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2-Aryl-*N*-acyl indole derivatives as liver X receptor (LXR) agonists

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Abstract—Structure–activity relationship studies on a series of Boc-indole derivatives as LXR agonists are described. Compound **1** was identified as an LXR agonist through structure-based virtual screening followed by high-throughput gene profiling. Replacement of the indan linker portion in **1** with an open-chain linker resulted in compounds with similar or improved in vitro potency and cellular functional activity. The Boc group at the N-1 position of the indole moiety can be replaced with a benzoyl group. The SAR studies led to the identification of compound **8**, a potent LXRβ agonist with an EC_{50} of 12 nM in the cofactor recruitment assay.

Liver X receptors (LXRα and LXRβ, or NR1H3 and NR1H2)) belong to the type 2 family of the nuclear hormone receptor superfamily that function as transcription factors.[1] LXRα is expressed at a high level in liver, adipose tissue, and macrophages, whereas LXRβ is ubiquitously expressed. LXRs form heterodimers with retinoid X receptors (RXR) and regulate the expression of a number of genes involved in cholesterol homeostasis[2] and fatty acid metabolism.[3] LXR was also recently reported as a glucose sensor involved in liver carbohydrate metabolism.[4] Upon agonist binding, the DNA binding domain (DBD) of LXR interacts with LXR response elements on target genes to initiate the transcription process. One LXR target gene is the ATP-binding cassette transporter ABCA1, which is involved in reverse cholesterol transport (RCT) from macrophages to high-density lipoproteins (HDL) in the plasma.[5] Increasing RCT through LXR agonism is a potential therapeutic approach for a number of pathophysiological states including dyslipidemia and atherosclerosis.[6] Since cholesterol accumulation in pancreatic β-cells is reported to result in β-cell dysfunction in type 2 diabetes,[7] increasing expression of β-cell ABCA1 through LXR agonism would reduce the cholesterol accumulation, improve insulin secretion and glucose homeostasis in β-cell, and would be a viable approach for diabetes. A number of small molecule LXR agonists such as GW3965 (I)[8a,b] and T0901317 (II)[8c] have been described (Fig. 1). Some LXR agonists were shown to raise plasma HDL levels in mice and showed anti-diabetic activity in rodent models of type 2 diabetes.[9,10] However, most LXR agonists also induce the expression of sterol response element binding protein-1c (SREBP1c) gene, which controls the entire fatty acid biosynthetic pathway and increases lipogenesis and hepatic steatosis.

In order to identify selective LXR modulators that would have the potential to increase reverse cholesterol transport with minimum or absence of lipogenic activity, we first conducted a structure-based virtual screening using LXRβ homology model based on crystallography. Compounds (~1500) with high docking score were selected and profiled on twelve LXR regulated genes including LXRα gene using high-throughput genomic technology (HTG, Inc., Tucson, AZ).[11] An indan derivative **1** (Fig. 1) was found to up-regulate ABCA1 gene expressions to the extent similar to T0901317 or GW3965 (Table 3), while showing less regulation on SREBP-1c genes in THP-1 differentiated

Keywords: Liver X receptor; LXR agonist; Indole; ABCA1; SREBP-1c.
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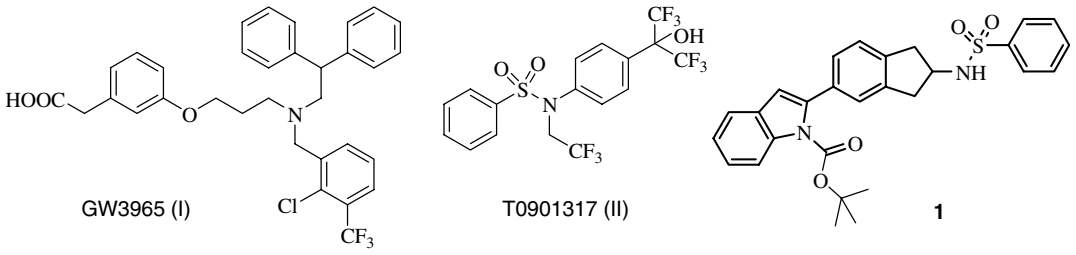


Figure 1. LXR modulators.

