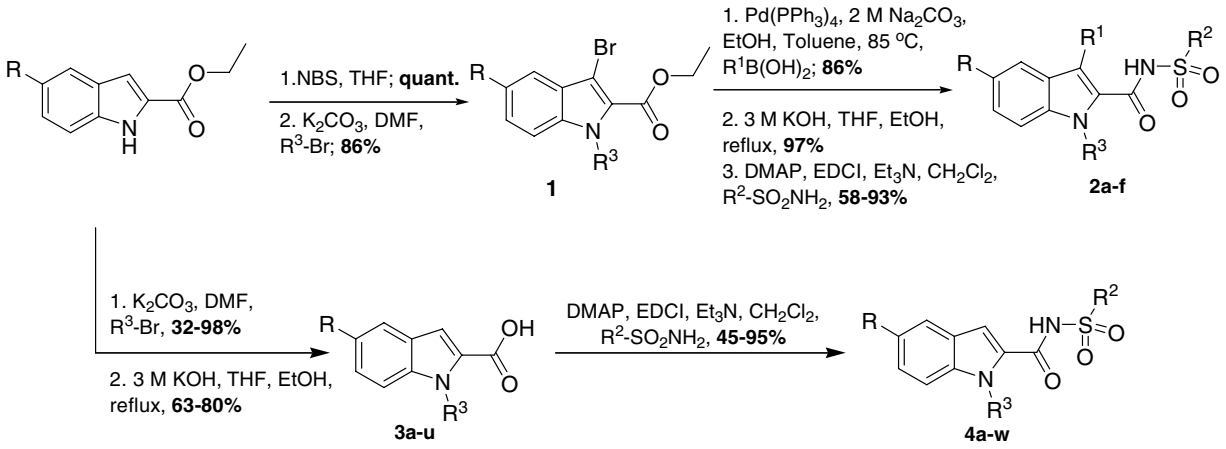


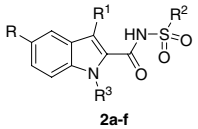
Scheme 1. Synthesis of *N*-sulfonyl-2-indole carboxamides.[10]

of an appropriate sulfonamide (EDCI, DMAP, Et_3N, CH_2Cl_2) giving the 3-H-substituted compounds **4a**–**w** in satisfactory overall yield (20–78%).

Our first efforts concentrated on evaluating 3-Ar *N*-sulfonyl-2-indole carboxamide compounds (**2a**–**f**). It was soon apparent (Table 1) that these were very potent ligands for PPAR-γ. Substitution at R^2 made very little impact on the binding data, as a variety of substituents were well tolerated (i.e., aryl vs alkyl, **2a** vs **2f**). The only drop in activity came when the phenyl group was substituted at the 3-position (**2c**, 60 nM).

However, since very similar compounds had been published,[7] our next attempt was to see what influence the 3-Ar-substituent had on the activity. With this, a number of compounds were made with R^1 = H investigating the SAR around several positions (R, R^2, and R^3). While keeping R^3 constant (3-CF_3Bn), we first looked at changing the sulfonamide portion (R^2) to evaluate whether the activity could be maintained as compared to the 3-Ar-substituted compounds (Table 2). The first compounds synthesized gave a noticeable erosion of binding activity (**2a** vs **4a**, 2 nM vs 290 nM). This loss

Table 1. PPAR-γ binding[11] for 3-Ar-substituted *N*-sulfonyl-2-indole carboxamides **2a**–**f**

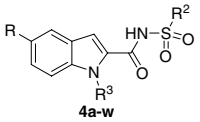


2a-f

Compound	R	R^1	R^2	R^3	IC_{50}[a] (nM)
2a	H	4-CH_3OPh	Ph	3-CF_3Bn	3
2b	H	4-CH_3OPh	4-F–Ph	3-CF_3Bn	7
2c	H	4-CH_3OPh	3-CF_3–Ph	3-CF_3Bn	60
2d	H	4-CH_3OPh	2-CO_2MePh	3-CF_3Bn	1
2e	H	4-CH_3OPh	2-CH_3Ph	3-CF_3Bn	7
2f	H	4-CH_3OPh	Me	3-CF_3Bn	2

[a] IC_{50} determinations are the average of three determinations on three separate days.

Table 2. PPAR-γ binding[11] for *N*-sulfonyl-2-indole carboxamides **4a**–**k**



4a-w

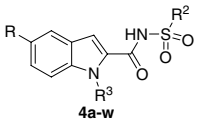
Compound	R	R^2	R^3	IC_{50}[a] (nM)
4a	H	Ph	3-CF_3Bn	290
4b	H	4-F–Ph	3-CF_3Bn	720
4c	H	4-Cl–Ph	3-CF_3Bn	280
4d	H	3-CF_3–Ph	3-CF_3Bn	80
4e	H	4-CH_3Ph	3-CF_3Bn	290
4f	H	2-CH_3Ph	3-CF_3Bn	180
4g	H	2-Naphthyl	3-CF_3Bn	90
4h	H	2-CF_3–Ph	3-CF_3Bn	80
4i	H	2-(5-Chlorothiophene)	3-CF_3Bn	80
4j	H	Me	3-CF_3Bn	680
4k	H	4-CF_3–Ph	4-CF_3Bn	700

[a] IC_{50} determinations are the average of three determinations on three separate days.

of activity was seen throughout most of the series with the exception, however, of a couple of compounds. First, the *N*-(3-(trifluoromethyl)phenyl)sulfonyl compound (**4d**) showed comparable activity to its 3-Ar counterpart (**2c**, 60 nM vs 80 nM). Other compounds that showed similar activity include **4g**–**i**, which all have substituents in either the *ortho*- or *meta*-position. One interesting observation was the loss of activity when going from the *ortho*- or *meta*-position to the *para*-position (**4d** and **4h**, 80 nM, vs **4k**, 700 nM). Also, unlike in the 3-Ar-substituted series, an aryl substituent on the *N*-sulfonyl group was essential to maintain good binding data (**4a** vs **4j**, 290 nM vs 680 nM).

Having established our best *N*-sulfonyl substituent (3-CF_3Ph, **4d**), we next evaluated the indole N-substituent (R^3) to see if there was any advantage for other groups (Table 3). The initial compounds made were closely related to the previously tested 3-trifluoromethylbenzyl compound (**4d**). The 3-methoxy- and 3-trifluoromethoxybenzyl compounds (**4l**,**m**) showed similar activity

Table 3. PPAR-γ binding[11] for *N*-sulfonyl-2-indole carboxamides **4l–t**



4a-w

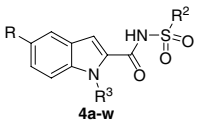
Compound	R	R^2	R^3	IC_{50}[a] (nM)
4l	H	3-CF_3–Ph	3-CH_3OBn	140
4m	H	3-CF_3–Ph	3-CF_3OBn	90
4n	H	3-CF_3–Ph	3-CF_3PhCO	9400
4o	H	3-CF_3–Ph	Et	2440
4p	H	3-CF_3–Ph	4-CF_3Bn	400
4q	H	3-CF_3–Ph	3-BnOBn	50
4r	H	3-CF_3–Ph	Bn	280
4s	H	3-CF_3–Ph	2,5-DiClBn	380
4t	H	3-CF_3–Ph	4-*t*-BuBn	330
Rosiglitazone				90
5[12]				80

[a] IC_{50} determinations are the average of three determinations on three separate days.

(140 nM and 90 nM, respectively). However, when the benzyl group was changed to a benzoyl group all activity was lost (**4d** vs **4n**, 80 nM vs 9400 nM). Alkyl substituents were also not tolerated in this position (**4o**, 2400 nM). The *meta*-substitution again proved to be the optimal compound; changing the 3-CF_3Bn to 4-CF_3Bn resulted in a significant loss of activity (**4d** vs **4p**, 80 nM vs 400 nM). The unsubstituted benzyl group proved to be less potent (**4r**, 280 nM) as well. However, the one change that proved beneficial was increasing the steric bulk at the *meta*-position of the benzyl group. Replacing the trifluoromethyl group with a benzyloxybenzyl group provided an increase in potency, albeit modest (**4d** vs **4q**, 80 nM vs 50 nM). We also evaluated the binding data of two known PPAR-γ modulators in-house, rosiglitazone (90 nM) and compound **5** (80 nM).