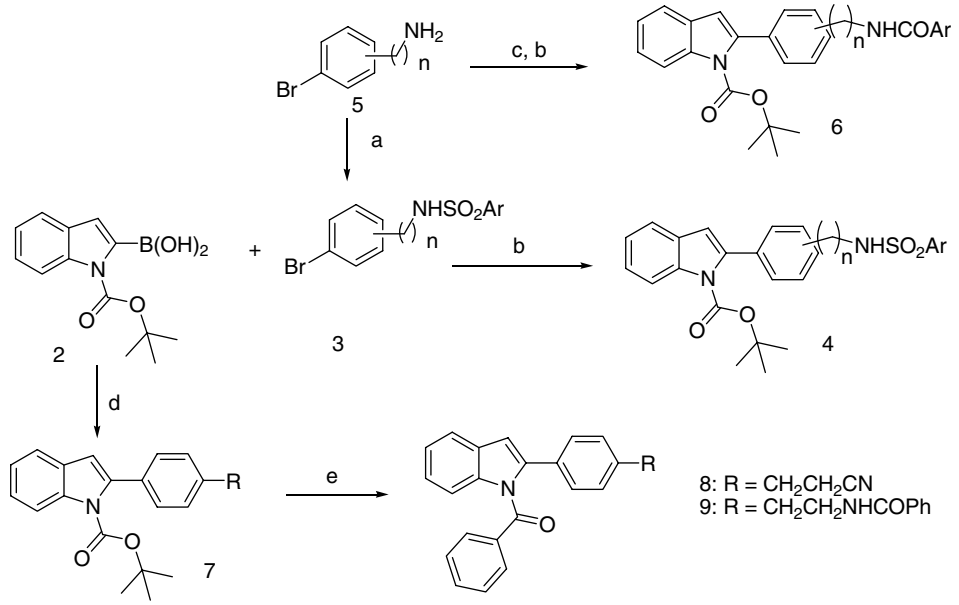
macrophages. Compound **1** was subsequently confirmed to be an LXR agonist in both a cofactor recruitment assay and a transactivation reporter assay (Table 3). Initial SAR indicated that an acyl indole moiety is essential for activity.[12] We herein describe the chemistry efforts on the indan linker modifications to explore the structure–activity relationship.

Indole sulfonamide compounds with an open-chain linker replacing the indan ring were prepared according to Scheme 1. The requisite substituted bromobenzene sulfonamides (**3**) were obtained from commercially available amines **5** via sulfonylation. Suzuki coupling of intermediates **3** with *N*-Boc-2-indole boronic acid **2** gave rise to the desired products **4**. Amide derivatives **6** were prepared in a similar fashion.

Suzuki reaction of *N*-Boc-2-indole boronic acid **2** with para-substituted bromobenzene provided compounds **7** with functionality other than sulfonamides or benzamides. Removal of the Boc group at the N-1 position of the indole moiety followed by benzoylation resulted in benzamide derivatives **8** and **9**.

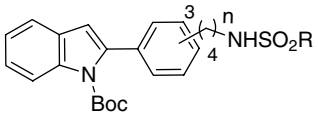
The compounds prepared above were tested in LXRα and β HTRF cofactor recruitment assays[13] and were rank-ordered by EC_{50} value for LXRβ (Tables 1 and 2). As observed with indan linker compounds,[12] these compounds tended to show slight selectivity for LXRβ over LXRα in the HTRF assay (Table 3). SAR compounds with good potency against LXRβ and/or with good selectivity were further profiled in a transactivation reporter[14] assay and cellular functional assays[15,16] (Table 3). Early SAR indicated that the indan moiety in **1** serves only as a linker to bring the Boc-indole and the sulfonamide functionalities together in a certain shape. We envisioned that a ring open system would be a viable surrogate for the indan ring. Indeed, phenethyl amine linker at either C-3 or C-4 position retained the potency with the C-4 substitution being slightly more potent than the corresponding C-3 substituted analog (**11** vs **10**) (Table 1). Compounds with shorter (CH_2) and longer [$(CH_2)_3$] spacers were tolerated (**12**, **13**), whereas aniline derivative (**14**) suffered a 7-fold loss in potency. The 2-fold increase in potency seen with 2-thienyl sulfonamide **15** (EC_{50} = 58 nM) followed a similar SAR trend observed in the aminoindan linker series.[12] Substitution on the thiophene ring (**16**) reduced the potency.