Lastly, we investigated substitutions on the phenyl ring of the indole (i.e., R), due to reports in the literature regarding this substituent.[7b] Keeping R^2 and R^3 constant, we were interested to see if these compounds would maintain the activity shown for the other compounds (Table 4). Starting from commercially available precursors we were able to obtain compounds **4u–w**. Unfortunately, these compounds showed much less activity than the parent R = H compound. Even small substituents (**4u**, R = Cl and **4u**, R = OH) showed a marked loss of binding.

Table 4. PPAR-γ binding[11] for *N*-sulfonyl-2-indole carboxamides **4u–w**



4a-w

Compound	R	R^2	R^3	IC_{50}[a] (nM)
4u	Cl	3-CF_3–Ph	3-CF_3Bn	330
4v	OBn	3-CF_3–Ph	3-CF_3Bn	770
4w	OH	3-CF_3–Ph	3-CF_3Bn	540

[a] IC_{50} determinations are the average of three determinations on three separate days.

To better understand the binding interactions of these sulfonyl indole carboxamides with PPAR-γ, compound **2a** was co-crystallized with the ligand-binding domain of the protein (PPAR-γ LBD) and the co-activator peptide fragment (SRC-1), and subjected to X-ray structure determination (PDB-id, **2HFP**).[13] Figure 1 illustrates the observed binding interactions between **2a** and PPAR-γ LBD. Interestingly, two molecules of compound **2a** were seen to span the binding pocket. Such a 2:1 stoichiometry of binding is not typical for reported PPAR-γ modulators.[14,15]

One molecule of **2a** (molecule B of Fig. 1) bound near the Helix-12 region and was shown to make hydrogen bonds with His449 through the carbonyl and sulfonyl oxygens. Also noteworthy with regard to molecule B is the close proximity to Tyr473, which has been implicated in the mechanism of action of PPAR-γ,[14a] although no distinct interaction is observed. The second molecule of compound **2a** bound at the opposite end of the PPAR-γ LBD (molecule A). This molecule occupied the area in PPAR-γ binding pocket similar to other known compounds, such as compound **5**,[12] and the *N*-methylaminopyridine portion of rosiglitazone.[13b] Hydrogen bond interactions are observed between the carbonyl and sulfonyl oxygens of this molecule and Ser342 and Lys265. The rest of the molecule makes productive hydrophobic interactions in the binding domain with residues Arg288, Leu330, and Ile341. The two bound ligand molecules interact with one another via their benzindole portions.

Having established a series of potent ligands for PPAR-γ, we next evaluated a number of these compounds in a cell-based assay to assess their potential to stimulate osteogenesis. For this measurement, we used the alkaline phosphatase (ALP) assay, where pre-osteoblastic cells were induced to produce alkaline phosphatase, a marker for osteoblast differentiation, on exposure to test compounds. Potency (EC_{50}) in this cell-based assay would indicate that a compound could potentially exert an osteogenic response in vivo. Such compounds may therefore be anabolic to bone, and thus offer a novel therapeutic utility to osteoporosis.

Compounds **2a**, **2b**, **4c**, and **5** (a reported PPAR-γ antagonist) as well as rosiglitazone were evaluated in the ALP assay (Table 5). Compared to rosiglitazone (a PPAR-γ full agonist) which was inactive in this assay, all compounds stimulated an alkaline phosphatase response, indicating the compounds induced osteoblast differentiation. Parallel cell count and viability measurements indicated the ALP response was not due to cell proliferation (data not shown). The 3-Ar analog, **2a**, proved to be the most potent with an ALP $EC_{50} \sim 100$ nM. An additional 3-Ar analog, **2c**, along with the 3-H analog, **4c**, were active in the 1–10 μM range.