As shown in Table 2, benzamide derivatives appear to be less potent than the corresponding sulfonamides if the spacers are methylene or ethylene (**11**, **12** vs **17**,



Scheme 1. Reagents and conditions: (a) $ArSO_2Cl$, DIEA, CH_2Cl_2, rt; (b) $2Pd(PPh_3)_4$, Cs_2CO_3, toluene/ethanol/water (10:1:1); (c) ArCOCl, DIEA, CH_2Cl_2, rt; (d) *p*-R-PhBr, $Pd(PPh_3)_4$, Cs_2CO_3, toluene/ethanol/water (10:1:1); (e) TFA, CH_2Cl_2, rt then PhCOCl, DIEA, CH_2Cl_2, rt.

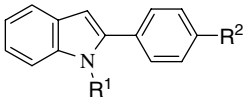
Table 1. Open-chain analogs of compound **1**



Compound	Attachment position	*n*	R	EC_{50} (μM)[a] LXRβ
1	—	—	—	0.19
10	3	2	Ph	0.42
11	4	2	Ph	0.12
12	4	1	Ph	0.22
13	4	3	Ph	0.35
14	4	0	Ph	1.32
15	4	2	2-Thienyl	0.058
16	4	2	5-(2-Pyridinyl)-2-thienyl	0.23

[a] HTRF assay results are mean values of duplicate samples in a single experiment.

Table 2. Linear linker derivatives as LXR agonists



Compound	R^1	R^2	EC_{50} (μM)[a] LXRβ
17	Boc	$–CH_2NHCOPh$	1.8
18	Boc	$–(CH_2)_2NHCOPh$	1.75
19	Boc	$–(CH_2)_3NHCOPh$	0.36
20	Boc	$–CH_2NH_2$	5.6
21	Boc	$–CH_2CN$	0.3
22	Boc	$–(CH_2)_2CN$	0.13
23	Boc	$–(CH_2)_3OH$	0.38
24	Boc	$–O(CH_2)_2OH$	0.42
25	Boc	$–(CH_2)_2CO_2H$	0.22
26	Boc	$–(CH_2)_2CONH_2$	0.057
27	Boc	$–(trans\text{-}CH_2{=}CH)CONH_2$	4.9
28	Boc	$–(CH_2)_2CONHMe$	0.49
29	Boc	$–(CH_2)_2CONHCH_2CH(CH_3)_2$	1.51
30	Boc	$–(CH_2)_2CONHPh$	1.78
8	COPh	$–(CH_2)_2CN$	0.012
9	COPh	$–(CH_2)_2NHCOPh$	1.78

[a] HTRF assay results are mean values of duplicate samples in a single experiment.

18). However, when the spacer is extended to a propylene moiety, both sulfonamides and benzamide are equally potent (**19** vs **13**). Free amino group-containing compound **20** is about 10-fold less potent than the corresponding cyano derivative (**21**). Increase in the linker length in the cyano derivative improved the activity slightly (**22** vs **21**). A hydroxyl group (**23**, **24**) or a carboxylic acid group (**25**) on the terminal seems to be well tolerated. A dramatic improvement was observed with the primary amide (**26**), which showed a 4-fold improvement in the activity over the corresponding carboxylic acid. Substitutions on the amide nitrogen resulted in a decrease in the potency (**28**, **29**, and **30** vs **26**). Introduction of α,β-unsaturation (**27**) led to more than 80-fold loss of the activity (EC_{50} = 5.9 μM), indicating that the hydrogen-bond interaction on the terminal portion plays a key role in the binding to the LBD. It is worth noting that amide bond orientation in the linker portion did not affect the activity (**18** vs **30**).

Previously we found that the replacement of the Boc group with even closely related carbamate, amide or urea moieties led to a complete or near-complete loss of activity in the indan series.[12] However, in the current open-chain linker series, the Boc moiety can be replaced by a benzoyl group. For instance, compound **9** with an *N*-benzoyl group showed similar activity as its Boc analog (**30**). Compound **8**, which possesses a cyano group on the terminal and a benzoyl group on the indole nitrogen, demonstrated a 10-fold increase in potency over the corresponding Boc derivative **22**.