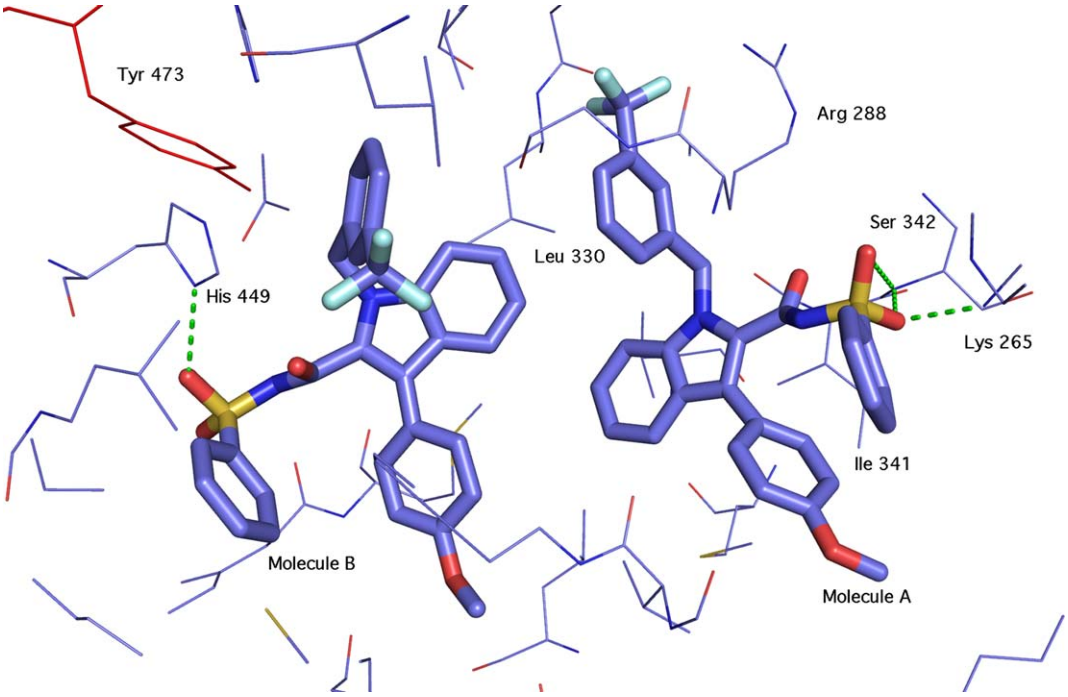


Figure 1. X-ray crystal structure of compound **2a** co-crystallized with PPAR-γ LBD and SRC-1. Ligands and relevant protein residues are shown as thick and thin sticks, respectively. The important Tyrosine 473 is highlighted in red; hydrogen bonds are represented by green dashes.

Table 5. Alkaline phosphatase (ALP) EC_{50} data for compounds **2a**, **2d**, **4c**, and **5**[16]

Compound	ALP EC_{50}[a] (μM)	Solubility (μg/mL)
2a	0.1	5
2d	1–10	ND
4c	1–10	ND
5[12]	0.1	ND

[a] EC_{50} determinations are the average of two determinations on two separate days.

In conclusion, we have described the synthesis and in vitro evaluation of novel *N*-sulfonyl-2-indole carboxamides as PPAR-γ binding agents. Several of these compounds were found to stimulate osteoblast differentiation in a cell-based assay, thus suggesting a potential application for the treatment of osteoporosis.