A few selected compounds with varying activity and selectivity profiles in cofactor recruitment assays were further tested in a reporter transactivation assay for LXRβ as well as other cell-based functional assays. In general, compounds with good potency in the cofactor recruitment assay maintained good potency in the cellular reporter assay and the cholesterol efflux assay with some exceptions (e.g., **25**). Most of compounds in the series are partial agonists as compared to GW3965 in the LXRβ reporter transactivation assay. Compound **8**, which showed similar potency in the cofactor recruitment assay but with a slightly better selectivity profile (LXRβ over LXRα) than GW3965 and T0901317, demonstrated almost identical gene expression profiles as GW3965 for ABCA1 and SREBP1c. LXRβ reporter transactivation assay appears to correlate with ABCA1 gene regulation as well as cholesterol efflux in THP-1 cells (Table 3). Compound **8** induced more significant lipogenesis than GW3965, indicating that the LXRα activity may be a more important contributing factor to the lipogenesis than the LXRβ activity. However, due to the lack of LXRα reporter transactivation data, no clear correlation can be drawn among LXRα potency and SREBP-1c regulation or lipogenesis.

Docking studies suggested that compound **1** fits nicely in the LXRβ ligand binding domain (Fig. 2). The indole aromatic ring system is close to Trp457 in the AF-2 (helix 12) and forms a π–π interaction while the Boc carbonyl group forms a hydrogen bond with the conserved His435 in helix 10/11, thereby locking it into an agonistic conformation.[17] Compound **8** overlaps well with **1** and fits nicely in the active site. The benzoyl group at the indole nitrogen occupies the hydrophobic region in a similar fashion as the Boc group. The 2-phenyl group in **8** or the indan ring in **1** seems to be close to the Phe 271, while the terminal cyano group is located in proximity to Leu314 via a hydrogen bonding interaction. The sulfonamide group may have an interaction with Arg319 (not shown).

In summary, we have described a series of potent LXR agonists based on the initial hit compound **1** identified by using a combination of virtual screening and high-throughput gene profiling. The rigid indan linker in the initial hit compound **1** could be replaced with an open-chain linker and the potency was retained or

Table 3. Profile of selected LXR agonists

Compound	HTRF EC_{50}[a,b] LXRα	HTRF EC_{50}[a,b] LXRβ	Reporter EC_{50}[a] LXRβ	ABCA1 induction[d] (THP-1)	SREBP-1c induction[d] (HepG2)	Efflux EC_{50}[a] (THP-1)	Maximum efflux[d]	Lipogenesis induction[d] (HepG2)
1	1.5	0.206	0.065 (43%)[c]	8.4	3.1	0.680	1.6	3.1
22	1.19	0.155	0.576 (45%)[c]	6.0	2.5	2.47	2.2	2.5
25	1.15	0.225	4.58 (76%)[c]	5.5	Nd[e]	7.0	1.4	1.2
26	1.11	0.057	0.285 (70%)[c]	5.7	4.0	2.4	1.9	2.7
8	0.22	0.012	0.042 (39%)[c]	10.3	5.3	0.051	1.7	4.0
GW3965	0.367	0.035	0.015 (100%)[c]	10.7	5.2	0.010	1.8	2.7
T0901317	0.04	0.015	0.011 (84%)[c]	8.4	6.7	0.033	1.6	3.6

[a] Values shown are in micromolar.
[b] All compounds including T0901317 are full agonists compared with GW3965 in both LXRα and LXRβ HTRF assays.
[c] Values shown are in percent of maximum induction by GW3965.
[d] Values shown are in ratio of compound (10 μM) versus control (vehicle only).
[e] Not determined.

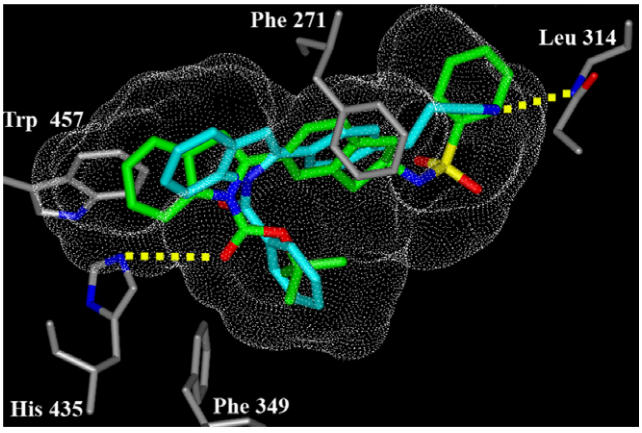


Figure 2. An overlay of compounds **1** and **8** docked into the active site of ligand-binding domain of LXRβ based on X-ray cocrystal structure of GW3965.

improved. The sulfonamide functionality, which may act as a hydrogen bonding donor in the initial hit, could be replaced by hydrogen bonding acceptors such as a cyano group. These compounds tend to be moderately selective for LXRβ in HTRF cofactor recruitment assay. Replacement of *N*-Boc moiety in the indole portion with a benzoyl group not only retained or improved the potency, but also improved the metabolic stability (not shown). Compound **8** was identified as the most potent and stable compound with an EC_{50} of 12 nM. Although they are active in cell-based functional assays such as cholesterol efflux in THP-1 macrophages, they also upregulate the undesirable SREBP1-c gene expression and cause lipogenesis in the HepG2 cells.

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13. The HTRF cofactor peptide recruitment assay was modified from a previous report. Briefly, polyhistidine-tagged human LXRα (2 nM) or LXRβ (1 nM) ligand-binding domain (Roche Diagnostics, Indianapolis, IN) was mixed with the test compound, 20 nM biotin-SRC1 peptide (Synpep, Dublin, CA), 5 nM streptavidin-allophycocyanin, and europium-labeled anti-polyhistidine antibody (1

and 0.5 nM for α and β, respectively) in 50 mM Tris, pH 7.5, 50 mM KCl, 1 mM EDTA, 0.1% BSA, and 1 mM DTT. The final volume of the mixture was 40 μl in a 384-well assay plate. The mixture was incubated at room temperature for 1 h with shaking. Time-resolved fluorescence was measured at 615 and 665 nm on an LJL Analyst plate reader. The ratio of 665/615 was used to calculate EC_{50} values of test compounds.

14. COS-7 cells were transfected for 24 h with plasmids of the human LXRβ receptor (OriGene, Rockville, MD), the LXR reporter +-GACCAGCAGTAACCTTGACCAGC AGTAACCTTGACCAGCAGTAACCT (prepared in house), and the RXRα receptor (OriGene, Rockville, MD). Subsequently, cells were treated with vehicle or compound for 16 h. LXR reporter activation was monitored by quantifying the luciferase activity in the cell lysate. EC_{50} values were calculated from mean values of quadruplicate samples in a single experiment.
15. Compound induction of cholesterol efflux was measured as described previously with small modifications. Briefly, THP-1 cells were differentiated into macrophages in 48-well tissue culture plates by 30-h treatment with 200 nM PMA. The cells were then labeled with 0.6 μCi of 1,2-^{3}H (*N*)-cholesterol for 18 h in the presence of 16 μg/ml acLDL and 200 nM PMA. Cells were then incubated with vehicle or compound for 6 h in serum-free media containing 2 mg/ml BSA. Subsequently, 5 μg/ml of APO-AI was added in fresh, serum-free medium along with vehicle or compound for an additional 18-h incubation. Cholesterol efflux was monitored by quantifying the radioactivity in the cell supernatant and the data are presented as fold-induction versus control (vehicle only). Results shown are mean values of triplicate samples in a single experiment.
16. To measure compound induction of lipogenesis, HepG2 cells in a 48-well tissue culture plate were pre-treated with vehicle or compound for 24 h. [^{14}C]-glycerol (1 μCi) was added and the cells were cultured for another 48 h. Cellular triglycerides were extracted, separated by TLC, and quantified on a Storm 820 phosphorimager (GE Healthcare, Giles, UK). Lipogenesis was presented as fold-induction versus control (vehicle only). Results shown are mean values of triplicate samples in a single experiment.
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