Acknowledgments

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8. All new compounds were characterized by ^{1}H NMR and high-resolution MS and found to be in agreement with their structures.
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10. Representative example. To a rt solution of acid **3q** (359.8 mg; 1.007 mmol) in CH_2Cl_2 (9 mL) were added DMAP (33.0 mg; 0.270 mmol), Et_3N (200 μL; 1.43 mmol), EDCI (232.3 mg; 1.212 mmol), and 3-(trifluoromethyl)benzenesulfonamide (285.6 mg; 1.269 mmol). The mixture was stirred at rt for 16 h. The reaction was quenched with 1 N HCl and then extracted with EtOAc. The organic extracts were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography (34 g SiO_2 pre-packed column from AnaLogix, 50% EtOAc in hexanes eluent) to yield 489.6 mg (0.8672 mmol; 86%) of the desired *N*-sulfonyl-2-indole carboxamide **4q** as a colorless, amorphous solid. ^{1}H NMR (DMSO-d_6) δ 8.31–8.27 (m, 2H), 8.10 (d, 1H, J = 7.8 Hz), 7.88 (t, 1H, J = 7.8 Hz), 7.75 (d, 1H, J = 7.8 Hz), 7.61–7.58 (m, 2 H), 7.38 (m, 6 H), 7.17 (t, 1H, J = 7.2 Hz), 7.03 (t, 1H, J = 8.1 Hz), 6.81 (dd, 1H, J = 8.1, 2.1 Hz), 6.58 (s, 1H), 6.40 (d, 1H, J = 7.5 Hz), 5.65 (s, 2 H), 4.95 (s, 2 H). HRMS (EI) *m/z* calcd for $C_{30}H_{23}F_3N_2O_4S$ (564.57), found 565.14 $(M+H)^+$.
11. PPAR-γ competitor assay. Twenty-seven nanomolar ligand binding domain of PPAR-γ (aa193-475) was added to 2.5 nM Fluormone, a high affinity, fluorescent-tagged PPAR-γ ligand (K_d = 9 nM). The PPAR-γ LBD/Fluormone complex yielded a high fluorescence polarization value (mP). Competitor compound was added to the complex, displacing Fluormone, yielding a lower fluorescence polarization value. Compounds that do not compete for PPAR-γ LBD binding will not reduce the fluorescence polarization value.
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13. (a) Two microliters of protein-peptide complex solution (10 mg/mL 206-477 His_6-PPAR-γ, 1 mM SRC-1 peptide fragment) was added to two microliters of well solution (2% Peg400, 1.6 M ammonium sulfate, and 100 mM Mes 6.5) and suspended over 1 mL of well solution in a hanging drop crystallization setup. The X-ray coordinates have been deposited with the Protein Data Bank, www.rcsb.org, as entries 2HFP (PPAR-γ/**2a**). Resolution for reported structures 2.0 Å; (b) Nolte, R. T.; Wisely, G. B.; Westin, S.; Cobb, J. E.; Lambert, M. H.; Kurokawa, R.; Rosenfeld, M. G.; Willson, T. M.; Glass, C. K.; Milburn, M. V. *Nature* **1998**, *395*, 137.
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16. Cell culture. MC3T3-E1 cells were obtained from ATCC (CRL-2594) and were grown in standard growth medium containing α-MEM and 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 and 90% air at 37 °C. Cells were plated at 2500 cells/well in 96 half-well plates and were grown to confluence in standard growth media. Once confluent, cells were cultured in differentiation medium containing α-MEM, 10% fetal bovine serum, 25 μg/mL ascorbic acid 2-phosphate, 10 mM β-glycerol phosphate, and compounds were resuspended in DMSO for 6 days. Fifty nanogram per milliliter of rhBMP-2 from R&D Systems was utilized as the positive control. Alkaline phosphatase activity. To assess alkaline phosphatase (ALP) activity, 10 μL Cell Titer 96® Aqueous One Solution Reagent (Promega) was added to each well of the 96 half-well plate containing 50 μL of culture medium. The cells were incubated for 1 h in a humidified atmosphere of 5% CO_2 and 90% air at 37 °C. Absorbance was read at 490 nm on a Molecular Device's SPECTRAmax PLUS 384 plate reader. After removing the culture medium, cell layers were washed once with D-PBS at room temperature. Fifty microliters ALP substrate solution (one tablet of Sigma 104® Phosphatase Substrate in 3.75 mL of substrate buffer containing distilled water, 50 mM glycine, and 1 mM $MgCl_2$ (adjust to pH 10.5) was added to each well and the cells were incubated at room temperature for 20 min. Absorbance was read at 405 nm on a Molecular Devices SPECTRAmax PLUS 384 plate reader. The enzyme activity was expressed as pNP nmols/min/million cells.